Project no.	*023044*
Project acronym	***NUTRA-SNACKS***
Project title	*Ready to eat food for breakfast and sport activity with high content of nutraceutics reducing a disease risk and promoting public health*
Instrument	*SPECIFIC TARGETED RESEARCH*
Thematic Priority	*Food Quality and Safety*

NUTRA-SNACKS Final Activity Report
"Publishable Final Activity Report"

Period covered	*From 1st May 2006 To 31th October 2009*
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Project coordinator name	*Maria Teresa Giardi*
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Content

1. Project execution

Objective	Major Achievements
1. Strategic Objective 1	Extraction of various metabolites with antilipidemic, anticholesterol, antimicrobial, antibacterial, anticancer, anti-inflamatory and antioxidant activity etc.
2. Strategic Objective 2	Improvement of yield and quality by application of elicitors and applying the techniques of the plant cell growth in vitro toward nutrition. Application of the transformants as "biological factories" for further extraction of the functional metabolites.
3. Strategic Objective 3	Feasibility study for the realization of a bioreactor to produce Nutraceutics; a set of sensors to monitor the content of the metabolites during the production was preliminarily developed.
4. Strategic Objective 4	Contribution to define a standard for new regulations on Nutraceutic, Safety and Quality Control Methods, Risk Assessment, Manuals and Tests, Certifications by applying a set of control analyses
5. Strategic Objective 5	We provided to offer on the market a wider range of new high quality ready-to-eat products for breakfast, snacks and sportsman nutrition that will result in a short-term in an improvement in the industrial economical balance. A lot of interest was obtained by mass-media on the nutra-snacks commercialization

The Project wants to develop the application of plant cell and in vitro culture systems together with a biotechnological approach to provide facilities for the production of new high quality ready-to-eat food with functional activity useful for promoting public health. Nutra-Snack aims at the production of food at high content of natural metabolites with the following recognized health activities: anticancer, antilipidemic, anticholesterol, antimicrobial, antibacterial, antifungal, antiviral, antihypertensive, antiinflammatory and antioxidant activity etc. The team, which is composed of 5 European countries and a candidate country (7 research institutes and three SMEs) has the main objectives in the following:

1. Selection of organisms producing natural biologically active metabolites with recognized anticancer, antimicrobial, antibacterial, antifungal, antiviral, antihypertensive, anti-inflamatory, anticholinergics and antioxidant activity.
2. Realization of bioreactor/sensors for innovative plant cell and in vitro systems suitable for nutrient production; protocols for production of biological material enriched on functional metabolites which counteracts the main health risks.
3. Use of genetic transformants in order to enhance the synthesis of the desired metabolites as "biological factories" for further extraction of functional metabolites for food additives
4. The production of new ready-to-eat food for breakfast, snacks and for the nutrition of those involved in sports will receive highest priority
5. Development of safety and quality control protocols for the new ready-to-eat-food
6. Contribution to define a standard for new regulations on Nutraceutics

Many traditional food products such as fruit, vegetables, soy, whole grain cereals and milk have been found to contain components with potential physical health. These components are now being incorporated in new food products.
The NUTRA-SNACKS' approach utilized various strategies and incorporated a variety of methodologies and disciplines relevant to the whole food production chain by covering all the areas outlined in the Specific Programme for this priority. "T *5.4.1.3 is on improving the quality and safety of ready-to-eat products and semi-prepared foodstuffs by the exploitation of new and innovative technologies".* The objective was to improve the quality, taste, safety and nutritional content of ready-to-eat products and semi-prepared foodstuffs by monitoring more carefully the quality and safety of the raw material and the development of innovative processes all along the production chain.

The work wanted to encompass consumer demands for a greater choice and a cut down in the use of some unhealthy ingredients such as saturated fats and greater attention paid to the safety and quality of the raw material used. The involvement of SMEs was of great importance. The results included new products (including improved traditional products) based on innovative manufacturing processes and improved, constant monitoring techniques to eliminate chemical and pathogenic contamination along the entire food production chain. NUTRA-SNACKS prime area of research was targeted towards major public health issues relevant to diets (e.g. cardiovascular disease, cancer or diabetes) or to issues regarding dietary supplementation or fortification. Consumers' demands are of vital importance and must be met and it will be necessary to find a mechanism that can combine these requests and key public health problems. Identification of research most likely to yield fast progress towards tangible results. Giving priority to such areas should (i) justify the need for the new nutrigenomic approaches, (ii) hasten the development of generic approaches applicable to a wide range of diet-related issues of socio-economic significance, and (iii) enhance the profile of nutrition research in Europe. The project *considered social and policy objectives* where the primary role of a diet is to provide sufficient nutrients to meet the nutritional requirements of an individual. There is now increasing scientific evidence that some food components have beneficial physiological effects over and beyond the provision of basic nutrients. Today, nutrition science has moved on from the classical concepts of overcoming nutrient deficiencies and implementing basic nutritional adequacy to the concept of "positive" or "optimal" nutrition. NUTRA-SNACKS research focused attention on the production of food, rich in biologically active components, which have the potential to optimize physical well being and reduce in some cases the risk of disease. The Project developed the application of plant cell and in vitro culture systems together with a biotechnological approach to provide facilities for the production of new high quality ready-to-eat food with functional health promoting properties. Nutra-Snacks aimed at the production of food with a high content of natural metabolites with the following recognized health promoting activities: anticancer, anti-lipidemic, anti-cholesterol, antimicrobial, antibacterial, antifungal, antiviral, antihypertensive, anti-inflammatory and antioxidant. The project consortium included 6 EU countries with participants from the public (7 research institutes) and private sector (3 SMEs) teamed together in an effort to combine different sets of skills and competences culminating in the production of innovative ready-to-eat food products. The consortium's main objectives were as follows:

- Selection of organisms producing natural biologically active metabolites with recognized anticancer, antimicrobial, antibacterial, antifungal, antiviral, antihypertensive, anti-inflammatory, anticholinergics and antioxidant health promoting activities.

- The construction of bioreactor/sensors for innovative plant cell and in vitro (production) systems suitable in nutrient production; protocols for the production of biological material enriched with functional metabolites which counteract major health risks.

- Use of genetic transformants in order to enhance the synthesis of the desired metabolites as "biological factories" for further extraction of functional metabolites to be used in food additives

- The production of new ready-to-eat food suitable for breakfast, snacks and for sports people that will receive the maximum priority

- The drawing up of safety and quality control protocols for the new ready-to-eat-food.

- Contribution to define new standards for the nutraceutics industry completes with regulations and risk assessment documentation. Besides, the consortium is set to devise new methods to assess the potential health hazards of these innovative food products.

List of Participants

1. Consiglio Nazionale delle Ricerche (Italy)
2. Martin-Luther-Universitaet Halle-Wittenberg (Germany)
3. University of Crete (Greece)
4. Institute of Soil Science and Plant Cultivation (Poland)
5. Université di Pisa (Italy)
6. National Institute for Biological Sciences (Romania)
7. Université de Perpignan (France)
8. NéoSENS SA (France) (present in the consortium until 24th Months)
9. ENERVIT S.p.A (Italy)
10. DAS S.r.l. (Italy)
11. University of Milan (Italy) (Subcontractor of P 9)

Concerning the production of metabolites from various organisms, we worked with various plant species such as soybean, mint (*Mentha piperita* and *Mentha longifolia*), dandelion, basil and clover. Finally, plants known to be safe for human nutrition were selected. However, after analysis of antioxidant properties of extracts from those plants which has been done at the University in Milan, we decided to focus on mint and basil since the extracts from those plants were the most effective to protect cell lines from damage provided by free radicals.

The goal was to establish and characterize the suspension cultures, examine whether the biosynthesis of selected compounds e.g flavonoids and polyphenols, occurs in cells and finally to optimize an efficient system of elicitation which can improve synthesis of desired secondary metabolites in those cultures. This approach was pursued by University of Pisa and Institute of Soil Science and Plant Cultivation in Poland.

Therefore, leaves coming from *in vivo* plants as well as plants germinated *in vitro* were tested for callus induction. To establish proper calli (fragile, loose and soft) we tested leaves, leaf stalks and fragments of stem all coming from 3-weeks old *in vitro* plants. Callus tissues were formed mainly from the leaves explants. After transfer to liquid medium and submitted to constant agitation callus begin to dissociate into single cells, clusters, clumps and small as well as big aggregates and all cultures were generated. Sterile meshes were used in order to separate large aggregates and established a suspension culture only with single cells, clusters and small aggregates. Typically, two months are needed before cell suspension cultures become stable and to examine secondary metabolism of *in vitro* plant cells, suspension cultures are used only when culture cycle is stabilized, usually after at least 5 generations.

As it concerns the analyses of metabolites, different plant subcultures were cultivated *in vitro* to establish a growth curve either for the whole plants or for callus of four different cultivars of Basil (*Ocimum basilicum* L. var. Dark Opal, Superbus, Red Rubin and Gecom). From a phytochemical point of view basil essential oils (EO) and volatile constituents were analysed from *in vitro* plant material in comparison with plants coming from greenhouse. The EO yield was very abundant from *in vitro* seedlings, but Methyleugenol and Eugenol were the main compounds identified in both extracts. Therefore, on the basis of the detected presence in basil essential oil of Methyleugenol –a potential carcinogen, that must not be in food products as stated by the Council of Europe (1999)-, we have established to focus on antioxidant extracts with the highest activity, such as Sage and Mint extracts.

An elicitation program of the selected *in vitro* plant cultures was performed in order to increase the production of secondary metabolites and several elicitors were tested. For instance it was found that Methyl jasmonate increase the synthesis of Rosmarinic acid of 11 %. Monoterpene and sesquiterpene derivatives were present in minor percentage. In particular the oxygenated monoterpenes represented the predominant monoterpene class in all sample analyzed, while sesquiterpene percentages ranged from 2 to 16.%. The oxygenated hydrocarbon compounds, 2,4-nonadienal and 2,6-nonadienal were present in relevant percentage with the oxygenated monoterpene compounds borneol and (E)-methyl isoeugenol (1-16.%).

Callus cultures of *Ocimum basilicum* L., Dark Opal (DO) and Red Rubin (RR) cultivars were subjected to UV irradiations as elicitation method. The headspaces of DO irradiated callus were characterized by a high percentage of the monoterpenes. In particular the non-oxygenated monoterpenes represent the predominant chemical class in all analyzed samples (24-44%). The aroma of RR irradiated callus was characterized by a high percentage of hydrocarbons and oxygenated monoterpenes. Eugenol was present in minor percentage in both DO and RR callus. The EO yields of *O. basilicum* in vitro plant material were lower than those obtained from the adult plants. However the *O. basilicum* callus was already able to produce the typical volatile constituents of *O. basilicum* adult plants. SPME-

GC-MS analysis confirmed the presence of the main constituents methyleugenol, 1,8-cineol and trans-b-bergamotene. Turmeric *in vitro* cultures (*C. longa*) were obtained using sprouting buds as initial explants. The first goal was the proliferation of shoots to be further used for regenerating plants, as well as for dissecting organs. Our experiments showed that one of the important factors which influenced callus tissues induction was the type of explants, since only callus tissues were achieved in sufficient amount from basal part of shoots. The analysis of curcuma rhizome extracts showed a good amount of curcumin derivatives. An induction of microrhyzomes from *in vitro* turmeric plantlets was obtained by choosing selected media and environmental conditions. Different samples showed different content in secondary metabolites curcumin, demethoxycurcumin and bisdemethoxycurcumin.
The use of plant cell culture was found to produce essentially one main component (e.g. rosmarinic acid), changing the composition of the natural extracts from plants. It was considered that this methodology leads to pure compound that own to the pharmaceutical approach more than a nutritional one; therefore, it is important to observe that can be useful for precious metabolites e.g. Taxol –a natural anticancer metabolite- .

Concerning use of transformants for nutrition purposes, it is has a social limitation but this point of view is still under discussion. This approach in the project was basic research waiting for more clarification/approval in Europe. The transformants seem to have a future as biological farms and particularly *C. reinhardtii* that grows at high rate and can be easily transformed. The Nutra approach regarding transformants was innovative and we believe that in the long term can give important results. As outlined in the project description, a major goal is the development of algae strains enriched with bioactive peptides that could be used as nutraceutical additives. A wide range of activities has been described for such bioactive peptides, including for example antithrombotic and antimicrobial properties, blood pressure and cholesterol lowering effects, immunomodulatory activities and opioid activities. Moreover there are peptides with antioxidative properties that prevent oxidation of essential cellular compounds like DNA, proteins and fatty acids by reactive oxygen species. Food derived peptides with antioxidant activity have been identified from many protein hydrolysates, especially from milk and whey. After protein hydrolysis from such sources different peptides were isolated with individual antioxidative properties. The highest radical scavenging activity of all peptides investigated could be determined for the WYSLAMAASDI sequence. The antioxidant activity of this peptide is even higher than that of butylated hydroxyanisole (BHA), which is currently used in the food industry as a powerful synthetic antioxidant.
In order to exploit the antioxidant effect of this peptide we have started to construct an alga, which should be able to produce the foreign peptide. *C. reinhardtii* mutant strain (termed MCS8) was generated by Martin-Luther-Universitaet Halle-Wittenberg (Germany) partner without involving antibiotic resistance markers. He succeeded in engineering a cassette system for the introduction of foreign peptides/proteins into the green alga *C. reinhardtii* in a marker-gene-free transgenic approach. Expression and correct processing of the transgene product was achieved by a translational fusion of the cassette with a highly abundant chloroplast protein, which is proteolytically "cut-to-size" at its C-terminal end involving a protease naturally occurring inside the cell. The multipurpose cassette contains two separate multiple cloning sites and three different tags for easy identification and isolation procedures. Results from these experiments demonstrate that the newly generated *C. reinhardtii* mutant MCS8 has growth rates equivalent to the control strains, the 8 kDa peptide encoded by the cassette is cleaved to the precursor and the peptide appears to be stable. Furthermore, in the selection of transformants no antibiotic resistance genes were involved.
The cassette system was employed for the introduction of peptides or proteins of nutritional value into the green alga *Chlamydomonas.* Several advantages can be envisaged in this innovative molecular approach. For example the fusion of foreign proteins/peptides to the D1 protein can be obtained, instead of just producing them directly. Moreover, coupling a foreign polypeptide (transcriptionally/traslationally) to the highly expressed D1 protein in the chloroplast, will allow an increased production of the foreign protein.
In order to characterize the *psbA*-Antiox *C. reinhardtii* strain in greater detail, the decrease of the chlorophyll content under high light conditions (2000 µmoles/m^2/s) was compared to the reference strain. Under these stress conditions increased amounts of ROS are generated, which in turn could be inactivated by the antioxidant peptide. In accordance we found that the mutant is significantly more stable than in the reference strain. This is a first and strong indication that the peptide has protecting effect on the pigment inside the cell and potentially could be used for human health (in any case after approval of the molecular approach by EU).
A lot of work was performed by German partner in collaboration with Consiglio Nazionale delle Ricerche (Italy) and University of Crete (Greece) partners, to induce in *C. reinhardtii* the synthesis of healthy antioxidant pigments like xanthophylls and correlated metabolites (such as Zeaxanthin and Lutein). In an attempt to induce the over-production

of pigments with high antioxidant activity in *C. reinhardtii* strains IL, A250R and S264K were treated as elicitation strategy by high light and high temperature (1000 µmol photons/m^2/s and 37°C). Samples were harvested to analyze cell density, viability, PSII quantum yield, chlorophyll content and pigment composition. Measurements of cell densities and total chlorophyll content revealed no changes in both IL and D1 mutant strains after exposure. Interestingly, we observed a significant decrease of cell viability just 15 min following exposure in IL strain, but not in the D1 mutants. The maximum quantum yield of PSII photochemistry was strongly affected. In particular, the most relevant decrease was noticed in S264K strain, in which the aminoacid substitution modifies the binding of Q_B quinone, hence the photosynthetic efficiency. HPLC analyses indicated activation of the xanthophyll cycle and of zeaxanthin levels in IL and A250R strains but not in the S264K mutant. The S264K mutant reveals the lowest accumulation levels compared to the other strains. Results concerning other carotenoid and chlorophyll accumulation levels followed undefined trends. This experiment indicates IL strain as a good candidate for the preparation of xanthophyll enriched particles for the Nutra project.

For nutritional purposes, new protocols have been developed, using safe reagents, for the extraction of thylakoid membranes enriched in Zexanthin and Lutein from *Chlamydomonas reinhardtii* cells and for their subsequent immobilization into calcium alginate beads by university of Creete in collaboration with Natioanl Council of Research-Rome. The reagents used for thylakoid extraction were safe for humans (such as phosphates instead of tricine buffers). Also three essential oils from *Salvia officinalis*, *Mentha officinalis* and *Ocimum basilicum*, provided by University of Pisa and Institute of Soil Science and Plant Cultivation (Poland), highly active as antioxidants, have been immobilized into calcium alginate beads. Two different immobilization conditions were used for each sample. The essential oils were immobilized either in the presence or in the absence of sodium cholate which can form micelles and thus the essential oil can be better dissolved and stabilized. The antioxidant activities of the immobilized samples have been evaluated by the partners in collaboration.

For the final Snack, three product formats have been developed and considered by Enervit Company in Italy, cluster, chips, extruded snacks. Since the cluster format is very interesting from the point of view of commercial interest, new flavor versions different from chocolate have been developed. Production by specialized manufacturer is suitable from 9 tons production batches which are a large quantity. Enervit developed a neutral version to which several flavors and additives can be added. This has the clear benefit of cumulative production volumes since one big production of neutral surrogate can be done, this last can be divided into minor flavored fractions during cluster production. Furthermore neutral surrogate has been developed to decrease fat content to about 27% vs an average level of 32% in standard surrogates.

We already established for nutra-snack, 40% calories from carbohydrates, 30% from protein, 30% from fat. For this product, soy protein concentrates, maize starches, rice starches water and salt are combined by the use of an extrusion technology. The resulting intermediate is a so called “pellet” which is a hard granule with a typical 3% moisture level (not edible). The resulting pellets are exploded in a special machine which applies high temperature and vacuum. The industrial trials led to 2 prototypes: neutral and pizza. As it concerns the production of the final snack, the following chocolate format was selected

Due to the demand of ensuring a suitable taste for NUTRASNACK bars, ensuring at the same time the necessary antioxidant efficacy of the product, the use of other possible plants was analysed. Taking into account the results obtained for the hydro-alcoholic either Mint or Sage extracts and those envisaged using Basil, we selected the extracts combining the highest antioxidant activity and safe composition.

After positive comments of Ethical Committee, nineteen men and seventeen women (for a total of 36 subjects) were recruited for assessing the safety, quality and effectiveness of human dietary supplementation with an antioxidant containing snack. The study applies to healthy volunteers, that regularly attend a gym center and that are not interested in losing weight. They were randomly divided into two groups for a double bind, placebo controlled, and cross over study. During the study each of the subjects has eaten two types of snacks: a placebo-snack and an antioxidant added snack, in random order. Each snack was eaten for 4 consecutive weeks and the two periods were separated by one day interval. The snacks were consumed twice a day, around midmorning and in the afternoon. Food intake and physical activities during the study had to be reported on diaries. All the subjects were subjected to 4 medical examinations: two weeks before the study start (visit 1) for a general anamnesis; at the beginning of intake (visit 2), after the first four weeks (visit 3), and after the second four weeks of supplementation (visit 4). At visit 2, 3 and 4 blood samples were collected. Blood chemistry analyses were performed on samples collected at visit 2 and 4 by Centro Diagnostico Senese (Siena, Italy), while oxidative stress evaluation was performed by Università degli Studi di Milano (Milan, Italy) as subcontractor of Partner ENERVIT. The BAP (Biological Antioxidant Potential) test, successfully validated in humans, allows evaluating the plasma antioxidant biological potential as the capacity of the plasma sample to reduce ferric ions to ferrous ions. The d-ROMs test allows assessing, in a biological sample, the concentration of hydroperoxides (ROOH). Oxidative stress status, evaluated by d-ROMs and BAP tests shows very interesting results. All the subject show normal and very low d-ROMs values, and even lower results for the four that are out of range. These latter values have been confirmed by performing the analysis in triple and on two different serum batches and statistics is in progress. On the basis of these results, other analyses that can complete the oxidative stress scenario (i.e. glutathione peroxidase, glutathione reductase, superoxide dismutase and the fatty acid composition), are planned by ENERVIT in the phase of commercial snack production. It would be interesting for the commercial snack to test it on a population with a higher basal oxidative stress.

The studies on development of biosensors were performed by National Institute for Biological Sciences (Romania) in collaboration with Université de Perpignan (France). It concerned the optimisation of recognition element immobilisation (poly-phenol oxidases-PPoxs), the development of sensor for antioxidant capacity determination based on the low-density lipoproteins, LDL; this wad to assess the antioxidant effect, and the application of the developed antioxidant capacity determination methods to various real samples. Another biosensor based on *Laccase* immobilisation during electro-deposition, in a composite chitosan or in Nafion –carbon nanotubes matrix was developed and applied to real samples analysis.

The performance parameters were assessed for both types of biosensors that were designed, in term of sensitivity, linearity range and reproducibility, stability and life-time for both designed biosensors; the biosensors being applicable with proper results on real samples e.g. extracts from *Mentha piperica*.

The operational stability of Laccase-Nafion biosensor is good, taking into account that single use is envisaged and stability was proven for at least 5 successive measurements, an optimisation of storage stability being required. Three procedures for antioxidant capacity determination were settled and applied to provide the TEAC values of some standard secondary metabolites and to real samples.

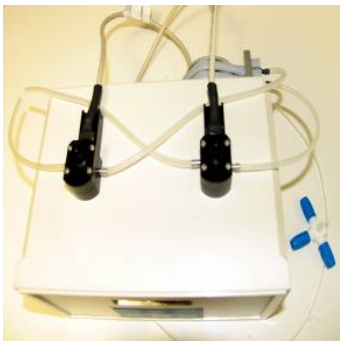
A new, suitable model able to be used in sensor production devoted to reliable assessment of antioxidant effects, focussed on low-density lipoproteins was proposed. The experiments were developed in order to test the LDL model applicability using secondary metabolites standards – rosmarinic acid; caffeic acid; chologenic acid etc., and to approach the potential assessment of preventive /reparatory action of secondary metabolites against LOO formation.

Activities were performed by Romanian group in collaboration with University of Perpignan-France, University of Pisa-Italy, Neosens-France and terminated thanks to the collaboration of DAS Company-Italy.

A set of biosensors was fabricated by DAS under management of the scientific partners to fulfill the main query of this project, namely to perform as sensitive, versatile and reliable analytical tool for the measurements of *in vitro* cell cultivation based on the amount of accumulated polyphenolic metabolites. This is an easy method to recognize the effective activity of nutritional products, able to control claims of antioxidant activity. It was proved the biosensor versatility against different polyphenolic metabolites that may occur in tested plants (basil, sage, mint,) like chlorogenic acid and caffeic acid.

The optimization of the biosensor built up by DAS Company in Italy, was performed by Rumania and Italian groups and in terms of: the amount of the immobilized bio-recognition element (*Laccase*) the biosensor operational and storage stability.
Results obtained testing Laccase-Nafion biosensors proved that with proper adjustments (use of two electronic schemes for two sensibility ranges), the prototype was applicable to accomplish the main goal and to develop an appropriate method of secondary metabolites and antioxidants analysis by a set of sensors. The adjustment was performed on level of double cell design, flow rate, volume cell, and accuracy of applied potential and amplifier characteristics and to adjust the existent software used by DAS Company with the actual model.

Another goal was to develop a bioreactor (photobioreactor) able to standardize the commercial production of secondary metabolites from plant cell cultures. The existing reactors available on the market are automatic systems designed and developed for bacterial and yeast fermentation (biofermentor); therefore, due their peculiar features required for the bacterial growth these systems are not fully satisfactory for plant cell culture purposes. This essential requirement led to the necessity to create a new bioreactor fully equipped to meet all the requirements to produce commercial, plant cell cultures with automatic synchronization of the cells.
The newly designed photobioreactor was called PhotoBioLab.



The automatic prototype has 6-20 vessels for contemporary growth, Internal and external controls of variables, visualization software, and real time controls. The automatic photobioreactor system was conceived for the continuous of cell culture with control of the major variables, both internal and external. Internal variables are automatically controlled and measured directly by the system; the survival LED lights utilize flexible rings and have the possibility to set intensity conditions ranging from 10 to 500 µmol photons//m/s. The temperature can be regulated in a range between 15 and 30°C. To achieve a homogeneous culture, the device was provided with an oscillating plate which can be set between 80 and 300 rpm. This innovative choice guarantees an equal distribution of oxygen and nutrients within the bioreactor, thereby preventing cell sedimentation and death. Optionally, the device can be equipped with a system for quantitative fluorescence measurements. The system possesses a sterilizing antibacterial light (an UV source) to be used before starting the in vivo culture. Moreover, the pressure of 1 bar could be applied to the device to avoid contamination of other pathogenic agents. External variables, always within the system, are measured by the user using external devices or special sensors. The measurement of external variables, such as pH, turbidity, fluorescence and gases, was carried out by using the Biogeochemical microsensor technology in separate containers.
The different phases of the culture growth were identified and analyzed and optimized in deep. Everything worked properly on cell and suspensions cultures (mother suspension, pre-filtered 500 microm suspension and synchronized cells suspensions).
The experiments were performed on *Salvia officinalis* and *Ocimum basilicum* callus cultures, previously prepared by University of Pisa laboratories: three repeated extractions, each of them measured in three replicates, the obtained SD ranged between 0.95 and 0.03 for level of equivalent rosmarinic acid of 10-6 molL-1. Results were further confirmed by HPLC-DAD-MS analysis of same callus in Rumanian lab in collaboration with Poland and University of Pisa.
In conclusion, the NUTRA-SNACKS project got positive results from several points of view:

- For basic research on the use of transformants for production of antioxidants
- For semi-basic research on elicitations methods able to improve the content of antioxidants on algae and plant cells cultures
- Establishment of plant cell cultures as a promising method to produce “pure active compounds”
- Production of safely extracted antioxidants with controlled content of metabolites (absence of unhealthy compounds like Methyleugenol) and of antioxidant activity
- Industrial analysis of the type of extracts useful for large production
- Production of various types of snacks with low glicemic index
- Positive testing of antioxidant activity on humans (after approval of ethical committee)
- Construction of a bioreactor for plant cell cultures with automatic synchronization of the cells
- Construction of a biosensor for monitoring the content of antioxidant metabolites

Nutraceutics constitutes a growing sector of the food industry dedicated to defining food components that can improve human health, the NUTRA-SNACKS project, with the support of the European Commission, utilized a combination of technologies to produce innovative ready-to-eat food products with functional health-promoting activities.
During the initial stages of the project, partners from research institutes and SMEs used biotechnology to isolate natural metabolites with specific health promoting proprieties. One of the main objectives of the project was to develop microbial or plant-based bioreactors-biosensors, which can be used to produce the desired metabolites in bulk quantities.
These systems were further improved to increase yield and enhance quality of safe extracted nutraceutics.

Creating a new paradigm in the food industry: NUTRA-SNACKS can boast the correct framework through which a new generation of food products can reach the European consumer. The objective was to create a favourable economic climate that would promote the marketing of a new wide range of health-promoting products.

- ENERVIT Company registered the mark NUTRA-SNACKS and established a further collaboration with the group for the improvement of the snack and final production
- DAS Company established a further collaboration with the group for the scale production of the system Biosensor/Bioreactor for antioxidant metabolites that will be commercialized by its spin off Company Biosensor in Italy
- A spin off company from DAS, National Council Of Research-It, University of Creete specialized researchers, called PHOTOFARM, was established waiting for approval of CNR, it is expected to be official in January 2010.
- Several scientific publications have been written and the Nutra-Book is published on line (printed on January 2010)
- Several other companies required the collaboration of the Consortium for safe production of Nutraceutics and five new collaborations were established in Europe.

http://www.nutra-snacks.com/
https://www.landesbioscience.com/curie/chapter/4519/

2. Dissemination and use

A Book on the subject will be published in January of 2010:
https://www.landesbioscience.com/curie/chapter/4519/

"Bio-Farms for Nutraceuticals: Functional Food and Safety Control by Biosensors"

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This book is dedicated to the Nutritionist P. Sorbini

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For more information about Nutra.Snacks book you can see page:
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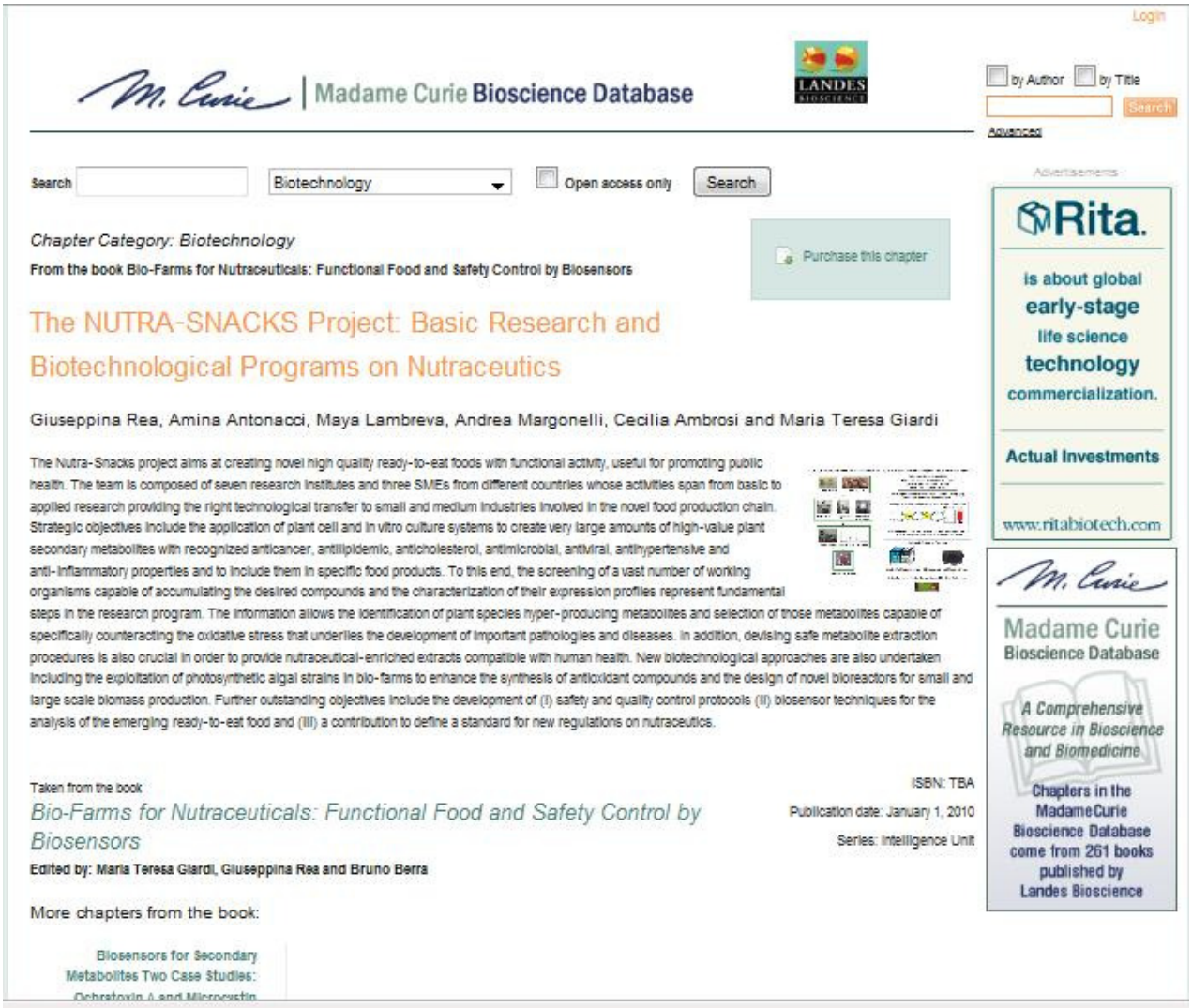

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Giuseppina Rea, Amina Antonacci, Maya Lambreva, Andrea Margonelli, Cecilia Ambrosi and Maria Teresa Giardi

The Nutra-Snacks project aims at creating novel high quality ready-to-eat foods with functional activity, useful for promoting public health. The team is composed of seven research institutes and three SMEs from different countries whose activities span from basic to applied research providing the right technological transfer to small and medium industries involved in the novel food production chain. Strategic objectives include the application of plant cell and in vitro culture systems to create very large amounts of high-value plant secondary metabolites with recognized anticancer, antilipidemic, anticholesterol, antimicrobial, antiviral, antihypertensive and anti-inflammatory properties and to include them in specific food products. To this end, the screening of a vast number of working organisms capable of accumulating the desired compounds and the characterization of their expression profiles represent fundamental steps in the research program. The information allows the identification of plant species hyper-producing metabolites and selection of those metabolites capable of specifically counteracting the oxidative stress that underlies the development of important pathologies and diseases. In addition, devising safe metabolite extraction procedures is also crucial in order to provide nutraceutical-enriched extracts compatible with human health. New biotechnological approaches are also undertaken including the exploitation of photosynthetic algal strains in bio-farms to enhance the synthesis of antioxidant compounds and the design of novel bioreactors for small and large scale biomass production. Further outstanding objectives include the development of (I) safety and quality control protocols (II) biosensor techniques for the analysis of the emerging ready-to-eat food and (III) a contribution to define a standard for new regulations on nutraceutics.

Taken from the book

Bio-Farms for Nutraceuticals: Functional Food and Safety Control by Biosensors

Edited by: Maria Teresa Giardi, Giuseppina Rea and Bruno Berra

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