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N°40 - October 2011



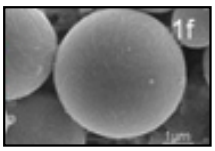
- AGRICULTURE /FOOD & NUTRITION / ENVIRONMENT -

LABORATORIES - RESEARCH RESULTS

Controlled release of active substances

The efficiency of a functional ingredient depends on its stability throughout the industrial process and during storage, and also on its absorption in the digestive tract. Researchers from INRA (Toulouse, France) and Laboratoires Phodé (Tersac, France) have developed a patented process based on glycerol, a "green" by-product of diester ,which ensures the effective protection of polyphenols. One example is curcumin, which is widely used in both human and animal diets.

Stabilised surfactant-free emulsions



Emulsions have gradually invaded our everyday living: foods, cosmetics, lubricants, medicines, etc. They are everywhere! But these "oil-in-water" mixtures have an unfortunate tendency to lose their stability over time, hence the frequent recourse made to surfactants to preserve them. Use of the latter is often criticised because of their negative environmental footprint. An ecological alternative is now being proposed by INRA researchers in Nantes: the use of crystals from plant resources, such as cellulose, to stabilise mixtures. These so-called Pickering emulsions open the way to hitherto unexplored applications.

Towards new methods to control mushroom diseases

Agaricus bisporus is commonly referred to as the cultivated mushroom and is the most widely produced and consumed mushroom in the world. Commercial strains are highly productive and display an attractive morphology and texture, but they are susceptible to a variety of viral, bacterial or fungal diseases. Control of these diseases is becoming increasingly difficult. To protect consumers, the regulations ban the use of antibiotics and continue to limit the number of authorised fungicides. Furthermore, the resistance of pathogens to approved agents is constantly increasing. Knowledge of the mechanisms of host-micro-organism interactions, and their genetic determinism, will enable the development of new control measures and open the way towards genetic improvement. With this in mind, the Microscopic Fungus Biology and Genetics research team in the INRA Mycology and Food Safety Research Unit (MycSA) in Bordeaux has focused on the three principal diseases affecting *Agaricus bisporus*.

Metabolomics: new prospects for melon breeding

Melon is a fruit of economic importance and its consumption is recommended in the diet, in compliance with the nutritional guidelines of the "Five fruits and vegetables a day" programme. It contains essential nutrients such as isoprenoids, vitamins and minerals. To a considerable extent, its acceptance by consumers is based on its aroma and sugar content. In the context of the European META-PHOR project, a study by the INRA Fruit Biology and Pathology Unit in Bordeaux, in collaboration with other teams, has demonstrated that the spatial and developmental variability of the metabolite and mineral composition of this fruit has major effects on the organoleptic and nutritional quality of melon, and identified groups of compounds with coordinated compositional changes.

Determinants of vegetable consumption in France

Since the end of the 20th century, the consumption of fresh vegetables in France has fallen, even though this has partly been compensated for by an increase in the consumption of processed vegetables. Initially, this substitution did not appear to affect all households equally. The 1980s saw the early signs among wealthier classes of a higher consumption of processed, and thus more expensive products. At the end of the 20th century, a diversification was observed in the supply of processed products, in line with the reduction in time devoted to food preparation because of the increase in the number of working women. Has the use of processed vegetables thus become an alternative in all types of household? The INRA Food and Social Sciences Research Unit in Ivry-sur-Seine has tried to answer these questions in the context of the ANR project: "Governing consumer behaviour: the case of policies to control obesity and encourage sustainable consumption."

The prevention of allergies to cypress pollen (cypress, juniper, thuja, etc.)

In the context of a partnership with Hôpital Nord Marseille-University of the Mediterranean (Denis Charpin, Lung Diseases and Allergology Department), Montpellier SupAgro (Michel Calleja and Isabelle Farrera, Palynology Unit) and the Pollen and Micro-Environment Laboratory in Valence (Vincent Penel), the INRA Research Unit on the Ecology of Mediterranean Forests at the PACA Centre (Christian Pichot) has been carrying out research on the prevention of cypress-induced pollinosis during the past fifteen years.

Environmental pollution and the quality of free range eggs

The consumption of foods contaminated by organic pollutants compounds is the principal cause of human exposure to these compounds. These substances are likely to enter the food chain, notably via the products of livestock farming. Scientists in the "Animal and Animal Products Functionalities" Research Group, working in the context of a CIFRE doctoral contract co-funded by the Technical Institute for Poultry Farming (ITAVI), studied the transfer kinetics of polycyclic aromatic hydrocarbons (PAH) included in the feed of laying hens to the yolks of the eggs they produced. The team has validated models to predict the level of egg contamination after the ingestion of organic pollutants. These models could be of considerable value to assessing product quality.

Water-saving fish farms

Fish farming is strongly dependent on the natural environment. Indeed, the water used for farming is taken from nature and subsequently returned. A pilot trout farm capable of reducing water requirements by 90% was set up at the INRA Experimental Fish Farm in Monts d'Arrée (Peima), in collaboration with the INRA SAS (Agricultural Soil and Spatialisation of Water Systems) and SCRIBE (Fish Physiology, Biodiversity and the Environment) Joint Research Units. This system may be of potential value to European fish farming that is less environmentally-dependent in the future.

TECHNOLOGY OFFERS

A novel method for preparing dicaffeoylquinic acids, natural molecules with high added value for pharmaceutical, nutritional, cosmetic and agronomical uses

Numerous publications describe the chemical and biological properties of dicaffeoylquinic acids (diCQ), natural substances that notably have a considerable antioxidant activity. This fact makes the diCQ very interesting from a nutritional perspective. The diCQ are only recently available on the market, but at high prices.A method for preparing high yield of diCQ would open up interesting prospects for the valuation of these molecules coming from the secondary metabolism of plants, and presenting many properties. Under the leadership of Dr. Jean-Luc Poëssel of the research unit “Génétique et d’Amélioration des Fruits et Légumes“ of INRA in Avignon, France, researchers have developed a method for producing 3,5-dicaffeoylquinic (3,5-diCQ), a diCQ isomer, from non-tuberous roots of sweet potato (Ipomoea batatas). The method of extraction of dicaffeoylquinic acids from non-tuberous roots of sweet potato or other species of the Convolvulaceae family, and the use of this compound family for combating aphids are protected under a patent application filed by INRA (see the patent family FR2926955, CA2712645, WO2009/095624, US2011/0054022 or EP2237665). INRA Transfert is in charge of the development of this innovation through licensing for commercial applications targeting companies with the ability to produce diCQ compounds, active ingredients intended for pharmaceutical, cosmetic, agro-food and agriculture, as well as companies of agronomical sector that develop alternative biocide formulations for combating crop pests.

EUROPEAN PROJECTS

IHMS – International Human Microbiome Standards

At a time of major initiatives relative to deciphering the genome of bacterial communities associated with humans, a new European project aims to harmonise practices and facilitate the comparison of data between projects of different origins. This project, launched on 1st February 2011 for a period of 4 years, is being coordinated by S. Dusko Ehrlich at INRA in Jouy-en-Josas and involves eight partners, all international experts in the microbiota, in order to ensure a global scale for its results.

AQUAEXCEL

The development of sustainable aquaculture is today a high priority for Europe. Coordinated by INRA, and launched on 1st March 2011, the AQUAEXCEL project (Aquaculture Infrastructures for Excellence in European Fish Research) will set up a new European platform of excellence for research and experimentation in aquaculture. The overall objective is to enable innovation to drive the development of high-quality aquaculture with a low environmental impact.

INFOGEST

This Action aims at building an international network of institutions working on the digestion of dietary proteins and the effects on human health of the digestion products released in the gut. So far, 37 institutions from 20 countries (from Europe but Canada and New-Zealand as well) are involved in this Action, but the network will be fully open for welcoming new partners who wish to join it.

Office of Industrial Relations (DPE)
The Information Service for companies

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Dietary supplements based on functional ingredients are widely used to prevent deficiencies in humans or to improve production yields in animals. The effects of these ingredients can be significantly reduced by various factors: thermal damage, light exposure, early degradation in the digestive tract, etc., to say nothing of the risk that potentially toxic new compounds may appear during production.

A natural physicochemical process to obtain stable structures

A combination process using non-covalent and reversible bonds was chosen to control the release of functional ingredients based on plant molecules. The active substances, from the polyphenol family, are associated with glycerol; the complexes obtained are then emulsified and stabilised on an inert material such as silica, the result being a dry powder. In this way, the active ingredient is protected against ambient attack and can be carried more efficiently to its target organs.

A validated application in animal feed

The researchers chose curcumin, an antioxidant known for its beneficial nutritional effects, as the first active substance. Application of the process enabled preservation of the chemical identity of this compound. Better, its physicochemical properties were markedly improved: curcumin became more stable with respect to light, temperature and physiological pH. Its antioxidant potential was multiplied 2.5-fold compared to the native curcumin. When tested in broilers feed, this new material improved the growth performances of poultry and led to a reduction in the mortality of animals subjected to cold stress.

An eco-design approach that takes account of industrial constraints

The process thus developed, which benefits from international patent protection, is energy-sparing. It does not require any organic solvents, so it is not polluting. This effective process is achieved under mild temperature conditions (<60°C) without a controlled atmosphere. It has been extrapolated successfully to the industrial scale.

An extension to human applications

Other active substances could profit from this process. More in-depth studies need to be planned first of all, to gain a clearer understanding of the nature of the combination mechanisms in play.

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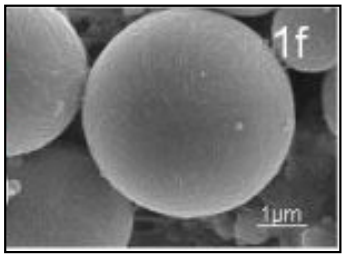
Stabilised surfactant-free emulsions



Emulsions have gradually invaded our everyday living: foods, cosmetics, lubricants, medicines, etc. They are everywhere! But these "oil-in-water" mixtures have an unfortunate tendency to lose their stability over time, hence the frequent recourse made to surfactants to preserve them. Use of the latter is often criticised because of their negative environmental footprint. An ecological alternative is now being proposed by INRA researchers in Nantes: the use of crystals from plant resources, such as cellulose, to stabilise mixtures. These so-called Pickering emulsions open the way to hitherto unexplored applications.

The formulation of an emulsion enables modulation of the texture of everyday products used in fields as varied as the agrifood industry, cosmetics, lubricants, chemical synthesis or even pharmaceuticals. An emulsion is generated by the dispersal of two, non-miscible liquids which, but over variable time scales they will ultimately and undoubtedly separate… unless a third element is included, a stabilising compound that has affinity for both phases and positions itself at the interface. This role has historically been played by surfactants, but they have one major drawback: a large quantity of surfactants is necessary to maintain stability at the interface because of the rapid sorption/desorption dynamics in this area.

Pickering emulsions: atypical emulsions

In the case of Pickering emulsions, stability is assured by the presence of solid particles that are firmly fixed at the interface. The materials obtained are much more stable than their standard counterparts stabilised by surfactant molecules, and are endowed with greater interfacial elasticity. These macroscopic properties can be understood from the change to the nature of the interface. In the case of standard emulsions stabilised by surfactants, the interfaces are "liquid", while with Pickering emulsions, the presence of particles renders the interface "solid" and very robust. These systems are currently the subject of renewed interest, notably for ecological reasons, the aim being to limit the use of synthetic surfactants.

Cellulose nanocrystals: efficient stabilisers

Scientists in the INRA "Biopolymers, Interactions and Assemblies" Research Unit in Nantes-Angers have demonstrated that it is possible to produce Pickering emulsions using polysaccharides from plant resources, such as non-modified cellulose, as the stabilising element (patent filed). These crystals with different morphologies are obtained either from the managed hydrolysis of natural structures (cotton, algal cell walls, bacterial cellulose) or from polysaccharide chains that are isolated and then re-crystallised (e.g. valorisation of fabric residues). The different methods used to prepare the crystals tested enabled the demonstration that their surface characteristics were determinant in stability of the emulsion, and that the latter could be modulated by the surface charge of the crystals. The stability studies carried out showed that these emulsions were resistant to major mechanical deformation and were stable throughout a broad range of temperatures (from -20°C to 80°C) and periods of time (at least one year).

Applications to be invented

The use of polysaccharide crystals to stabilise Pickering emulsions could replace particles arising from organic synthesis in existing applications, but could also open the way to new areas of application for these emulsions because of the functional properties specific to biopolymers and the food-quality

or biocompatibility of the crystals. Given the stability and rigidity of the interface and the biological nature of the crystals, this type of emulsion applied in foods could, for example, be used in the longer term for innovations in the encapsulation of nutrients and/or the drying of emulsions.

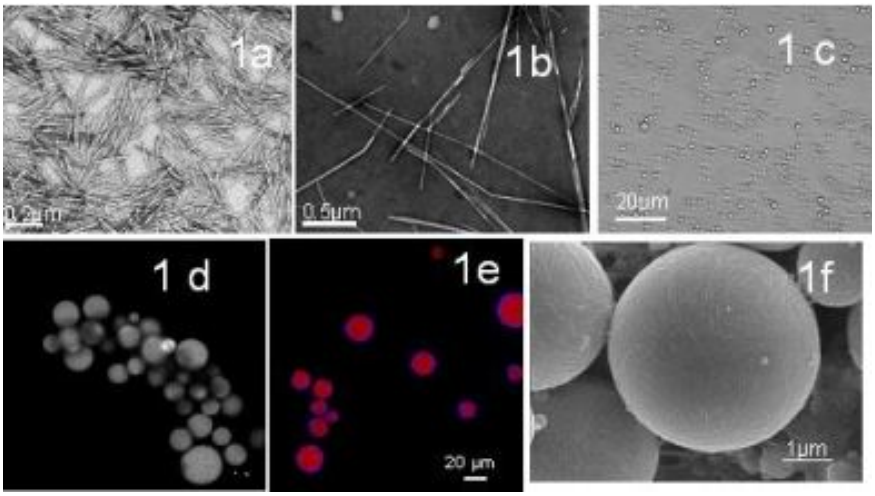


Figure 1. Cellulose nanocrystals obtained by the managed hydrolysis of cotton linters (1a) or cladophora (algae) (1b). Emulsion formed by cellulose microcrystals under the optical microscope (1c); imaging under confocal laser scanning microscopy with labelling of the hydrophobic phase with bodipy (1d) and double cellulose-specific bodipy and calcofluor labelling (1e) and scanning electron microscopy (1f).

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Towards new methods to control mushroom diseases

***Agaricus bisporus* is commonly referred to as the cultivated mushroom and is the most widely produced and consumed mushroom in the world. Commercial strains are highly productive and display an attractive morphology and texture, but they are susceptible to a variety of viral, bacterial or fungal diseases. Control of these diseases is becoming increasingly difficult. To protect consumers, the regulations ban the use of antibiotics and continue to limit the number of authorised fungicides.**

Furthermore, the resistance of pathogens to approved agents is constantly increasing. Knowledge of the mechanisms of host-micro-organism interactions, and their genetic determinism, will enable the development of new control measures and open the way towards genetic improvement. With this in mind, the Microscopic Fungus Biology and Genetics research team in the INRA Mycology and Food Safety Research Unit (MycSA) in Bordeaux has focused on the three principal diseases affecting *Agaricus bisporus.*

Among the bacterial diseases, brown blotch disease, mainly caused by *Pseudomonas tolaasii*, is characterised by deep brown lesions on the cap and stipe of harvested mushrooms. Work at INRA has shown that *Pseudomonas tolaasii* produces extracellular toxins called tolaasins, whose efficiency in inducing symptoms is controlled by an extra-genomic factor. The susceptibility of the mushroom to the bacterium depends on its gamma-glutamyl transferase activity and its content in gamma -glutaminyl-4-hydroxybenzene. Pre-treatment of the mushroom with WLIP lipodepsipeptide produced by *Pseudomonas reactans* can protect the mushroom against brown blotch disease. However, no commercial WLIP-based agent has so far been developed. Other studies have also led the research team to propose use of the TO1 bacteriophage isolated from the surface of the cultivated mushroom in order to control brown blotch disease. More recently, an Egyptian team observed that incorporation of the medicinal plant *Salvia miltiorrhiza* in the cultivation substrate reduced the risks of mushroom infection by this bacterium.

In terms of fungal diseases, the most serious are green mould and *Lecanicillium* (*Verticillium*) disease ("dry bubble")

Green mould is caused by *Trichoderma aggressivum* which competes with *Agaricus bisporus* to colonise the cultivation substrate. Contaminated areas are rapidly covered with Trichoderma spores and no longer produce any mushrooms. It has been shown that *Agaricus bisporus* stimulates the spore formation of *Trichoderma* and this in turn inhibits the fungal development of the mushroom. Adaptation of the competitor to the cultivation substrate is due to its greater tolerance than other fungi to the inhibitory effects of the bacteria present. However, *Trichoderma aggressivum* remains susceptible to some bacteria, mainly of the *Bacillus* family, isolated from the compost, which opens the way to the use by French mushroom growers of a biofungicide to control green mould. In addition, several authors have studied the in vitro effects of essential oils versus *Trichoderma*, but the inhibitory effects observed need to be confirmed under cultivation conditions.

Verticillium disease (or "dry bubble") is caused by *Lecaniccilium* (*Verticillium*) *fungicola*. It is mainly characterised by globular masses (bubbles) that form instead of the mushrooms, and also by mushrooms whose stipe splits and peels or bears spots. The MycSA team has shown that this disease is linked to the recognition and/or penetration of the pathogen during the very early stages of development of the fruiting body of *Agaricus bisporus*. Because of their potential at this critical stage, the use of bacteria (*Pseudomonas fluorescens*) or essential oils displaying an in vitro inhibitory effect on *Lecanicillium fungicola*

is proposed, thus suggesting the possibility of control if this effect is confirmed during cultivation. Studies on the regulation of several genes, and the on production of oxidative compounds, have highlighted the involvement of oxidative processes in the resistance of *Agaricus bisporus* to *L. fungicola*, and have oriented research towards resistant varieties. Some mushroom varieties that are highly tolerant to *L. fungicola* have been identified among the genetic resources from natural environments in the INRA CGAB collection, but no totally resistant variety has yet been found. This tolerance is of a polygenic type. Groups of genes with partial effects (QTL) on resistance have been revealed. They cover large parts of the genome, but their position must be specified for the development of marker-assisted breeding.
Full sequencing of the cultivated mushroom genome by an international consortium, including INRA, will soon enable improvements to these varietal selection tools, and should throw new light on the defence mechanisms of mushrooms, thus opening the way to other innovations to control diseases in cultivated mushrooms.

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- Microbially induced diseases of Agaricus bisporus : biochemical mechanisms and impact on commercial mushroom production. Michèle L. Largeteau, Jean-Michel Savoie, Appl. Microbiol Biotechnol (2010) 86, p. 63-73.

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Metabolomics: new prospects for melon breeding

Melon is a fruit of economic importance and its consumption is recommended in the diet, in compliance with the nutritional guidelines of the "Five fruits and vegetables a day" programme. It contains essential nutrients such as isoprenoids, vitamins and minerals. To a considerable extent, its acceptance by consumers is based on its aroma and sugar content. In the context of the European META-PHOR project, a study by the INRA Fruit Biology and Pathology Unit in Bordeaux, in collaboration with

other teams, has demonstrated that the spatial and developmental variability of the metabolite and mineral composition of this fruit has major effects on the organoleptic and nutritional quality of melon, and identified groups of compounds with coordinated compositional changes.

The melon variety chosen for this research was "Escrito", which was cultivated in an open field (at a density of 9200 plants/hectare) in Moissac, France, between April and August 2008. Management of the crop complied with current professional practices. The melons were harvested at three developmental stages: just before the appearance of the suberized net on the fruit (stage 1), at the very beginning of ripening (stage 2) and at the beginning of fruit abscission (stage 3, or "commercial maturity"). At each stage, three homogeneous batches of three melons were made up. Two, 1-cm thick slices were cut in the equatorial plane of the fruit. After removal of the skin and seeds, the analysis concerned five concentric rings of flesh from the periphery to the centre. At the three developmental stages of the fruit, fleshy tissues from the outer flesh to the seeds underwent extractions and then analyses, which enabled the quantification of 2000 metabolite signatures and 15 mineral elements.

Using six complementary analytical platforms, the profiles of the metabolites and minerals in each fruit section, at each stage, could be mapped. This concerned primary metabolites, secondary volatile metabolites, secondary non-volatile metabolites and mineral elements. The results confirmed that metabolism is re-programmed during ripening and displays spatial changes in the composition of metabolites and mineral elements in the fruit. These spatial changes may be linked to the distribution of vascular bundles and to the production of phytohormones by the seeds and their gradual diffusion across the melon flesh. If this is confirmed, then varietal breeding for fruits with a more homogeneous composition could focus on homogenising the vascular structure of tissues, and possibly target seedless fruits.

This research also demonstrated numerous correlations, firstly between primary and secondary non-volatile metabolites, and secondly between the latter and volatile compounds. Visualisation of the correlations between metabolites, in the form of association networks, was able to highlight points of "metabolic cross-talk", involving sucrose, aspartic acid, 2-isopropylmalic acid, β-carotene, phytoene and dihydropseudoionone, which may become candidate metabolites to be monitored during breeding programmes targeting fruit quality.

With respect to aroma, the research confirmed the close links between the concentrations of certain primary metabolites and that of certain volatile compounds with different chemical properties (esters, aldehydes, alcohols and sulphur-containing compounds). Furthermore, unexpected links between the levels of certain mineral elements and certain metabolites were demonstrated and should be taken into account during selection.

This work on melon, partly pursued in the context of another European project, SAFQIM (Plant KBBE 2010),

has demonstrated the importance of taking account of a metabolomic approach in selection programmes, with respect to both nutritional and organoleptic quality. By exploring the relationships between the presence of metabolites and fruit quality, new opportunities are now available for breeding programmes.

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Determinants of vegetable consumption in France

Since the end of the 20th century, the consumption of fresh vegetables in France has fallen, even though this has partly been compensated for by an increase in the consumption of processed vegetables. Initially, this substitution did not appear to affect all households equally. The 1980s saw the early signs among wealthier classes of a higher consumption of processed, and thus more expensive, products. At the end of the 20th century, a diversification was observed in the supply of processed products, in line with the reduction in time devoted to food preparation because of the increase in the number of working women. Has the use of processed vegetables thus become an alternative in all types of household? The INRA Food and Social Sciences Research Unit in Ivry-sur-Seine has tried to answer these questions in the context of the ANR project: "Governing consumer behaviour: the case of policies to control obesity and encourage sustainable consumption."

In order to measure the determinants of vegetable consumption in France, the research team made use of data on the grocery and fruit and vegetable purchasing of 2765 households belonging to the 2007 TNS-Secodip consumer panel. During this study, all vegetables were included (except potatoes), whatever their degree of transformation: raw vegetables bought in bulk, washed and peeled vegetables in sachets, tinned vegetables, soups, frozen vegetables (cooked or not) and prepared meals containing at least one portion of vegetables.

Vegetable purchases in France reached 64 kg per consumption unit (CU) and per year, including 55.5% of fresh vegetables. However, this mean hid some major disparities, insofar as 50% of households purchased less than 54 kg. The study then refined the results by age, household structure and social position. It thus showed that the most discriminating consumption determinants were age and composition of the household. The largest consumers are people aged over 50 years (where consumption exceeded 74 kg/CU/year, and even 85 kg/CU/year among those over 60). A person aged over 60 years consumed almost twice as many vegetables as a 30-year old person. Similarly, living in a couple appeared to favour vegetable consumption (72.7 kg/CU/year), but this consumption dropped among couples with children (nearly 48 kg/CU/year). Women living alone consumed more than men in the same situation (nearly 82 kg versus 51 kg).

The effect of income was also preponderant, insofar as the richest 15% of the French population had a vegetable consumption that was higher than the mean (69 kg/CU/year), while the poorest 15% only consumed 51.5 kg. By contrast, during this study, the effect of educational level did not appear to be significant. A higher consumption of fresh vegetables seemed above all to be correlated to age. Thus, because they were older, less qualified households purchased more of these products. The older generations were always more inclined to consume non-processed vegetables, as were households with a vegetable garden.

The research team also focused on the structure of vegetable purchasing as a function of the gain in time and degree of culinary simplification provided by these products: from the fresh product to the fully-prepared, composite dish. Overall, the total quantity of vegetables consumed was closely correlated to the consumption of fresh vegetables. In France, the profile of a "high" consumer (128 kg/CU/year) was above all that of a consumer of fresh vegetables (68% of these purchases), who tended to be older, usually living in a couple and wealthier than average. Indeed, the more a household purchased vegetables, the more it purchased them in a fresh rather than processed form. Purchases by the households purchasing the least vegetables (21 kg/CU/year) were divided between 40% fresh and 10% composite dishes.

The composition of the purchases of processed products also varied as a function of age and household structure. Young and single people preferred ready washed and peeled raw vegetables, while soup was more appreciated by those over the age of 60. Finally, composite dishes (providing a full meal containing meat or fish and vegetables) presented as a "convenience food" were the preference of those below the age of 40 and men living alone, as well as retired people, whatever their level of income and education.

This study tended to show that the consumption of processed vegetables is no longer a marker of social position. They are no longer reserved for higher social categories or a sign of popular consumption. The relative fall in the prices of industrial products, which has made them available to all groups in the population, is one of the reasons for this. Inversely, the consumption of fresh vegetables may have become the preserve of wealthier groups who seek to avoid industrialised foods or consider the freshness of vegetables as proof of their quality.

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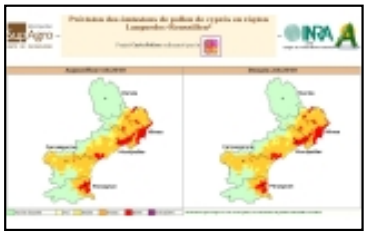
The prevention of allergies to cypress pollen (cypress, juniper, thuja, etc.)



In the context of a partnership with Hôpital Nord Marseille-University of the Mediterranean (Denis Charpin, Lung Diseases and Allergology Department), Montpellier SupAgro (Michel Calleja and Isabelle Farrera, Palynology Unit) and the Pollen and Micro-Environment Laboratory in Valence (Vincent Penel), the INRA Research Unit on the Ecology of Mediterranean Forests at the PACA Centre (Christian Pichot) has been carrying out research on the prevention of cypress-induced pollinosis during the past fifteen years.

Pollen allergies are a real public health problem and the prevalence of pollinosis continues to rise. In the Mediterranean region, cypress pollen is particularly abundant, representing a third of the pollen spectrum each year, and causes allergies that range from slight discomfort to incapacitating rhinitis and conjunctivitis. This allergy manifests itself at the end of the winter but can extend over nearly 6 months (November-April) because of the large number of plant species producing pollen (cypress, juniper, thuja, etc.).

Work at INRA mainly focuses on predicting the risk of exposure to pollen through the development of an information system for the spatial and temporal forecasting of pollen emissions (Cartopollen*), on reducing the amount of pollen produced by trees thanks to the trimming of hedges and on searching for non-pollinating varieties. Their research also concerns the development of a new method to evaluate exposure, based on analysing pollens trapped in the hair, studying their penetration into homes and their allergenic persistence**.

A tool to enable the daily and spatial prediction of cypress pollen emissions in the Languedoc-Roussillon region has been developed. It is based on integrating the factors that determine pollination within a GIS: the presence and abundance of vegetation, phenological equations on pollination and daily meteorological variables. The daily forecasts are accessible at: http://www.france-pollen.com. Mainly intended for healthcare professionals and allergy patients, they enable a reduction in exposure and the initiation of preventive therapies.

By eliminating some of the male cones that produce pollen, the trimming of hedges can also contribute to reducing exposure. Its effect has been evaluated at -30% in an urban hedge in Montpellier***.

**"CartoPollen: development of a spatial and temporal mapping tool for allergic risk based on phenological equations of pollination: application to Cypress trees in Languedoc-Roussillon". Regional Public Health Group, Languedoc-Roussillon (GRSP LR) 2009*
***"Towards a clearer understanding of exposure to cypress pollen". AFSSET 2007-2010*
****M. Calleja, C.. Pichot, D. Charpin, 2011. Trimming cypress tree hedges and its effect on subsequent pollination. Ann Allergy Asthma Immunol. 106-3): 259-260.*

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Environmental pollution and the quality of free range eggs

The consumption of foods contaminated by organic pollutants is the principal cause of human exposure to these compounds. These substances are likely to enter the food chain, notably via the products of livestock farming. Scientists in the "Animal and Animal Products Functionalities" Research Group, working in the context of a CIFRE doctoral contract co-funded by the Technical Institute for Poultry Farming (ITAVI), studied the transfer kinetics of polycyclic aromatic hydrocarbons (PAH) included in the feed of laying hens to the yolks of the eggs they produced. The team has validated models to predict the level of egg contamination after the ingestion of organic pollutants. These models could be of considerable value to assessing product quality.

Ubiquitous pollutants

Persistent Organic Pollutants (or POP) are chemical compounds resulting from different human activities. Defined and listed in the Aarhus protocol of 1998, these toxic substances can spread in the environment and accumulate in living organisms, thus causing numerous risks to human health (cancer, immune deficiencies, reproductive disorders) in the event of long-term exposure. Released into the atmosphere, POP can contaminate any environment at a considerable distance from their site of emission. Polycyclic aromatic hydrocarbons (PAHs) resulting from urban combustion systems and road transport form part of POPs. They display a particular affinity for fat-rich environments (lipophilic).

Possible contamination of the food chain

The consumption of contaminated foods is the principal route of human exposure to POPs. Contamination occurs via animal feed, either through the introduction of a batch of feed contaminated by one of the raw materials in its composition, or through prolonged contact between the animal and recurrent pollution of its environment. This latter mode of exposure particularly mays affect hens farmed under a free-range system. In this case, the birds may ingest significant quantities of soil or plants, which are potential sources of chemical contaminants. With growing consumer demands for eggs produced under alternative and free-range systems, the issue of pollutant transfer to eggs – in the yolk because of the lipophilic nature of PAHs – becomes crucial. Few data are available on the transfer of PAHs to eggs. It is necessary to clearly assess the risks of contamination (transfer kinetics, bioconcentration factors) to avoid any over- or under-estimation that might engender additional costs (through the adoption of excessively stringent precautions in a sector subject to strong competition) or risks to human health.

Towards a model to simulate transfers

Scientists in the INRA Animal and Animal Products Functionalities Research Group have worked on characterising the transfer kinetics of PAHs and modelling the deposition of these substances in egg yolk. To achieve this, they fed several batches of hens with a mixture of different compounds (phenanthrene (Phe), pyrene (Pyr) and benzo-[a]-pyrene (B[a]P)) representative of the PAH family and which have already been found in eggs during field surveys. Two batches of hens were exposed to this mixture, for either one day or three days, at a rate of 2 mg of each PAH per kg body weight. The eggs were then collected for the 12 days that followed the initial exposure. These PAHs and their principal metabolites (2-OH Phe, 3-OH Phe, 1-0H Pyr and 3-OH (B[a]P) were then assayed using high-performance liquid chromatography (HPLC).

The scientists observed that under these two experimental conditions, peak concentrations of PAHs

and their metabolites in the eggs were reached between 3 and 6 days after exposure to the pollutants. The levels of contaminants found in the eggs were higher after three days of exposure than after one day, ranging from 2- to 4-fold higher depending on the compound considered (after three days of exposure: 34, 12 and 15 ng/g yolk for Ph, Pyr and B[a]P, respectively). The amounts assayed demonstrated that only a small proportion (less than 1%) of the PAHs ingested was excreted via egg yolk. It then took about ten days for the Phe, Pyr and B[a]P levels to return to their pre-exposure values. This rapid return to baseline was characteristic of compounds that accumulate little. These findings thus emphasised that PAHs do not accumulate and are either little absorbed or are excreted in a metabolised form via the bile or urine. In parallel, mathematical models were developed, based on the mode of lipid deposition in egg yolk. The experimental results were in accordance with those predicted by the mathematical simulations.

Additional studies were performed during this thesis project in order to determine the role of soil matrices in the bioavailability of POP, and to evaluate the relationship between the daily dose of exposure to POPs and the amount excreted in egg yolk. This work will enable an improved assessment of the risk of transfer under real-life conditions and will soon lead to an "in silico" model for pollutants transfer in laying hens.

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- Fournier, A. ; Feidt, C. ; Dziurla, M.A. ; Grandclaudon, C. ; Jondreville, C.Transfer kinetics to egg yolk and modeling residue recovered in yolk of readily metabolized molecules: Polycyclic aromatic hydrocarbons orally administered to laying hen. Chemosphere. 2010, 78 (8) : 1004-1010

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Water-saving fish farms

Inra C. Maitre

Fish farming is strongly dependent on the natural environment. Indeed, the water used for farming is taken from nature and subsequently returned. A pilot trout farm capable of reducing water requirements by 90% was set up at the INRA Experimental Fish Farm in Monts d'Arrée (Peima), in collaboration with the INRA SAS (Agricultural Soil and Spatialisation of Water Systems) and SCRIBE (Fish Physiology, Biodiversity and the Environment) Joint Research Units. This system may be of potential value to European fish farming that is less environmentally-dependent in the future.

In a standard, open-circuit fish farming system, the water supply is taken from the natural aquatic environment, crosses through the rearing ponds and is then returned to the environment. This type of fish farming raises several problems linked to the large quantities of water used. The high throughputs of waste water and the considerable dilution of matter to be treated means it is technically difficult to purify the water from such farms. A large proportion of soluble wastes, such as the ammonia and phosphorus produced by the fish in rearing ponds, is thus emitted into the natural environment. The extraction of water from rivers also poses problems regarding the free circulation of migrating salmonids (salmon, brown trout, etc.) at certain periods. Finally, open-circuit farming systems remain highly dependent on the quality or availability of water, which may be deficient during a drought. The rearing of salmonids (salmon, trout, whitefish, etc.) in farms is indeed subject to stringent environmental constraints laid down in European Directives (EU Water Framework Directive 2010-2015 and LEMA).

In order to comply with this regulatory framework and offer an alternative to open-circuit fish farming, INRA has developed a system for salmonid farming based on the recirculation of water, the aim being to control interactions between farmed fish and the aquatic ecosystem.

http://www.inra.fr/internet/Projets/Diaporama/Pisciculture_pilote/
A pilot fish farming system that uses 10 times less water

Farming high-quality fish at lower economic and environmental costs

In this experimental system, 90% of the water is recycled by means of a system that involves re-injection of the water, after treatment, into the rearing ponds. In this way, only 10% of the amount of water necessary is extracted from the natural environment. Before the water leaving the ponds is pumped back into the circuit, it is filtered to treat the toxic substances produced by the animals: solid waste (matter in suspension) is eliminated by mechanical filtration, ammonia is transformed into nitrate by biological filtration and dissolved CO_2 is evacuated via an air injection system. The low flow rate of waste water from the circuit facilitates the treatment of waste before it is emitted into the natural environment.

The aim of the pilot plant set up at the INRA Experimental Fish Farm in Monts d'Arée is to define the conditions for the viability and equilibrium of this production system, assessed using several criteria: reduction in water needs and waste, maintenance of zootechnical performance, fish welfare, fish flesh quality and the preservation of production potential.

Successful early results

The early results showed that in operating mode, the pilot was able to maintain a permanent stock of 2 tonnes of fish, equivalent to an annual production of 7 tonnes of salmonids, thus exceeding the

initial target by 20%. The amount of "new" water required to achieve this yield was only 7 m3 per kilo of fish produced, versus 100 m3 under an open system. The quality of the water in the circuit was always compatible with the rearing of salmonids.

Zootechnical performance was compared with that of rainbow trout reared in a standard, open-circuit system. The results were more than encouraging. Indeed, they showed that not only was the zootechnical performance of the animals not degraded, but it was improved. After 10 months of operation, fish produced under the recirculated system were 30% fatter than those from the open-circuit system. Finally, from the health point of view, no bacterial or parasitic diseases occurred during trial.

Research to be pursued

Numerous points still need to be clarified, and particularly the impact of this new rearing system on the physiological functions of fish, the quality of their flesh and their welfare. Problems with acclimatization to the environment, behaviour and responses to stress may indeed occur because of the increased number of fish in the ponds.

These analyses will focus in particular on:

- fish health, behaviour, morphology and growth as a function of the different aquatic parameters modulated by the recirculated rearing system,
- the characteristics and reproductive performance of fish, such as age at puberty, gamete quality and embryonic development,
- the impact of rearing conditions on carcass quality and flesh quality.

Finally, an environmental analysis will be made of the system using tools such as Life Cycle Analysis (LCA).

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A novel method for preparing dicaffeoylquinic acids, natural molecules with high added value for pharmaceutical, nutritional, cosmetic and agronomical uses

Numerous publications describe the chemical and biological properties of dicaffeoylquinic acids (diCQ), natural substances that notably have a considerable antioxidant activity. This fact makes the diCQ very interesting from a nutritional perspective. The diCQ are only recently available on the market, but at high prices.A method for preparing high yield of diCQ would open up interesting prospects for the valuation of these molecules coming from the secondary metabolism of plants, and presenting many properties. Under the leadership of Dr. Jean-Luc Poëssel of the research unit "Génétique et d'Amélioration des Fruits et Légumes" of INRA in Avignon, France, researchers have developed a method for producing 3,5-dicaffeoylquinic (3,5-diCQ), a diCQ isomer, from non-tuberous roots of sweet potato (*Ipomoea batatas*). The method of extraction of dicaffeoylquinic acids from non-tuberous roots of sweet potato or other species of the Convolvulaceae family, and the use of this compound family for combating aphids are protected under a patent application filed by INRA (see the patent family FR2926955, CA2712645, WO2009/095624, US2011/0054022 or EP2237665). INRA Transfert is in charge of the development of this innovation through licensing for commercial applications targeting companies with the ability to produce diCQ compounds, active ingredients intended for pharmaceutical, cosmetic, agro-food and agriculture, as well as companies of agronomical sector that develop alternative biocide formulations for combating crop pests.

Background

Dicaffeoylquinic acids (diCQ) are phenolic compounds of the phenylpropanoid family. They are a group of isomeric compounds identified in many species of agronomic interest belonging to different botanical families: Rosaceae (pome fruit and stone fruits), Solanaceae (tomato, potato), Asteraceae (sunflower, artichoke, lettuce), Rubiaceae (coffee), Convolvulaceae (sweet potato), etc. They are derived from chlorogenic acid (5-caffeoylquinic acid), a phenolic compound widely distributed in plants.

Numerous publications describe the chemical and biological properties of these natural substances, notably a considerable antioxidant activity which makes them very interesting from a nutritional perspective. Many pharmacological properties have been also described: analgesic, hypouricemic, anti-inflammatory, hepatoprotective, antidiabetic, anticancer and antiviral properties.

The diCQ production by extraction from various plant sources (agricultural or medicinal plants) has already been described. However, these compounds are often difficult to obtain with a high yield due to the limited diCQ content of the plant material used, the formation of isomers during extraction process, and the presence of many other phenolic compounds that hinder the isolation of these molecules. The diCQ are only recently available on the market, but at high prices.

A method for preparing high yield of diCQ would open up interesting prospects for the valuation of these molecules coming from the secondary metabolism of plants, and presenting many properties.

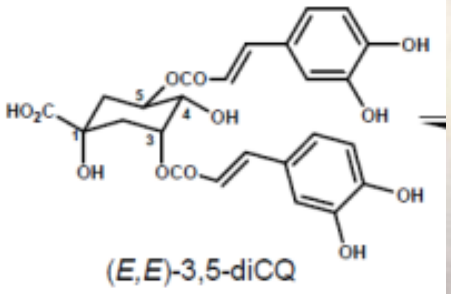
Description of the innovation & industrial applications

Under the leadership of Dr. Jean-Luc Poëssel of the research unit "Génétique et d'Amélioration des Fruits et Légumes" of INRA in Avignon, France, researchers have developed a method for producing 3,5-dicaffeoylquinic (3,5-diCQ), a diCQ isomer, from non-tuberous roots of sweet potato (Ipomoea batatas). The plant source may contain, under certain culture conditions, a 3,5-diCQ content representing 5% to 10% of the root dry matter. The process of extraction and purification of the substance allows the production of 3,5-diCQ at a high level of purity (>98%) and with a yield that is very much higher than those described so far in the literature. For example, INRA's protocol allows the production of 200mg of 3,5-diCQ purified from only 10 g of root dry matter. The protocol for production of 3,5-diCQ has been optimized thanks to the identification of the plant sources, the use of favorable growing conditions, and

the development of a efficient process of extraction and purification by high performance liquid chromatography.
Applying this new process on sweet potato, a cheap and potentially abundant vegetable source, will open the way for the production of large amounts of 3,5-diCQ at low cost. Thus, this molecule can be offered to certain companies of the pharmaceutical, nutritional and cosmetic sector, which are interested to use high added value molecules for their research and in the formulation of their products. Moreover, in vitro experimental results have also demonstrated the toxic properties of diCQ on aphids, major pests of many crops and pathogen vectors, thus opening the way for opportunities to develop new aphicide formulations, which are strongly requested in agriculture. The aphicide activity of these molecules by application to plants has to be demonstrated, and the search for effective formulations must be developed in partnership with specialized companies.

Intellectual property and technology transfer

The method of extraction of dicaffeoylquinic acids from non-tuberous roots of sweet potato or other species of the Convolvulaceae family, and the use of this compound family for combating aphids are protected under a patent application filed by INRA (see the patent family FR2926955, CA2712645, WO2009/095624, US2011/0054022 or EP2237665).
INRA Transfert is in charge of the development of this innovation through licensing for commercial applications targeting companies with the ability to produce diCQ compounds, active ingredients intended for pharmaceutical, cosmetic, agro-food and agriculture, as well as companies of agronomical sector that develop alternative biocide formulations for combating crop pests.
Moreover, collaborative research projects, specifically focusing on aphicide applications of diCQ will be evaluated with interest by INRA.



(*E*,*E*)-3,5-diCQ

3,5-dicaffeoylquinic acid (3,5-diCQ) is a phenolic secondary metabolite of plants purified from non-tuberous roots of sweet potato (Ipomoea batatas). The 3,5-diCQ is particularly involved in the resistance of peach to the green aphid Myzus persicae (repellency and toxicity demonstrated in in vitro bioassay by INRA). The diCQ have also interesting properties in pharmacology, nutrition and cosmetic.

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IHMS – International Human Microbiome Standards

At a time of major initiatives relative to deciphering the genome of bacterial communities associated with humans, a new European project aims to harmonise practices and facilitate the comparison of data between projects of different origins. This project, launched on 1st February 2011 for a period of 4 years, is being coordinated by S. Dusko Ehrlich at INRA in Jouy-en-Josas and involves eight partners, all international experts in the microbiota, in order to ensure a global scale for its results.

Bacteria that want to do us good

With 10 to 100 bacterial cells for each human cell, according to the lowest estimate a human being is made up of 90% bacteria. And their diversity is such that, thanks to them, we possess approximately one hundred times more genes than the 20,000 genes of our genome. This bacterial community, or microbiota, appears to work very closely in maintaining the equilibrium of our bodies, hence the international race that has been under way in recent years to elucidate its role. A race, yes, but not very organised or methodical! This was therefore the aim of the new European IHMS (International Human Microbiome Standards) project. The challenge is considerable: to permit the comparison of data arising from different research studies, now and in the future, through the introduction of international standards. One key reason is that although major projects have already been engaged in Europe, the USA, China and Canada, none of them, however ambitious, can claim to have achieved an exhaustive characterisation of the microbiota and its role in health.

"Omic" approaches to generate a considerable mass of data

Today, metagenomics enables study of the genomics of bacterial communities through the mass sequencing of purified DNA. These studies have generated a complex series of sequences, but very few organisms have been wholly sequenced. Study of these series of sequences, combined with the sequences of complete genomes, raises numerous problems, not only of an informatics nature but also of a methodological and mathematical type.

A project to harmonise global practices and facilitate data comparisons

The European IHMS project aims to coordinate the development of standardised protocols and operational procedures in order to optimise the comparison of data in the field of the human microbiome and thus improve synergies between different projects. The IHMS is thus targeting three key aspects of data generation:

- The collection of human samples, their processing and identification
- The quality of the DNA sequences generated
- The analysis of DNA sequences.

The project aims to organise public access to standardised protocols and operational procedures and enable exchanges between the users and suppliers of standards.

Partners* who are international leaders in the field of the human microbiome*

The IHMS project brings together eight international partners who are involved in the most

ambitious projects currently under way on three continents: Europe, Asia and America. This project interfaces with other projects in Africa, Australia and Asia through privileged exchanges with the IHMC – International Human Microbiome Consortium – an international initiative born in 2008 in Heidelberg, Germany, which seeks to coordinate the major programmes exploring the human microbiota and undertaken by different institutions.

**Partners involved in the IHMS project:*

INRA; HUVH (Institut Catala de la Salut – Spain); BCM (Baylor College of Medicine, USA); SJTU (Shanghai Jiao Tong University, China); CEA – Genoscope (Atomic Energy and Alternative Energies Commission, France); BGI Shenzhen (Beijing Genomics Institute, Shenzhen, China); EMBL (European Molecular Biology Laboratory, Germany); Western (The University of Western Ontario, Canada).

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AQUAEXCEL

The development of sustainable aquaculture is today a high priority for Europe. Coordinated by INRA, and launched on 1st March 2011, the AQUAEXCEL project (Aquaculture Infrastructures for Excellence in European Fish Research) will set up a new European platform of excellence for research and experimentation in aquaculture. The overall objective is to enable innovation to drive the development of high-quality aquaculture with a low environmental impact.

European aquaculture covers a broad spectrum of fish species (such as salmon, trout, sea bass, gilthead bream or carp). An small-scale activity in the 1960s, this sector has rapidly become a true industry involving multinationals, SME and family enterprises.
According to Marc Vandeputte, Research Engineer at INRA and coordinator of the **AQUAEXCEL project**, "consumers are concerned about where their food comes from. The European aquaculture industry will only be able to respond to their demands through greater knowledge of fish biology and aquacultural technologies."

AQUAEXCEL is being carried out by a cross-disciplinary team of experts in fish nutrition, physiology, health and welfare, genetics, instrumentation and aquacultural engineering.

It will constitute a network of the best European aquaculture infrastructures (17 partners, 1O countries*), covering all types of production systems, environments, species, scientific expertise and disciplinary fields.

- The main goals of the project are:
- to offer European aquaculture research teams access to the best infrastructures.
- to combine resources and know-how in order to create conditions for the emergence of joint research projects.
- to develop projects aimed at improving the efficiency of infrastructures aquaculture research (remote access and follow-up, phenotyping, limitations on the use of live animals, validity of research at an industrial scale, development of biological models).
- closer links between the scientific community and end users through the development of mission-oriented research and improvements in technology transfer.

AQUAEXCEL (2011-2015) was granted funds of €9.2 million in the context of the "Infrastructures" section of the Seventh European Framework Programme (FP7).

At a European level, this project will enable improvements in competitiveness through the transfer of knowledge, innovation and technological development.
It will ensure a better fit between research results and the needs of industrial aquaculture.

* Belgium, Czech Republic, France, Greece, Hungary, Ireland, Netherlands, Norway, Spain, United Kingdom.

For more information:
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http://www4.jouy.inra.fr/gabi/les-Recherches/Projets-Transversaux/Projet-Aquaexcel

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INFOGEST

This Action aims at building an international network of institutions working on the digestion of dietary proteins and the effects on human health of the digestion products released in the gut. So far, 37 institutions from 20 countries (from Europe but Canada and New-Zealand as well) are involved in this Action, but the network will be fully open for welcoming new partners who wish to join it.

The links between food and human health was a top research priority for Europe. EU legislation, as advised by EFSA, now demands proper scientific data in nutrition and health claims. There is a lot of data being generated on the link between the food digestion and human health and a significant effort continues to be expended separately in each EU country on optimizing food for preventing the development of food-related diseases.
The INFOGEST Action will gradually build a European network that will spread and improve current basic knowledge on food digestion and promote harmonization of currently used digestion models used including validation with human data from different populations such as infants, elderly, sport professionals etc. A multidisciplinary scientific community will be built: food science, nutrition, physiology, immunology, cell biology, etc.
The Action will facilitate the transfer of new scientific advances to European food companies (large groups as well as SMEs) for developing new functional foods and reinforcing their competitiveness in a growing world market.
This 4-year Action will have a budget around 100 k€ / year only dedicated to the organization of meetings, workshops and conferences.

The INFOGEST Action (Improving health properties of food by sharing our knowledge on the digestive process) will be funded by COST.

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http://w3.cost.eu/index.php?id=181&action_number=FA1005