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Maria Teresa Giardi
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Bio-Farms for Nutraceuticals

Functional Food and Safety Control by Biosensors

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BIO-FARMS FOR NUTRACEUTICALS: FUNCTIONAL FOOD AND SAFETY CONTROL BY BIOSENSORS
Maria Teresa Giardi, Giuseppina Rea and Bruno Berra

Bio-Farms for Nutraceuticals

Functional Food and Safety Control by Biosensors

Edited by

Maria Teresa Giardi, PhD

Institute of Crystallography, Department of Agrofood and Department of Molecular Design, National Council of Research, Area of Research of Rome, Monterotondo Scalo, Rome, Italy

Giuseppina Rea, PhD

Institute of Crystallography, Department of Agrofood and Department of Molecular Design, National Council of Research, Area of Research of Rome, Monterotondo Scalo, Rome, Italy

Bruno Berra, PhD

Institute of General Physiology and Biochemistry, Department of Molecular Sciences Applied to Biosystems, DISMAB, University of Milan, Milan, Italy

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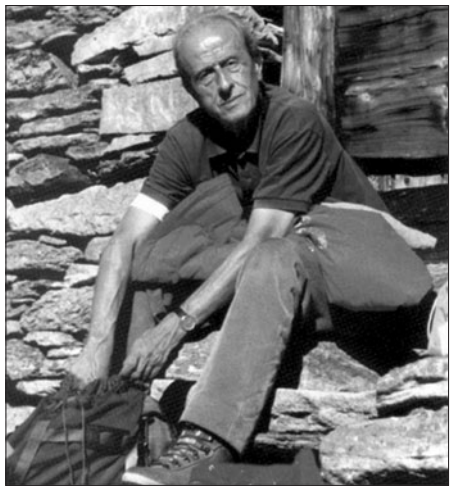


This book is dedicated to the nutritionist Dr. Paolo Sorbini, founder of Also-S.p.A., now known as Enervit S.p.A. Enervit S.p.A. is the foremost Italian company specialising in sports nutrition and wellness. It manufactures and distributes products satisfying specific needs for those involved in sports at the professional level and for those merely interested in preserving their own healthy lifestyle. Since the 1980s, Dr. Sorbini, an early contributor of great discoveries in this field, succeeded in completely revolutionizing the idea of nutrition before, after and while performing sports.

Supporting the opinion that good results can be obtained in sporting competition by using a suitable nutrition strategy while avoiding doping, he understood the importance of restoring the right hydro-saline balance in the blood and the necessary supplementation for muscle recovery after intense physical activity. Born in 1926 to an Italian family of Montepulciano 'apothecaries', he dedicated his life to scientific research and sports nutrition, collaborated with universities, sports institutions and placed his experience at the service of events and ventures across diverse disciplines and high level events: from Moser's hour record in the World Cycling competition to the exploits of Valentina Vezzali in fencing, from Messner's primacy in ascending all fourteen mountain peaks over 8,000 metres to Tomba and Di Centa in the Skiing World Cup, from Simeoni's world record in the women's high jump to the successes of Inter-Milan and Juventus in football. The

energy and enthusiasm of Paolo Sorbini also opened his entry into the field of medicine concerning obesity. He supported the idea that these worldwide nutritional disorders could be controlled by establishing the right balance of insulin in the blood and collaborated with the famous American biochemist Dr. Barry Sears, creator of the Zone diet. This collaboration brought about the development of a new important brand called EnerZona which combines a series a food supplements to establish the correct hormonal balance in the body. After his death in December 2006, his sons Maurizia, Pino and Alberto founded the 'Paolo Sorbini Foundation for Nutrition Science'. The foundation was established with the aim to promote scientific research and to communicate the knowledge acquired in the field of diet and nutrition through the organization of seminars, congresses, practical courses, work experience and editorial projects.

Finally, particularly close to the foundation's heart is to inform the younger generation of the importance of appropriate nutrition and diet when approching physical activity.

PREFACE

This volume can be said to have been born of the NUTRA-SNACKS project within the Sixth Framework Programme Priority on Food Quality and Safety. One objective of NUTRA-SNACKS was to improve the nutritional and eating properties of ready-to-eat products and semi-prepared foodstuffs through better monitoring of the quality and safety of raw materials and the development of innovative processes along the production chain. Another main objective of the project was the production of ready-to-eat snacks with high nutraceutic activity. Seven research institutes and three companies in six European countries were involved in this effort. The co-operation resulted in the production of food having a high content of natural metabolites with the following beneficial health effects: anticancer, antilipidemic, anticholesterol, antimicrobial, antibacterial, antifungal, antiviral, antihypertensive, anti-inflamatory and antioxidant activities.

Bio-Farms for Nutraceuticals treats comprehensively yet concisely the subjects of phytochemicals in food and feedstuffs, the production of phytochemicals by field crops and tissue cultures as important safety and quality issues in developing phytochemical products.

The early chapters describe the efficacy and safety of some medicinal, nutraceutic herbs and plants, providing a comprehensive review of the most notable phytochemicals being researched today. Information is given about carotenoids, polyphenols, sulfur-containing compounds and secondary plant metabolites showing antioxidant activity. Diet-gene interactions are treated as well as the mechanism and the therapeutic potential against cancer, obesity and aging disorders. Nutrition strategies are also proposed to prevent diseases and improve general well-being. The middle chapters expand the information on phytochemicals and focus on the production of useful secondary products by using molecular biological techniques. The most important methods to enhance the quantity of phytonutrients are discussed as well as the possibility of producing plant compounds on a large scale using industrial bioreactors.

The later chapters deal with testing the safety and quality of nutraceutics using both classical and new research methodologies and technologies. Special attention is given to the currently very active field of research into biosensors and their

applications. Different forms of these devices, which are able to detect the presence of active phytochemicals and toxic compounds or evaluate the antioxidant activity of bioactive species, are described. The final chapters add a comprehensive analysis of the main regulations about functional food and nutraceutical products. The most important ethical and safety issues are elaborated to provide a broader understanding of the field of functional foods, their pros and cons and the future prospects for a worldwide nutraceuticals market.

Maria Teresa Giardi, PhD
Department of Agrofood and Department of Molecular Design, CNR-IC National Council of Research, Institute of Crystallography, Rome, Italy

Giuseppina Rea, PhD
Department of Agrofood and Department of Molecular Design, CNR-IC National Council of Research, Institute of Crystallography, Rome, Italy

Bruno Berra, PhD
Faculty of Pharmacy, Department of Molecular Sciences Applied to Biosystems, University of Milan, Milan, Italy

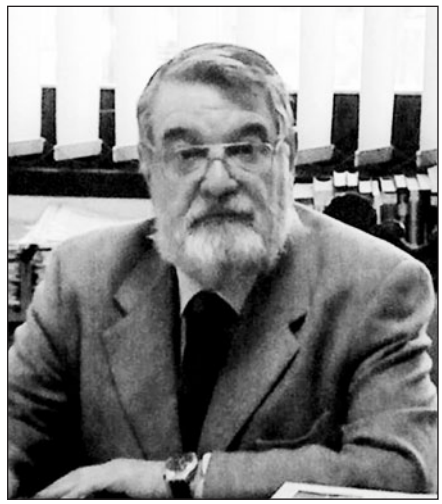
ABOUT THE EDITORS...

MARIA TERESA GIARDI, PhD, is Research Manager at the National Council of Research (CNR) in Rome, Italy. Her background is in organic-industrial chemistry. She has extensive experience in molecular biology; modification of photosystem II to increase its resistance to high temperature and sensitivity to radiation; signal transduction in optical and electrochemical biosensors; protein stabilization and utilization in biosensor design, and analytical procedures in general. She is a referee for the US Department of Agriculture for industrial projects and has worked in various research institutions and research and development companies both in Europe and the USA. She is a supervisor or coordinator of a number of national and international projects in the field of biosensors: European Agency Life V project, 20xx-2004; a European Space Agency project involving space flight in 2005; a NATO "linkage grant", dedicated to sensors based on plant and microrganisms.

ABOUT THE EDITORS...

GIUSEPPINA REA, PhD, is a molecular biologist and is on the permanent staff of CNR-IC. She is an expert in the molecular mechanisms involved in plant defense responses and has many years of experience in plant molecular biology, biochemistry, biotechnology and protein engineering techniques. She monitors the CNR project Biotechnological Applications of Oxygenic Photosynthetic Organisms in the Agro-food Field and Space Environment, and the EU projects Multibioplat and Sensbiosyn, aimed at producing bacteria and algae with improved photosynthetic performance to exploit them in biological farms for nutraceutical applications and biosensing elements in biosensor development. Her research activities are mainly focused on *Chlamydomonas reinhardtii* and *Rhodobacter sphaeroides*. She is advisor to several PhD students in Plant Biotechnology, in the fields of exobiology, nutraceutics and biosensors.

ABOUT THE EDITORS...



BRUNO BERRA, PhD, received his academic degree in Chemistry from the University of Pavia, Italy. He is Professor f.r. of Biochemistry at the University of Milan and works at the Department of Molecular Sciences Applied to Biosystems in the Faculty of Pharmacy. During his active teaching career he taught Biochemistry, Molecular Biology, Nutrition Biochemistry and Cosmetology. His most important research areas include: isolation, fractionation, purification and analysis of glycolipids from brain, extranervous tissues and biological fluids; biochemical diagnosis of sphingolipidosis and follow-up of Gaucher's patients receiving enzyme replacement therapy; nutritional biochemistry with reference to fats and antioxidants; membrane component studies in spontaneous or induced degenerative processes, with particular reference to solid and tumor cells; skin biochemistry and molecular biology of the skin; and space biology. He is a member of numerous national and international scientific organisations including the Italian Society of Biochemistry (1957-2004), the Italian Society for Fat Research, the International Conference of Biochemistry of Lipids (ICBL), the Euro Fed Lipid, the American Oil Chemist's Society, and the American Society of Diabetes.

PARTICIPANTS

William W. Adams, III
Department of Ecology
and Evolutionary Biology
University of Colorado
Boulder, Colorado
USA

Cecilia Ambrosi
Institute of Crystallography
Department of Agrofood
and Department of Molecular Design
National Council of Research
Area of Research of Rome
Monterotondo Scalo
Rome
Italy

Amina Antonacci
Institute of Crystallography
Department of Agrofood
and Department of Molecular Design
National Council of Research
Area of Research of Rome
Monterotondo Scalo
Rome
Italy

Lise Barthelmebs
IMAGES Group
Centre de Phytopharmacie
University of Perpignan
Perpignan
France

Giovanni Basile
Biosensor srl
Palombara Sabina
Rome
Italy

Bruno Berra
Institute of General Physiology
and Biochemistry
Department of Molecular Sciences
Applied to Biosystems
DISMAB
University of Milan
Milan
Italy

Patrizia Berselli
Institute of General Physiology
and Biochemistry
Department of Molecular Sciences
Applied to Biosystems
DISMAB
University of Milan
Milan
Italy

Alessandra Bertoli
DCBB-Department of Bioorganic
Chemistry and Biopharmacy
University of Pisa
Pisa
Italy

Iain Brown
Cancer Medicine Research Group
School of Medicine and Dentistry
Aberdeen University
Aberdeen, Scotland
UK

Vittorio Calabrese
Department of Chemistry
Clinical Biochemistry
and Clinical Molecular Biology
University of Catania
Catania
Italy

Carole Calas-Blanchard
BIOMEM Group
Centre of Phytopharmacy
University of Perpignan
Perpignan
France

Monica Campàs
BIOMEM Group
Centre of Phytopharmacy
University of Perpignan
Perpignan
France

Calogero Caruso
Immunosenescence Unit
Department of Pathobiology
and Biomedical Methodologies
University of Palermo
Palermo
Italy

Paola Corsetto
Institute of General Physiology
and Biochemistry
Department of Molecular Sciences
Applied to Biosystems
DISMAB
University of Milan
Milan
Italy

Montserrat Cortina-Puig
BIOMEM Group
Centre of Phytopharmacy
University of Perpignan
Perpignan
France

Anna Czubacka
Institute of Soil Science
and Plant Cultivation
Department of Biochemistry
State Research Institute
Pulawy
Poland

Barbara Demmig-Adams
Department of Ecology
and Evolutionary Biology
University of Colorado
Boulder, Colorado
USA

Sandra Eremia
Centre of Bioanalysis
National Institute for Biological
Sciences
Bucharest
Romania

Tahira Fatima
Department of Biology
University of Western Ontario
London, Ontario
Canada

Simone Ferrari
Department of Plant Biology
University of Rome La Sapienza
Rome
Italy

Dirk Fischer
Institut für Pflanzenphysiologie
Martin-Luther Universität
Halle-Wittenberg
Halle, Saale
Germany

Demetrios F. Ghanotakis
Department of Chemistry
University of Crete
Voutes-Heraklion
Greece

Maria Teresa Giardi
Institute of Crystallography
Department of Agrofood
and Department of Molecular Design
National Council of Research
Area of Research of Rome
Monterotondo Scalo
Rome
Italy

Annalisa Giovannini
Research Unit for Floriculture
and Ornamental Species
CRA-Agricultural Research Council
Sanremo
Italy

Roberto Giunta
Ethical Committee
ASL-Local Health Agency
Milan
Italy

Avtar K. Handa
Department of Horticulture
and Landscape Architecture
Purdue University
West Lafayette, Indiana
USA

Steven D. Heys
Cancer Medicine Research Group
School of Medicine and Dentistry
Aberdeen University
Aberdeen, Scotland
UK

Anja Hohtola
Department of Biology
University of Oulu
Oulu
Finland

Georges Istamboulie
IMAGES Group
Centre de Phytopharmacie
University of Perpignan
Perpignan
France

Udo Johanningmeier
Institut für Pflanzenphysiologie
Martin-Luther Universität
Halle-Wittenberg
Halle, Saale
Germany

Justyna Krzyzanowska
Institute of Soil Science
and Plant Cultivation
Department of Biochemistry
State Research Institute
Pulawy
Poland

Maya Lambreva
Institute of Crystallography
Department of Agrofood
and Department of Molecular Design
National Council of Research
Area of Research of Rome
Monterotondo Scalo
Rome
Italy

Teresa Lavecchia
Biosensor srl
Palombara Sabina
Rome
Italy

Simona Carmen Litescu
Centre of Bioanalysis
National Institute for Biological
Sciences
Bucharest
Romania

Mariella Lucchesini
Department of Crop Plant Biology
University of Pisa
Pisa
Italy

Andrea Margonelli
Institute of Crystallography
Department of Agrofood
and Molecular Design
National Council of Research
Area of Research of Rome
Monterotondo Scalo
Rome
Italy

Jean-Louis Marty
BIOMEM Group
Centre of Phytopharmacy
University of Perpignan
Perpignan
France

Autar K. Mattoo
Sustainable Agricultural Systems
Laboratory
The Henry A. Wallace Beltsville
Agricultural Research Center
United States Department of Agriculture
Agricultural Research Service
Beltsville, Maryland
USA

Anna Mensuali-Sodi
Superior School of Advanced Studies
Sant' Anna
Pisa
Italy

Gigliola Montorfano
Institute of General Physiology
and Biochemistry
Department of Molecular Sciences
Applied to Biosystems
DISMAB
University of Milan
Milan
Italy

Manuela Negroni
Institute of General Physiology
and Biochemistry
Department of Molecular Sciences
Applied to Biosystems
DISMAB
University of Milan
Milan
Italy

Thierry Noguer
BIOMEM Group
Centre of Phytopharmacy
University of Perpignan
Perpignan
France

Wieslaw Oleszek
Institute of Soil Science
and Plant Cultivation
Department of Biochemistry
State Research Institute
Pulawy
Poland

Paolo Petroni
Enervit S.p.A.
Zelbio
Como
Italy

Riccardo Pina
Enervit S.p.A.
Zelbio
Como
Italy

Laura Pistelli
Department of Plant Crop Biology
University of Pisa
Pisa
Italy

Luisa Pistelli
DCBB-Department of Bioorganic
Chemistry and Biopharmacy
University of Pisa
Pisa
Italy

Beatriz Prieto-Simón
BIOMEM Group
Centre of Phytopharmacy
University of Perpignan
Perpignan
France

Gabriel Lucian Radu
Centre of Bioanalysis
National Institute for Biological
Sciences
Bucharest
Romania

Giuseppina Rea
Institute of Crystallography
Department of Agrofood
and Department of Molecular Design
National Council of Research
Area of Research of Rome
Monterotondo Scalo
Rome
Italy

Angela Maria Rizzo
Institute of General Physiology
and Biochemistry
Department of Molecular Sciences
Applied to Biosystems
DISMAB
University of Milan
Milan
Italy

Giuseppe Rodio
Enervit S.p.A.
Zelbio
Como
Italy

Dino Rotondo
Strathclyde Institute of Pharmacy
and Biomedical Sciences
Strathclyde University
Glasgow, Scotland
UK

Régis Rouillon
IMAGES Group
Centre de Phytopharmacie
University of Perpignan
Perpignan
France

Barbara Ruffoni
Research Unit for Floriculture
and Ornamental Species
CRA-Agricultural Research Council
Sanremo
Italy

Giovanni Scapagnini
Department of Health Sciences
University of Molise
Campobasso
Italy

Vijaya Shukla
Sustainable Agricultural Systems
Laboratory
The Henry A. Wallace Beltsville
Agricultural Research Center
United States Department of Agriculture
Agricultural Research Service
Beltsville, Maryland
USA

Arianna Tibuzzi
Biosensor srl
Palombara Sabina
Rome
Italy

Eleftherios Touloupakis
Department of Chemistry
University of Crete
Voutes-Heraklion
Greece

Francesca Vergari
Institute of Crystallography
Department of Agrofood
and Department of Molecular Design
National Council of Research
Area of Research of Rome
Monterotondo Scalo
Rome
Italy

Klaus W.J. Wahle
Cancer Medicine Research Group
School of Medicine and Dentistry
Aberdeen University
Aberdeen, Scotland
UK

Surender K. Yachha
Department of Pediatric
Gastroenterology
Sanjay Gandhi Postgraduate Institute
of Medical Sciences
Lucknow
India

Stefania Zava
Institute of General Physiology
and Biochemistry
Department of Molecular Sciences
Applied to Biosystems
DISMAB
University of Milan
Milan
Italy

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Chapter 1

The NUTRA-SNACKS Project:
Basic Research and Biotechnological Programs on Nutraceutics

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

Abstract

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.

Introduction: Rationale of the Nutra-Snacks Project

In recent years, food quality and safety have become a fundamental issue in public opinion and the media. Compelling evidence indicates inappropriate dietary habits as a leading cause of poor health that results in a worldwide increase in health care costs. These concerns prompted policymakers to declare a thematic priority to Food Quality and Safety Programmes, in an attempt to improve the health and well-being of European citizens through higher quality food and improved control of food production and related environmental factors. The supported program gave priority to identifying

*Corresponding Author: Maria Teresa Giardi—Institute of Crystallography, Department of Agrofood, CNR, 00016 Monterotondo Scalo, Rome, Italy. Email: giardi@mlib.cnr.it

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the major concerns for consumers along the food production chain, outlining issues associated with primary production, animal feeds, processing, distribution, consumption and environmental health risks (http://ec.europa.eu/research/research-eu).

The Nutra-Snack project found application in this social research context. The project aims at the realization of ready-to-eat food for breakfast and sport with a high content of compounds endowed with functional activity useful for promoting public health. There is already a wide range of foods available to today's consumer but now the impetus is to identify functional foods that have the potential to improve health and well-being, reduce the risk from—or delay the onset of major diseases such as cardiovascular disease, cancer, osteoporosis and so on. Combined with a healthy lifestyle, functional food can make a positive contribution to health and well being.

The Influence of Various Life Style and Environmental Factors on Human Health

Human health is determined by the interaction of genetic, nutritional, socio-cultural, economic, physical infrastructure and ecosystem factors that, in turn, are all influenced by the environment and by its changes. Currently, several disciplines, including geography, gender studies, health and social sciences, are bringing to light the complex pathways by which factors in the environment influence patterns of health. Environmental changes arising from urbanization, population increases, industrial and agricultural activities have resulted in thermal fluctuations, reduced quality of air, water and soil, a greater exposure to radiation and persistent chemical pollutants that can trigger disease processes. In particular, environmental degradation and chemical overload coming from agriculture practices have been linked to diseases such as respiratory and cardiovascular disease, neurological and physiological disorders, and increased incidence of many cancers.[1] In addition to the built environment, several life style choices such as smoking habits, decrease of physical activity and consumption of alcohol and drugs can have profound impacts on our health and have been associated with the abovementioned pathologies.

Among lifestyles, dietary factors also affect health and well-being. The growing epidemic of obesity is one of the major public health issues in the world, and is often a consequence of high calorie intake compared to energy expenditure. Poor eating-habits leading to inadequate intake of calories can negatively affect human health and have been unequivocally and causally associated with the risk of obesity, cardiovascular disease, type 2 diabetes, stroke, cancers and neurodegenerative disorders.[2]

Oxidative Stress Is an Important Health Risk Factor

The term oxidative stress describes a harmful process resulting from an imbalance between the excessive production of reactive oxygen (ROS) and/or nitrogen species (RNS) and limited action of antioxidant defenses. Oxidative stress is implicated in the development of many important pathologies including atherosclerosis, hypertension, ischemia-reperfusion injury, inflammation, cystic fibrosis, diabetes, cancer, Parkinson's and Alzheimer's diseases. [3-9]

Due to its bi-radical nature, molecular oxygen can accept unpaired electrons giving rise to a series of dangerous partially reduced species that include superoxide ($O^{\cdot}_2$), hydrogen peroxide, hydroxyl radical and peroxyl and alkoxyl radicals. In addition, $O^{\cdot}_2$ can react with other radicals including nitric oxide yielding products, the dangerous peroxynitrites, which have a very potent oxidant activity. These compounds may be involved in the initiation and propagation of free radical-mediated chain reactions that can involve proteins, lipids, polysaccarides and DNA, leading to biological damage. [10]

ROS and RNS can be derived either from such essential metabolic processes in the human body as aerobic respiration, peroxisomal β-oxidation of fatty acids, microsomal cytochrome P450 metabolism of xenobiotic compounds, stimulation of phagocytosis by pathogens or lipopolysaccharides, arginine metabolism and activity of tissue specific enzymes, or from external sources such as exposure to ozone, cigarette smoking, air pollutants and chemicals. In physiological conditions, ROS are efficiently scavenged from the cell mainly by the activity of detoxifying enzymes like superoxide dismutase, catalase, or glutathione peroxidase or electron carriers like ubiquinol.[11]

In addition to deleterious effects, ROS and RNS also perform several useful functions.[12] Moreover, sub-toxic levels of ROS and RNS serve as important intracellular signalling molecules that can influence cellular survival.[13] In fact, it has been shown that numerous cellular processes in several cell types can be regulated by slight changes in redox balance. Examples of these processes include the activation of certain nuclear transcription factors, determination of cellular fate by apoptosis or necrosis, modulation of protein and lipid kinases and phosphatases, membrane receptors and ion channels.[14,15]

As oxidative stress is believed to play a major role in the ageing process and in several diseases there is considerable public interest in the anti-oxidative effects of dietary factors.[2,16] Indeed, with proper nourishment, the body can, on its own, make sufficient quantities of antioxidant enzymes and substrates for those enzymes. These can facilitate the quenching of excess free radicals by antioxidants. An enhancement in dietary intake of antioxidants and phytochemicals with related functions can counteract oxidizing species and potentially restore a healthy cellular redox balance.[17] Apart from traditional components with potential health benefits, new food is being developed to enhance or incorporate these beneficial nutraceutics for their health benefits or desirable physiological effects.

Why Novel Nutra-Snacks?

In this context, the research developed in the Nutra-Snack project is focused on the creation of new foods capable of acting in concert with human defense responses to provide protection against oxidative stress. The idea was the development of a new generation of healthy dietary formulations containing natural but bolstered ingredients able to meet and satisfy the consumer's taste and eating-habits.

Faced with a highly diverse food supply, consumers can take advantage of a wide variety of dietary intake of micro- and macro-nutrients. Nowadays, the consumption of midday and/or midnight snack food is becoming one of the most popular eating-habit trends, despite its close correlation with several metabolic disorders. Eating small amounts of food on a regular basis is considered healthy, but often available snacks in the market provide excess calories and fats and little or no nutrient value to the diet. Moreover, most snacks contain preservatives, sweeteners and flavoring that can have negative effects on health.

Healthy diets include factors such as making proper food, keeping track of meal times and regulating the amount of food intake. There are many different healthy eating diet plans available today. Among these, the Zone diet is one of the most popular focused on food management rather than weight loss. The Zone diet suggests the 40:30:30 ratio for carbohydrates, proteins and fats that a person should consume. With this ratio of components, the body is able to balance insulin and glucagon, as well as provide a more effective internal metabolism. One of the key partners in the Nutra-Snack project is Enervit®, a well-known firm in the development of nutrition programs to enhance the performances of athletes and sportsmen. As the manufacturer of several products for breakfast, everyday meals, snacks and food supplements, the company is a leading supporter of the Zone diet. Currently, experts from the Enervit® team are involved in the development of modern alimentary strategies for those who believe in health through correct nutrition and food supplementation (http://www.enervitwellness.it).

Plant Secondary Metabolites as High-Value Compounds for Human Nutrition

In essence, healthy eating refers to the idea of maintaining a well-balanced food intake comprised of food types from different food sources. Much epidemiological and experimental evidence supports the hypothesis that vegetables and fruits in the diet can reduce risk of diseases; this has led to the use of a number of phytometabolites as preventive and/or protective agents, promoting a dramatic increase in their consumption as dietary supplements. The therapeutic potential of plant-based formulations has been ascribed to various plant secondary metabolites whose biological activities are beneficial at the cellular and molecular levels. In addition, plant-based extracts are the most favorable choice, although a large dosage of a single compound is not considered advantageous to deliver it to many tissues.[18,19]

Plants are basic ingredients of our diet whose nutritional and medicinal value have been recognized since ancient times. In addition to essential primary metabolites, like carbohydrates, lipids and amino acids, plants are capable of synthesizing an amazing variety of low-molecular-weight organic compounds, called secondary metabolites, usually with unique and complex structures. Compared to the main and most abundant molecules found in plants, these secondary metabolites are defined by their low abundance, often less than 1-5% of the dry weight. Secondary metabolites have no recognized role in the maintenance of fundamental life processes; nevertheless, these molecules contribute largely to plant wellbeing by interacting with the ecosystems. Their production, in fact, is often enhanced or induced in response to biotic and abiotic stress conditions, after that, they can be stored in specific cells and/or organs of the plant.

Some of these secondary metabolites are endowed with functional activity useful for promoting public health as they provide health benefits beyond basic nutrition.[18] Some of the aforementioned compounds and their functional roles are briefly described below and will be treated in detail later in this book.

Carotenoids

Photosynthetic carotenoids are red, orange, and yellow lipid-soluble pigments found embedded in the membranes of chloroplasts and chromoplasts. They are polyisoprenoid compounds containing 40 carbon atoms formed by the condensation of eight isoprene units. They possess a long chain of conjugated double bonds whose linkage order is reversed in the central part of the molecule giving rise to a symmetrical molecule. This set of conjugated double bonds is responsible for the absorption of light in the visible region of the spectrum. Left and right end groups are variable and different levels of hydrogenation and introduction of oxygen-containing functional groups create a large family of over 600 natural compounds.[20]

In photosynthetic organisms, carotenoids play a vital role in the photosynthetic reaction center. They either participate in the energy-transfer process, or protect the reaction center from auto-oxidation. In non-photosynthesizing organisms, carotenoids have been linked to oxidation-preventing mechanisms. Animals are incapable of synthesizing carotenoids and must obtain them through their diet. Carotenoids absorbed through the diet, and often metabolized into other compounds, are responsible for the color of familiar animals such as lobster, flamingo, and red fish.

However, carotenoids may have many physiological functions. Apart from the carotenoid, β-carotene, the primary vitamin A source, carotenoids are of physiological interest in human nutrition, since they exhibit radical or singlet oxygen trapping activity,[21] and as such have potential antioxidant effects in vivo.

Given their structure, carotenoids are efficient free-radical scavengers, capable of enhancing the vertebrate immune system. Indeed, epidemiological studies have shown that people with high β-carotene intake and high plasma levels of β-carotene have a significantly reduced risk of lung cancer. However, studies of supplementation with large doses of β-carotene in smokers have shown the opposite effect—an increase in cancer risk—possibly because excessive beta-carotene results in breakdown products that reduce plasma vitamin A and induce the lung cell proliferation caused by smoke.[19]

Moreover, there is evidence supporting a protective role for lutein and zeaxanthin in delaying chronic diseases, including age-related vision loss via macular degeneration and cataract formation, cancer and heart disease. Lutein and zeaxanthin are the only carotenoids present in both the macula and lens of the human eye and are also referred to as macular pigment. Functions of these pigments include improving visual function, quenching free radicals and thereby acting as an antioxidant to protect the macula from oxidative damage. The involvement of lutein and zeaxanthin in protection against photo-induced damage is as blue light filters, antioxidants and shielding potentially harmful short-wave radiation. The retina is susceptible to oxidative stress because of its high demand for oxygen, the high proportion of polyunsaturated fatty acids, and aerobic metabolism. Age-related macular degeneration is the leading cause of blindness in the elderly in several countries. Many researchers have found significant associations between lutein and zeaxanthin concentrations in ocular tissues, serum, and plasma, with a possible reduced risk of macular degeneration.[22]