INNOVA HEALTH FOOD LABS
Trophometry Experts

INNOVATION & HEALTH FOODLABS

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INNOVAHEALTH FOODLABS

INTRODUCTION

iNNovaHealth Foodlabs is a laboratory for nutritional research and development of innovative functional foods resulting in increasing competitiveness in the agri-food sector and the food industry. We are aiming in the accurate qualitative and quantitative identification of the ingredients of any liquid or solid food, of plant or animal origin, even food supplements, and thus in the creation of their unique “molecular identity”.

Creation of a laboratory for nutritional research and development of innovative foods with the consequence of increasing competitiveness in the agri-food sector and the food industry.

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ABOUT

Trophometry, a new branch of food and nutrition science, is the set of methods used to study a food and the identification of all proteins, peptides, metabolites, lipids and all other groups of bioactive molecules it contains.

Trophometry can lead to the creation of microstructures of food that will allow their adaptation and absorption by the human body at a higher personal level, depending on the nutritional requirements of each individual to achieve a personalized nutrition with maximum efficiency, quality and identity but also having a strong scientific signature and validity.

What we do

Basic Research

which will contribute substantially to the production of new knowledge based on modern principles of quality assurance of existing and new food products in order to investigate their potential role in the health and quality of life of modern human.

Applied Research

an area in which new knowledge will be applied to improve the quality and safety of existing and new food products on a semi-industrial- industrial scale leading to market.

Technological Research & Development

which will include systemic work that is either based on existing knowledge and aims to prepare for the production of new products, services, or to improve existing ones.

The particular progress of cutting-edge holistic technologies as well as their applicability in food technology provide for the first time the opportunity for precise qualitative and quantitative determination of the components of any liquid or solid food, of vegetable or animal origin, even nutritional supplements, and thus to create their unique "molecular identity". Already this progress and its applications have led to the development of a new branch of food and nutrition science, trophometry, the set of methods used to study a food and identify all of its proteins, peptides, of metabolites, lipids and all other groups of bioactive molecules it contains.

Combined, all these molecules create the trophic footprint of each food, which is unique, as is the case with the genetic profile of each person. Trophometry in combination with 3D printing can lead to the creation of food micro-structures that will allow adaptation and absorption by the human body on a highly personal level, depending on the nutritional requirements of each individual in order to achieve personalized nutrition with maximum effectiveness but also quality identity having a strong scientific signature and validity.

At the same time, it is possible to precisely identify the products of higher quality with beneficial actions for human health, the geographical origin of each and which of them can have the "stamp" of the functional food, i.e. that which, beyond its natural nutritional value contains additional bioactive ingredients (endogenous or additives of natural origin), which benefit the body and its specific functions, reducing at the same time the possibility of chronic diseases.

All of the above are expected very soon to break new ground in the food industry as they will offer a valid scientific seal of identity and quality to every product that reaches the table of consumers around the world. In addition, they will also provide added value to the producer, who is the initial link in the production chain of a food product since he is the one who provides the raw material, which will now be able to be characterized and identified, making the Greek agri-food chain competitive on a global level . In addition, in the long run, the national economy will be strengthened through the production of excellent, competitive products.

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EXPERTISE

iNNovaHealth Foodlabs implements the following techniques / technologies in the laboratory

Extraction methodologies

Extraction of bioactive substances from food (e.g. fruits, vegetables) in order to isolate bioactive substances such as vitamins, ω fats, etc.

The extraction method Pressurized hot water extraction (PHWE)] is one of the most modern and promising techniques in the extraction of bioactive substances. The PHWE method is based on using water at high pressure and sometimes at a temperature higher than the boiling temperature of water at that pressure. The advantage of this method is based on the fact that under these conditions, the physical and chemical properties of water change dramatically and for example the dielectric constant of water changes from 80 at room temperature (25°C) to 33 at 200°C, which is close to that of organic solvents such as methanol. Furthermore, both the surface tension and the viscosity of the extractant decrease with increasing temperature while the diffusivity increases thereby promoting extraction both in efficiency and speed. In addition, the solubility parameter of the various bioactive substances in water is modified with temperature and the selectivity of the extraction of the various substances is altered. The use of a combination of high pressure and temperature ensures a faster extraction which can be completed in as little as 20 min instead of 10-48 h and using a much smaller amount of solvent.

Encapsulation of bioactive compounds and ingredients

Encapsulation technologies of bioactive substances with specific functional properties in food products. The encapsulation of bioactive substances in food products aims at preserving their bioactivity and at their more efficient absorption by the human body.

Bioactive substances are usually sensitive and deteriorate if not encapsulated in a suitable excipient for use in food, cosmetics, dietary supplements and/or other uses. The answer to this problem is the encapsulation of the active substance of the extract in a suitable excipient such as maltodextrin, various cyclodextrins, whey protein, lecithin, starch and modified starch, etc. In addition, encapsulation causes masking of unpleasant odors and color as well as modifying the solubility of initially insoluble materials. The production of an encapsulated derivative from an extract enriched by industrial chromatography is based on mixing the extract with a solid excipient and creating a solution with a solids content of 15% to up to 60%. This solution then undergoes particle size reduction at the micro- (>1 μm) or even nano- (<100 nm) level using an ultrasonic homogenizer or a high-pressure homogenizer and is then converted into a stabilized powder using freeze drying or spray drying, which is better preserved than the liquid enriched extract.

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EXPERTISE

Holistic High-Throughput Technologies (-omics toolbox) in food and nutritional ingredients for the design of "personalized foods".

Today, in addition to the four main types of -omics analyzes (genomics, transcriptomics, proteomics and metabolomics), a variety of sub-types of -omics analyzes such as epigenomics, lipidomics, nosomics etc. have been developed. Through the use of these of high-performance holistic technologies, the possibility of connecting nutritional components, food, nutrition, health and human disease at a personalized level is now possible. This broad vision, however, needs not only the application of advanced holistic technologies that are already developed and constantly being improved but above all the ability to approach the question from a different point of view, that of food. In this context the term "foodomics" was developed to describe the integrated approach to food and nutrition through holistic high-throughput technologies to exploit food science in light of improving human nutrition and thus health. It is a new approach that studies food and nutrition as a whole with their nutritional value to achieve the main goal of optimizing human health and well-being.

Technological progress so far has succeeded in coupling various analytical techniques with the goal of developing holistic high-performance technologies. The most reliable qualitatively and quantitatively is liquid chromatography-mass spectrometry (LC-MS).

Liquid Chromatography - Mass Spectrometry (LC-MS) is an analytical chemistry technique that combines the physical separation capabilities of liquid chromatography (or HPLC) with the mass analysis capabilities of mass spectrometry (MS) through interface based on atmospheric pressure ionization (API), electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), and atmospheric pressure photoionization (APPI) strategies. Such an above-mentioned linkage is already successfully used in fields such as proteomics, metabolomics and lipidomics and can accurately analyze a huge range of biochemical, organic and inorganic compounds commonly found in complex samples of environmental and biological origin.

EXPERTISE

Food nutritional analysis techniques

Nutritional analysis of food based on the requirements of the legislation but also more detailed analyzes such as vitamin profiles, lipids, etc.

Few things in life are more important than the food we consume. Today, the food supply is more diverse but also more processed than ever. To ensure food safety and nutritional quality, many countries have established regulations that set acceptable levels for individual chemical additives, residues and impurities in food products. Other regulations require that ingredients related to their nutritional content, such as unsaturated and saturated fat, be listed on food packaging. In this direction, food manufacturers and processors themselves must be able to assess the quality of the food product. Meeting all these requirements is the function of food analysis.

High performance liquid chromatography

Real-time polymerase chain reaction (Real-Time PCR)

Recently, the concept of genetic identity has been introduced in food quality control and it is expected that this information will be included on their label soon in the future. The genetic identity of a food is nothing more than recording the existence of specific DNA sequences or the very characteristic expression of genes for each food and is carried out by the molecular technique of real-time polymerase chain reaction (Real-Time PCR). This is a common method of amplifying (amplifying) DNA outside of living cells.

In order to quantify the expression of a gene, its mRNA should be collected, then reverse transcribed into complementary DNA (cDNA) using the enzyme reverse transcriptase, and then the single-stranded cDNA converted to double-stranded and the chain reaction performed on it polymerase, i.e. its in vitro amplification. Real-Time PCR, also known as quantitative polymerase chain reaction (qPCR), is a molecular biology laboratory technique based on the polymerase chain reaction and can monitor the amplification (amplification) of a targeted DNA molecule during its in vitro replication (i.e., in real time), and not just at the end of the process, like conventional PCR. It can use either cell DNA or that resulting from the reverse transcription of mRNA and thus provide quantitative information on the expression of specific genes in selected cells at a time.

PCR generally consists of a series of temperature changes repeated 25-50 times. These cycles usually consist of three steps: the first, at about 95 °C, allows the separation of the DNA double strand. The second, at a temperature of about 50-60 °C, allows the binding of primers to the DNA template and the third, at 68-72 °C, facilitates the polymerization carried out by DNA polymerase. The last step is usually omitted in Real-Time PCR as the DNA polymerase enzyme can increase the number of DNA copies during the transition between the DNA alignment step and the DNA unwinding step. The temperatures and timings used for each cycle depend on a wide variety of parameters, including: the enzyme used to synthesize the DNA, the concentration of divalent ions and deoxyribonucleotides (dNTPs) in the reaction as well as the optimal temperature of binding the primers to the DNA.

Two common methods for detecting Real-Time PCR products are 1) non-specific fluorescent dyes that intercalate between any double-stranded DNA and 2) specific DNA probes consisting of oligonucleotide sequences labeled with a fluorescent molecule, and which allow detection of the products only after their hybridization with their complementary sequence on the reaction product thus significantly increasing the specificity of the method for a single DNA molecule even in the presence of other double-stranded DNA molecules. As the process progresses, increasing the product targeted by the reporter probe in each PCR cycle causes a proportional increase in fluorescence.

By using different color labels, fluorescent probes can be used in multiplexed assays to monitor multiple DNA sequences in the same tube. As the reaction begins, during the annealing step both the probe and primers are annealed to the DNA target. real-time allows the identification of specific, amplified DNA fragments by analysis of the characteristic melting temperature of each DNA molecule, which is the same for all replicated DNA molecules, which is the same for all replicated DNA molecules produced from it. The results of this technique are obtained by comparing the separation curves of the analyzed DNA samples.

Polymerization of a new DNA strand is initiated by the primers, and once the polymerase reaches the probe, an increase in fluorescence is induced. Fluorescence is detected and measured in a real-time PCR machine, and the geometric increase corresponding to the exponential increase in product is used to determine the quantification cycle in each reaction. Real-time PCR allows the identification of specific, amplified DNA fragments by analysis of the characteristic melting temperature of each DNA molecule, which is the same for all replicated DNA molecules, which is the same for all replicated DNA molecules produced by this. The results of this technique are obtained by comparing the separation curves of the analyzed DNA samples.

In the health and food sector the applications of Real-Time PCR are constantly increasing and include:

Diagnostic uses

Diagnostic Real-Time PCR is applied to the rapid detection of nucleic acids that are diagnostic of, for example, infectious diseases, cancer and genetic diseases. The introduction of qualitative PCR assays into the clinical microbiology laboratory has greatly improved the diagnosis of infectious diseases, [30] and is being developed as a tool for the detection of emerging diseases, such as new strains of influenza and coronavirus.

Microbiological uses

Real-Time PCR is also used by microbiologists working in the fields of food safety, food spoilage and fermentation and for microbial risk assessment of water quality (drinking and recreational waters) and public health protection.

Detection of phytopathogens

The agricultural industry is constantly trying to produce propagating plants or seedlings that are free of pathogens to prevent economic losses and protect health.

Detection of genetically modified organisms

Real-Time PCR using reverse transcription can be used to detect genetically modified organisms given its sensitivity and dynamic range in DNA detection. Alternatives such as DNA or protein analysis are usually less sensitive. Specific primers are used that do not amplify the transgene but the promoter, terminator or even intermediate sequences used during the vector construction process. As the process of creating a transgenic plant usually results in the introduction of more than one copy of the transgene, its quantity is also usually estimated. This is often performed by relative quantification using a control gene from the treated species that exists only as one copy.

DESCRIPTION OF INSTRUMENTS

Encapsulation: Spray dryer

Spray drying, which is an important and special dehydration technique that is responsible for the production of droplets. With the development of technology, a wide range of spray dryers is available and depending on the desired result, fine or coarse powders or agglomerates or particles can be produced.

Initially the material is placed in a drying chamber in the form of a fine mist or by spraying in the form of small droplets where it comes into contact with hot air, if the size of the droplets is small this is considered a key advantage as rapid dehydration is achieved and the time needed to remain in the dryer is a few seconds. Dehydrated material in powder form is taken up by the base of the dryer and removed by a conveying screw or a pneumatic system, and the produced powder is continuously cooled.

The term atomization which refers to the breaking up of a liquid into many tiny droplets which appear as a form of cloud and hence the term fogging. A key feature of spray drying is the formation of a cloud or spray. In order to obtain economic production and the production of a quality product, an important role is played by the correct selection and operation of the nebulizer. To achieve the desired product in the atomization stage, a cloud must be produced for excellent vaporization conditions. The most common are rotary atomizers and nozzle atomizers.

The operation of rotary atomizers involves feeding the material to the center of the rotating disk which rotates at a speed of 90-200 m/s and from the edges of the wheel, droplets that have a width of 50-50 μm and their shape are uniform come out at high speed . They are low pressure systems. They are characterized by their ease of operation and can accept high feed rates.

In nozzle atomizers the liquid must pass through a nozzle with a very small opening at high pressure (700-2000 kPA) resulting in the creation of droplets with a diameter of 120-300 μm. Inside the nozzle there are ridges that give a cone-shaped spray effect and for this the entire volume of the chamber is used. Compared to that from rotating discs the produced cloud is less homogeneous and voluminous.

DESCRIPTION OF INSTRUMENTS

Organology of High Performance Liquid Chromatography

High performance liquid chromatography (HPLC) is increasingly used in the analysis of food samples for the separation and detection of additives and impurities. This method breaks down complex mixtures into individual compounds, which in turn are identified and quantified by suitable detectors and data handling systems. In essence, HPLC is a form of column chromatography that pumps a sample mixture or analyte in a solvent (known as the mobile phase) under high pressure through a column packed with chromatographic packing material (stationary phase). The sample is carried by a moving stream of helium or nitrogen gas. HPLC has the ability to separate and identify compounds present in any sample that can be dissolved in a liquid at trace concentrations as low as parts per trillion. Because of this versatility, HPLC is used in a variety of industrial and scientific applications, including pharmaceutical, environmental, forensic and chemical.

Because separation and detection occurs either at room temperature or at slightly higher temperatures, the method is ideal for compounds of limited thermal stability. The retention time of the sample on the chromatography column will vary depending on the interaction between the stationary phase, the analytes and the solvent or solvents used. As the sample passes through the column, it interacts between the two phases at a different rate, mainly due to the different polarity of the analytes. Analytes that have the least amount of interaction with the stationary phase or the most interaction with the mobile phase will exit the column more quickly.

The main components of an HPLC instrument include the solvent reservoir or multiple reservoirs of different solvents, a high-pressure pump, a column, an injector system, and the detector. The reservoir contains the solvent, which is referred to as the mobile phase. There are usually at least two tanks in a system, each holding up to 1000 cc of solvent and usually fitted with a gas diffuser through which the gas (mostly helium or nitrogen) can be passed. A pump is used to create a defined flow of the mobile phase. Although manual injection of samples is still possible, most HPLCs are now fully automated and computer controlled. The injector introduces the solvent into a phase stream that transports the sample to the high pressure column (up to 400 bar), which contains special chromatographic material required to perform the separation.

The chromatographic material is referred to as the stationary phase because it is held in place by the column material. Finally, a detector is required to observe the separated composite bands as they elute from the high pressure column. The information is sent from the detector to a computer that generates the chromatogram. The mobile phase exits the detector and is either sent to waste or collected. In modern HPLC instruments, the ability to inject large amounts of sample (up to 1-2 ml per injection) makes HPLC a very sensitive analytical technique. In addition, the non-destructive detection techniques included in the instrument allow fractions to be collected for further analysis. Finally, modern sample preparation techniques such as solid-phase extraction (SFE) and supercritical fluid extraction allow high-throughput, sensitive, and selective analysis by the HPLC instrument.

Other equipment and micro instruments

The accompanying equipment of the above large instruments requires a number of micro instruments for each stage of processing and analysis of the samples which are presented by category of use below.

Sample preparation

- Precision scale (0.0001gr)
- Simple scale
- Drying oven
- Magnetic Stirrer Hot Plate
- Homogenizer
- Digital Refractometer eg Brix
- Water bath
- Table top centrifuge
- Vacuum filtration system
- Speed vacuum condenser for deaeration of liquids
- Ultra-pure water system with UV filter
- Water deionization column
- Grinding mill

Measurements of physical and chemical properties of foods

- Activity counter
- All consumable glassware for the application of the Kjeldahl method for protein determination
- pH-meter
- Conductivity meter
- Acidity meter
- UV-Vis spectrophotometer
- Microorganisms and glass consumables for the application of Soxhlet extraction and determination of total fat

Microbiological analyses

- Laminar flow cabinet to prevent the spread of microbes from and to the samples
- Solid culture incubator
- Shaking wet culture incubator
- Buchner lamp
- Desktop microcentrifuge
- Floor centrifuge
- Liquid sterilization oven
- Dry sterilization oven

EQUIPMENT JUSTIFICATION
LABORATORY ANALYSES

Food ingredients

Vitamins

- Fat-soluble vitamins (A, D, E, K)
- Water-soluble vitamins (C, B)

Sugars

- e.g. Fructose, Glucose, Lactose

Lipid analysis

- eg ω3, ω6, ω7

Metals

- e.g. Iron, Lead, Zinc, Copper

Trace elements

- e.g. Magnesium, calcium, potassium, sodium

Heavy metals

- e.g. Lead, Arsenic, Aluminum, Mercury

Food additives

Food contaminants

- e.g. Acrylamide, Dioxins

Mycotoxins

- e.g. Aflatoxin

Allergens

- e.g. Nuts, eggs, milk

Biological hazards

- e.g. Genetically modified products

Microbiological hazards

- e.g. E.coli, Salmonella, yeasts and fungi