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FRONTIERS IN BIOACTIVE COMPOUNDS

Volume 2

AT THE CROSSROADS BETWEEN NUTRITION AND PHARMACOLOGY

Editors:
M. Victorina Aguilar
Cristina Otero

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Frontiers in Bioactive Compounds

(Volume 2)

(At the Crossroads Between Nutrition and Pharmacology)

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At the Crossroads between Nutrition and Pharmacology

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CONTENTS

FOREWORD

Nutrition is the science that deals with the role of nutrients and other substances in food in relation to growth, development, metabolism and function, often in the context of health and disease. Pharmacology is the science of the study of drug action; a drug is a molecule which has a biochemical or physiological effect within an organism. Typically, in the context of pharmacology, the molecule (i.e., the drug) is used to treat, cure, prevent, or diagnose a disease or to promote well-being and is referred to as a medicine. It is important to note that many drugs are natural substances or derivatives of natural substances. It is immediately evident that there is likely to be some overlap between nutrition and pharmacology, since both are concerned with molecules that exert biochemical and physiological effects within the organism. However, in modern times, neither the teaching nor the scientific practice of nutrition and pharmacology have been considered to have much in common, and they exist quite separately from one another. This is different from earlier times, where the boundaries between these two academic disciplines were not clear and indeed may not have even existed. For example, many foods, food extracts and food-based potions have been used in traditional medicine to prevent and treat diseases and to promote well-being; this practice continues today in many, perhaps most, non-Western cultures. In other words, food can be medicine (i.e., a drug) and medicine can be food. Fortunately, the artificial barrier between nutrition and pharmacology is once more being removed. The pharmaceutical industry is becoming increasingly interested in food components as functional agents that have potential as drugs, while the food industry and nutrition scientists are expected to mainly adopt the practices of pharmacology and the pharmaceutical industry as part of their normal research and development activities.

This blurring of the boundaries is likely to become greater over the next years, and will certainly increase the chances of new discoveries being made by both the food and pharmaceutical industries and of translating those discoveries into new products, new claims, new preventative strategies and new treatments for human disease. In the contexts of these changing research and regulatory environments “At the Crossroads Between Nutrition and Pharmacology” is a timely offering. It brings together a series of articles dealing with bioavailability and bioactivity of a range of natural substances found in foods, suggesting that these nutritional substances have properties that will make them useful in health maintenance, disease prevention and, in some cases, disease treatment. The disease contexts being considered include those that pose an ever increasing threat to the global population, including diabetes, metabolic syndrome and cardiovascular disease. Thus, the contents of this book are extremely relevant, making it the most welcome addition.

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PREFACE I

This is the first volume of this eBook series entitled “Bioactive compounds: at the frontier between Nutrition and Pharmacology”. Functional Foods are emerging in the modern food industry. But their potential for preventive and therapeutic treatments must be safely determined. This way the field of Nutrition will be transformed into a knowledge-based science for the development of Functional Food products. This eBook presents the state-o--the art and most recent advances in the computational design of Functional Food products, their sources, detection, analysis, extraction or synthesis and their different biological effects. The book presents the most important and recent advances in chemistry, technology and health research of products with potential use as drugs, nutraceuticals, functional food ingredients, or cosmetics. This volume will be a great value to students, clinicians, nutritionists R&D scientists and food companies.

In Chapter 1, Younesi discusses the need of following the path of drug discovery and development to obtain new functional foods by the modern nutraceutical industry. He describes the potential of recent advances made by pharmaceutical stakeholders to evaluate the effects of bioactive compounds on human health. Targets identification for drugs and nutraceuticals are revised. Evidence-based modeling of the mode of action of functional ingredients influencing Alzheimer’s disease is presented as an example. The author describes how the fundamentals of systems biology and *in silico* target identification can be applied to the field of nutrition in support of the development of new functional food products.

The most recent developments for the extraction, identification and quantification of bioactive peptides in foods are described in Chapter 2 by Puchalska *et al.* More than 2600 bioactive peptides have been discovered. They are specific protein fragments with favorable effects on human health, and their different bioactivities are described. The general workflow for their identification is presented and an overview of the most modern strategies for their recovery from food protein hydrolysates is given. This chapter covers from the standard analytical and electrophoretic methods to new alternatives for the identification of bioactive peptides in complex food matrices. These methodologies are essential for safety evaluation, establishment of health claims, policy and regulations.

The concepts of bioavailability, bioaccessibility, bioactivity, bioefficiency and bioconversion of bioactive foods are clarified in Chapter 3. *In vivo* and *in vitro* methods for evaluating the bioactivity and bioavailability of foods are reviewed. Methods employed to provide scientific evidence on the effects of food structure, food composition, dietetic factors and food processing on bioactive foods, are described.

Sugar fatty acid esters are another class of promising bioactive compounds with bioactivities such as antimicrobial, antitumor and anti-insect activities. These biodegradable emulsifiers are used in pharmaceutical, cosmetic and food industries. Ye and Hayes describe the most important synthetic routes for obtaining these biocompatible non-ionic and biodegradable sugar esters in chapter 4. This Chapter is also an overview of the bioactive properties of these

sugar esters, including the comparison of the bioactive characteristics of sugar esters synthetized *via* chemical and enzymatic reactions.

Li *et al.* analyzes the bioactivities of arabinoxylans in relation to their molecular structure in Chapter 5. Arabinoxylans -present in cereals cell walls- have several health benefits as mediators of physiological and immunological processes. Various *in vitro* immunological tests are discussed. This chapter also relates the molecular features of arabinoxylans to the different extraction technologies used to obtain and study them.

Kumar in Chapter 6, describes the potential of indigenous medicinal foods, particularly the species of genus Dioscorea available in India as future functional foods. Some rural and tribal communities of wild Odisha base their subsistence on these foods, where they also play a critical role in their conventional medicine. Kumar studied the ethnobotanical values and bioactive compounds present in these tubers from the literature. Their potential as functional foods and for the formulation of new drugs is highlighted.

In Chapter 7, Mantello *et al.* describes the important role of Nutrigenomics to identify key cellular functions by specific genetic and epigenetic interactions with a nutrient or a food component. Novel features of new nutrigenomic driven action plan strategy to develop specific pharmacological treatments for the reduction or prevention of diseases are described. In this chapter, the case of fermented papaya is presented as an example of functional food, with the most recent rational and evidence-based biotechnological progress.

Chapter 8 reviews the most recent investigations on the anti-cancer properties of saponins as important bioactive components of medicinal plants, used in traditional medicine. The Examples of different plants, molecular and cellular mechanisms of their anti-tumor activities, and their prospective use to elaborate personalized nutrition are described.

Chapter 9 covers the most important advances on the study of the effect of a diet based on different bioactive foods on the prevention and treatment of Diabetes. The bioactive compounds and the Mediterranean Diet (rich in this compounds) affecting glucose metabolism are described by Menacho-Román *et al.*

In Chapter 10, Becerra-Fernández *et al.* describe the evidences of the antioxidants effects on cardiovascular diseases. They review cohort studies and randomized controlled trials that related the frequent consume of fruits and vegetables, and the intake of antioxidants supplements with the lower incidence of cardiovascular diseases.

In Chapter 11, Pen *et al.* reviews the most updated and relevant evidences of the beneficial effects of bioactive foods on metabolic syndrome, which is known as the coexisting metabolic disorder that increases an individual's likelihood of developing type 2 diabetes, cardiovascular disease and stroke, as well as other chronic diseases.

In Chapter 12, Brites gives a comprehensive review on Tauroursodeoxycholic acid and urso- and glycoursodeoxycholic acids with detergent properties for the treatment of hepatobiliary diseases, along with their mechanisms of action, their potential application in the prevention

and recovery of diseases associated to the central nervous system dysfunction and pathology, and neurodegenerative diseases.

In Chapter 13, Yartseva and Ivanenkov review the most recent studies showing the beneficial effects of some foods such as, natural phytochemicals in prostate cancer. This chapter describes the state of the art about the scientific studies focused on dietary polyphenols and analogs that have anticarcinogenic properties. Controversial results are discussed together with major issues of developing naturally occurring compounds into clinically used agents.

In Chapter 14, the readers will find a rigorous description beneficial and deleterious effects of methylxanthines (caffeine and others) present in many foods and beverages. Factors that play a role in methylxanthine effects and metabolism are analyzed. This chapter summarizes the physiological and toxicological effects of these bioactive food constituents.

Finally, in Chapter 15, the effects of different culinary methods used at present on bioactive food properties are described by García Viguera and Soler-Rivas. Positive and negative effects of traditional and the most modern technologies for food cooking or processing are presented and discussed.

The editors are grateful to the authors for their excellent contributions and to Bentham Science Publishers for making the publication of this eBook possible.

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PREFACE II

The Prevalence of chronic-degenerative diseases is increasing among the world's population. Claiming 63% of all deaths worldwide, it is currently the world's main killer. According to the World Economic Forum (2011), the total costs of these diseases were expected to rise upto $ 47 trillion by the year 2030.

Interventions to reduce disease risks (for example, diet) would constitute the most economic, affordable and sustainable key elements for effective primary prevention.

Since Hippocrates reported the aphorism "Let food be the medicine and medicine be the food", there is strong evidence that reported links between diet and health. Healthy food actions are not only due to nutrients, but also to other constituents (bioactive compounds) with functional properties. These bioactive compounds are extranutritional constituents with beneficial effects and are typically present in small quantities in foods. They may promote optimal health. Actually, there are evidences of their effects on cancer, cardiometabolic syndrome, immunological system, nervous system, learning processes, sports performance. These evidences constitute new parts of the complex puzzle that is the Nutrition, in addition to demonstrating the permeability of the borderline between Nutrition and Pharmacology since these compounds can be used as drugs, nutraceuticals, functional food ingredients, or cosmetics. The relationship between bioactive food compounds and drugs is becoming closer. Moreover, bioactive products are considered as drug targets or physiological pumps and the technology traditionally used for drugs is being used to pioneer functional health ingredients from the bioactive compounds.

There are varied papers about bioactive compounds in which the most appropriate technological treatments (synthesis, concentration or purification from different natural sources, including food derivatives) are studied. Innovative ingredients and their healthy properties both in humans or animals are documented. Therefore, there are many areas of interest. Because of all of this, in this book, the first in a series, the latest knowledge on the different chemical or technological facets of bioactive compounds and their nutritional and pharmacological applications in prevention and treatment of different nosological entities are collected. The first volume of this eBook series is a compilation of several well written reviews on the state-of-the art developments in computational design of compounds with functional activity, sources, identification, analysis, technological treatments effect, or biological action. "Bioactive compounds: at the frontier between Nutrition and Pharmacology" focuses on this important area of chemical, technological and health research. This book will also be a valuable resource of information for professionals in this field that allows them to see the news of the topic and its potential for preventive and therapeutic application with safety, quality and efficacy.

This book has been possible by numerous co-authors for their collaboration to the task of synthesizing their knowledge on the subject in relatively concise chapters.

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CHAPTER 1

From Pharma to Food: Mechanistic Target Identification for Bioactive Compounds Using Nutritional Systems Biology

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Abstract: Advancements in molecular biology and the advent of high-throughput technologies for characterization of biological processes across multiple biological scales from cells and tissues to the whole organism have provided exciting opportunities to investigate and evaluate the influence of foods and their bioactive ingredients on the human health and disease. These advances, mainly developed by pharmaceutical stakeholders, are going to transform the way nutrition is addressed in relation to human health by the nutraceutical industry. Consumers now demand healthier food products and regulatory agencies have raised the bars for approval of health claims associated with functional foods. To tackle these issues, a knowledge-based, integrated systems biology strategy is required for successful production of functional foods, which can be adopted from experiences of pharmaceutical industry in this area. This strategy benefits from integrative mechanistic modeling of health and disease processes, which can support the process of health claim substantiation for bioactive food ingredients in new product development. Currently, there are thousands of natural compounds or functional food ingredients whose mode-of-action and their final impact on the human health or disease are unknown. Thus, identification of biological targets for thousands of bioactive compounds in food and their mode-of-action in the human body is of high priority. This chapter discusses how fundamentals of systems biology and *in silico* target identification, as is done in the pharmaceutical industry, can be applied to the field of nutrition in support of developing novel functional foods. The author foresees that identification of mechanistic targets for bioactive compounds extracted from foods and natural resources, and explanations for their pharmacological mode-of-action will play a crucial role in the future of healthcare and preventive medicine.

Keywords: Functional food, Integrated systems-based strategy, Systems biology, Target identification.

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M. Victorina Aguilar and Cristina Otero (Eds.)

INTRODUCTION

Avicenna and Rhazes, medieval Persian practitioners, were first to suggest the concept of "food as medicine" based on the use of natural products. This concept was transformed with the rise of drug development technologies so that the use of synthetic compounds in the form of supplements shadowed the concept of "food as medicine". Now after more than two centuries, for two reasons, the concept of functional food and its role in health promotion has again gained momentum: firstly, the current paradigm with synthesized drugs in the pharma industry, *i.e.* "one size fits all", has been seriously challenged by toxicity and unwanted effects of chemical compounds in the complex system of the human body (*i.e.* safety issue). Secondly, expensive and growing attrition rates in drug development pipelines plus the non-selective nature of chemical compounds (*i.e.* efficacy issues) together with the lack of theragnostic strategies for early diagnosis of and intervention in progressive chronic diseases like dementia or cancer has encouraged both pharmaceutical and food companies to bring the disease prevention and treatment using natural compounds into their business focus [1]. The term "reverse pharmacology" has been used to describe identification and evaluation of traditional recipes (*e.g.* herbal extracts) that have been used by ancient eastern societies for treatment of health problems for centuries. This process takes advantage of up-to-date, modern pharmacological techniques to convert those traditional recipes into health promoting products by following a target identification and clinical trial approach [2].

This emerging paradigm has generated both opportunities and challenges for the food industry. For instance, many countries have politically realized that prevention of chronic diseases through healthy life style including nutrition is a priority and this is a good opportunity for functional food businesses to step in. However, this is not a trivial task: the health and/or disease phenotype in humans results from complex interactions between biological molecules, environmental factors and disease-modifying entities. Hence, understanding how functional food ingredients are going to find their place in this complex picture is key to successful development of functional foods by the food industry. The food industry can benefit from expensive experiences that the pharma industry has accumulated during many years of heavy investment and scientific research in the area of "drug-like functional food" production. In contrast, addressing the complex biology of human health, which has been the focus of the pharmaceutical industry for long time, is considered as a new challenge posed to the food industry if the safety (side effects) and efficacy (mode-of-action) of the functional food products are to be shown for health claims. Regulatory authorities are gradually

increasing the pressure on food businesses to cope with stringent health claim regulations. For example, from the perspective of the Food and Drug Administration (FDA), functional foods including food and beverage products with health claims do not belong to the food category but considered as drug and must meet safety and efficacy requirements of the FDA's regulatory guidelines. Recently, several guidelines on scientific assessment of health claims have been published by the European Food Safety Authority (EFSA) has released guidelines on scientific assessment of health claims, which enforces food manufacturers to prove the quality, relevance and adequacy of scientific evidence supporting their health claims [3]. Perhaps, the most efficient solution for the food industry to keep up with the increasing amount of market demand and regulatory pressure is to attempt at bridging the technology gap with the pharmaceutical industry.

Technology Gap and the Role of Systems Biology

As mentioned above, the pharmaceutical industry has already established a sophisticated, strong technological infrastructure and scientific expertise in the area of drug discovery and development to deal with the complexities involved in human health and disease. However, it appears that the food industry lags behind such advancements when it comes to proving safety and efficacy of bioactive compounds in food products with health claims. It should be noted here that although drugs are different than nutrients, there are striking similarities between food and pharma pipelines (Fig. **1**).

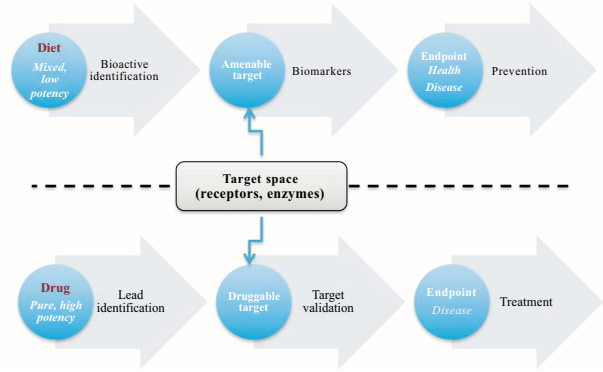

Fig. (1). Similarities between drug development and nutraceutical development pipelines.

Perhaps the main reason behind such discrepancy are structural differences so that the pharmaceutical industry has gone through a gradual transformation from a manufacturing-based traditional business to a knowledge-based, modern industry whereas the food industry has still preserved its manufacture-based traditional

CHAPTER 2

Advances in the Determination of Bioactive Peptides in Foods

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Abstract: Several studies have shown that food is an important source of many bioactive compounds that may have a beneficial health impact on human body. Among them, bioactive peptides have extensively been studied and have proved to possess a variety of activities such as antihypertensive, antioxidant, anti-inflammatory, antimicrobial, and many others. These peptides can be natural constituents of foods but they can also be released from a parent protein present in a food during gastrointestinal digestion or by an enzyme or microorganism during food processing. The analysis of bioactive peptides in food samples is a very challenging task due to their wide concentration dynamic range and the complexity of these samples. The general workflow usually employed in the investigation of the presence of bioactive peptides in foods is presented in this chapter. Some important characteristics of bioactive peptides that need to be taken into account during their analysis are highlighted. Additionally, an overview of novel strategies to obtain bioactive peptides from food protein hydrolysates is presented. A separate section is devoted to describe chromatographic and electrophoretic approaches successfully used for the analysis of food bioactive peptides. Mass spectrometric techniques dominate in the identification of bioactive peptides in complex food matrices. Some alternatives to tentatively assign a signal to a peptide sequence are displayed. In addition, a recent 'hot topic' such as the quantitation of bioactive peptides in complex matrices is included. Information given in this chapter is based on the most recent literature.

Keywords: Analytical methods, Bioactive peptides, Chromatography, Functional foods, Mass spectrometry, Protein hydrolysis, Quantitation.

INTRODUCTION

Hypertension, diabetes, and other chronic diseases are serious human health problems worldwide. To address these issues, nowadays research interest is

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M. Victorina Aguilar and Cristina Otero (Eds.)

focused on dietary strategies, since food is the fundamental environmental factor that influences human health. Functional foods are of special interest since beyond their nutritional purposes, they also demonstrate many physiological benefits like reduction of the prevalence of chronic diseases risk [1]. Bioactive compounds incorporated within functional foods are the components that can affect biological processes and/or substances at a molecular level and that can exert influence on body functions and conditions. Induced effects should be measurable at physiological level and must be beneficial for health [2]. Thus, discovery and characterization of novel food derived bioactive compounds have become an important approach in targeting important diseases. Among bioactive compounds, peptides are one of the most widely studied and appreciated.

Bioactive peptides are specific fragments of protein(s) that have positive influence on human organism functions and conditions, hence may influence health [3, 4]. It is important to highlight that some dietary peptides are particularly potent and even microgram amounts entering to the body circulation can have major physiological impact [5]. Increasing attractiveness of bioactive peptides in comparison with synthetic drugs is complemented by their lower production cost and, above all, the lack of the side effects. Although bioactive peptides from animal origin are the most widely studied, vegetable origin bioactive peptides are recently gaining a lot of interest [6]. Bioactive peptides have been discovered in various animal sources such as milk and dairy products (cheeses, yoghurt, kefir), marine food (oyster, giant squid, shrimps, blue mussels), eggs (yolk, whole egg), fishes (Alaska Pollock, bonito, pacific hake, tuna, salmon), meat (pork, chicken, porcine) and many others. Among plant origin bioactive food, one of the most prevalent is soybean and its derived products (douche, miso paste, soybean sauce *etc.*), and other plants like wheat, maize, rice, chickpea, sunflower, amaranth and seaweeds as wakame [6].

According to the bioactive peptides database-BIOPEP, until now more than 2600 bioactive peptides were reported with more than 37 different bioactivities [7]. Bioactive peptides have shown antihypertensive, antimicrobial, antioxidant, opioid, immunomodulatory, antithrombic, metal-chelator, cytomodulator, hypocholesterolemic and many other bioactivities [6]. Importantly, some of these bioactive peptides are already incorporated into commercially available functional foods and food ingredients. Brand names, bioactivities, and incorporated bioactive peptides within commercial functional foods can be found elsewhere [3]. Moreover, some bioactive peptides can show more than one bioactivity at once. For example, it is very common among peptides that bind metal elements through certain amino acids (histidine, methionine, or cysteine) to possess at the same

time antioxidant activity [8]. In addition, as it is schematically presented on Fig. (**1**), peptides that claim a particular bioactivity may affect different major organism systems at the same time [3].

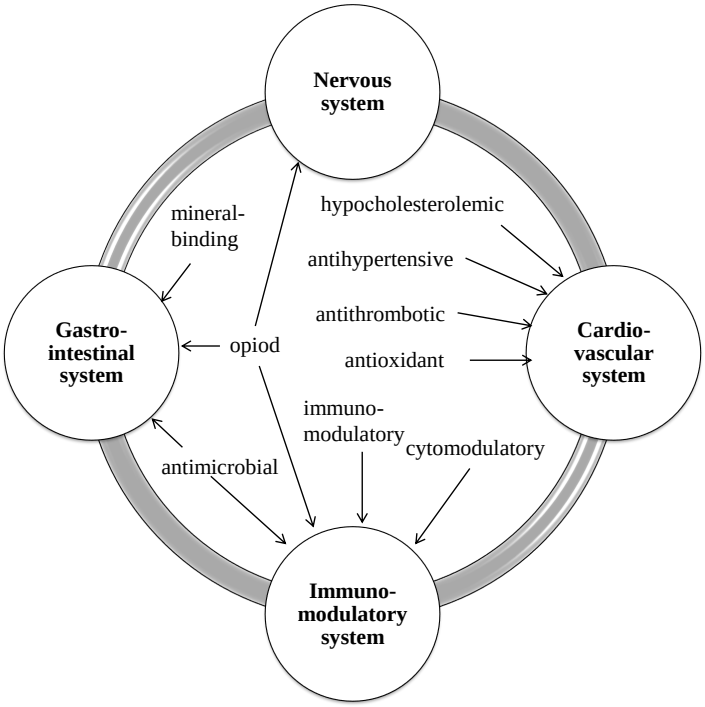

Fig. (1). Influence of bioactive peptides on different human systems. Adapted from [3].

Recently, the scientific interest within the area of bioactive peptide discovery is concentrated on the peptides showing multiple bioactivities or peptides that exert not extensively explored bioactivities. Among the newly studied peptides bioactivities are renin inhibitors [9], dipeptidyl peptidase (DPP) IV inhibitors [10], or platelet activating factor acetylhydrolase inhibitors (PAF-AH) [11]. Renin inhibitors block the first link in the renin-angiotensin system enzymatic reaction chain that plays a pivotal role in the regulation of blood pressure. As authors suggested, inhibition of renin over more popular target ACE (angiotensin I converting enzyme) can have some advantages [9]. On the other hand, DPP IV inhibitors can control the activity of the enzyme associated with the degradation of two insulinotropic incretin hormones and, therefore, can be an effective novel strategy for the prevention and control of type II diabetes [10]. Finally, PAF-AH is considered a promising therapeutic target for the prevention of atherosclerosis, an inflammatory disease and the most common cause of stroke and cardiovascular disease [11].

CHAPTER 3

Overview of *In vivo* and *In vitro* Methods for Assessing Bioavailability of Bioactive Food Compounds

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Abstract: The knowledge of bioavailability, *i.e.* the fraction of bioactive compound that can be absorbed and utilized, is a very relevant topic of food and nutraceutical research. The present review sheds light to the concepts of bioavailability, bioaccessibility, bioactivity, bioefficiency and bioconversion. Besides, it reviews the main *in vivo* methods such as balance techniques (chemical balances and isotopes methods), plasma biomarkers and animal models. Several *in vitro* methods that simulate physiological conditions of the human gastrointestinal digestion (static or dynamic models) and the usefulness of $Caco_2$ culture cells for evaluating bioactive compounds bioavailability in foods are also reviewed. The advantages and disadvantages of these methods are discussed, beside specific conditions applied for several bioactive compounds (minerals, carotenoids, α-tocopherols, folic acid, plant sterols and polyphenols) from recent studies. Human studies are the method of choice, and data obtained from these assays represent the gold reference because they provide the most accurate results and the highest scientific evidence regarding the bioavailability of a bioactive compound. *In vitro* models are reproducible, rapid and simple, due to the fact they allow tight control of the experimental variables than animal or human studies. These methods are particularly useful to check the impact of digestion conditions and to undertake studies on the positive or negative effects of food structure, food composition, dietetic factors and food processing. Furthermore, it is mandatory to promote more research into *in vivo-in vitro* correlations using well harmonized and standardized systems, so that more valid *in vitro* models can be designed to evaluate the bioavailability of bioactive food compounds.

Keywords: Animal models, Balance studies, Bioaccessibility, Bioactive compounds, Bioavailability, $Caco_2$ cells, Simulated gastrointestinal digestion.

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M. Victorina Aguilar and Cristina Otero (Eds.)

CONCEPTS OF BIOAVAILABILITY, BIOACCESSIBILITY, BIO-ACTIVITY, BIOEFFICACY AND BIOCONVERSION

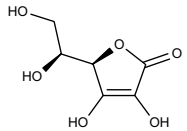
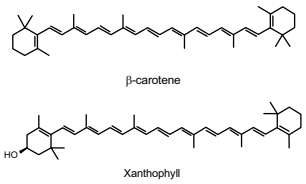
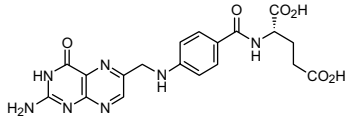
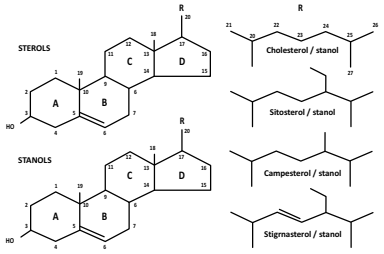
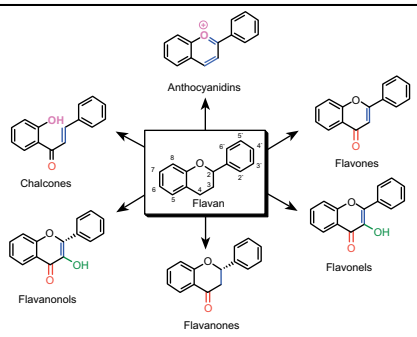
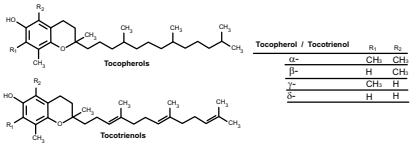
Considering a physiological standpoint, after oral intake food is digested prior to reach the proximal intestine. Here the bioactive compounds (BCs) present in foods can be converted into other compounds with distinct bioavailability (BAv), or they might not be released from foods - consequently affecting their native biological activity. Moreover, previously to perform their biological activities, the absorption and metabolism of BCs must be considered, since only those released from the food matrix by the action of digestive enzymes and colonic bacterial fermentation can become available [1]. The main bioactive compounds from foods assessed for bioavailability are shown in Table **1**.

Bioavailability is a pivotal term for nutritional effectiveness, independently of the type of food considered (functional or otherwise). Only few amounts of all BCs are available for exerting their action within the body in the target tissues.

The term BAv has different meanings depending on the scientific context. Nutritionally speaking, it is defined as the fraction of the ingested component available for utilization in normal physiological functions, and is determined by *in vivo* assays [2]. Bioavailability comprises three main steps: (a) digestibility and solubility of the element in the gastrointestinal tract; (b) absorption of the BCs by the intestinal cells and transport into the circulation; and (c) incorporation from the circulation to the functional entity [3, 4]. Besides, BAv has two additional terms: bioaccessibility (BAcs) and bioactivity (BAct) [5].

Bioaccessibility has received two alternative definitions. The first one is the fraction of a compound that is released from its food matrix in the gastrointestinal tract and thus becomes available for intestinal absorption (typically based on *in vitro* procedures). This definition thus does not encompass absorption across the intestinal wall or any metabolic processing [6], and refers only to digestion and release from the food matrix. The second definition is more stringent and much less widely used. It describes BAcs as the fraction of a compound that is released from its food matrix in the gastrointestinal tract and thus becomes available for intestinal absorption (*i.e.*, enters the bloodstream) - comprising the entire sequence of events that take place during the digestive transformation of food into material that can be assimilated by the body, absorption/assimilation into the cells of the intestinal epithelium and, lastly, presystemic metabolism (both intestinal and hepatic) [5].

Table 1. Chemical structure of main bioactive compounds from foods assessed for bioavailability.

Bioactive compound	Chemical structure
Ascorbic acid	
Carotenoids	
Folic acid	
Plant sterols	
Polyphenols	
Tocopherols	

Bioactivity in turn includes events linked to how the nutrient/BCs is transported and reaches the target tissue, how it interacts with biomolecules, the metabolism or biotransformation it may undergo, and the generation of biomarkers and the physiological responses it causes [5].

CHAPTER 4

Novel Nutrigenomics Avenues in Nutraceuticals Use: The Current Status of Fermented Papaya Preparation

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Abstract: Functional foods present a constantly growing research field, interrelating genomics, epidemiology and clinical investigations, followed by increased interest from the public and food supplement industry. As a matter of fact, the outcome of the implementation of functional foods is now amenable to be assessed by employing many of the most recent diagnostic tools.

Nutrigenomics is a relatively new discipline, which studies the genetic and epigenetic interplay with a nutrient or its functional component(s) in order to bring about a phenotypic modification of key cellular functions, such as, cell metabolism, differentiation or apoptosis. This represents one of the most expanding fields of research to unveil the health benefits of functional foods and their bioactive moieties which cannot be differentiated, as it happens in synthetic molecules devised by pharmaceutical industries.

Within this scenario, a specific functional food, *i.e.* fermented papaya preparation, coming from a controlled bio-fermentation process of papaya, is herewith reported and scientifically backed up by several experimental models and properly-designed clinical protocols.

The promising clinical health benefits provided by fermented papaya preparation are

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M. Victorina Aguilar and Cristina Otero (Eds.)

also discussed under the viewpoint of nutrigenomic mechanism understanding, and a significant antioxidant and effective transcriptomic property is described while ongoing investigations are warranted.

In general, this specific fermented papaya preparation represents a functional food, which closely meets the novel criteria of the new nutrigenomic-oriented strategic approach of preventive medicine, aimed to reduce the burden of chronic illnesses, while also offering potential adjuvant benefits within drugs regimens.

Keywords: Fermented papaya preparation, Functional food, Gene expression, Nutraceuticals, Nutrigenomics, Oxidative stress.

INTRODUCTION

The Ever-changing Nomenclature of Functional Foods

Such a new vision in the last 2 decades has led to continuous modifications in the functional food terminology which in 1999 an expert scientific European panel had defined it as follows "A nutrient can only be easily considered functional if it has been satisfactorily proven that it can beneficially change one or more target functions, besides nutritional effects per se, it can also significantly improve health, well-being while reducing disease risk. A functional food should ideally be a food and should not alter its efficacy when included in a diet, it should not be either a pill or a capsule" [1]. It was then concluded that, from a practical viewpoint, a functional food should meet the following features:

1) a natural food; 2) a food with one added component; 3) a food with no added component; 4) a food which the structure of one or more of its components have been changed; 5) a food which one or more components' availability has been changed; 6) a combination of the previous features. Besides its inner nutritional properties or physiological effects, it was necessary to offer a consistent administration safety profile. Such a condition is nothing but a prerequisite to further develop any functional food.

The recommendations of such European expert panel led to a definite resolution, stating that, "The design and development of a functional food are key factors, besides the scientific challenge, they should be primarily based on robust understanding of target functions and their potential modulations by nutritional components". Thus, it was later emphasized that, "functional foods are not universal, thus a conventional nutritional approach would no longer be satisfactory, but rather, a specific scientific approach will only be applicable". This points towards an innovative nutritional viewpoint regarding the role exerted

by “Functional Foods Science”, which now emerges as the leading vision towards deriving effective clinical inferences. An ancient Chinese proverb stated that “medicine and food are isogenic” and this may represent the traditional root of what in 1984, in Japan, a specific national working group had set up, under the patronage of the Ministry of Education, Science and Culture (MESC). This was called to explore the interconnections between nutrition and several aspects of molecular biology. Researchers investigated a number of foods and nutrients which were then officially categorized as “foods to be specifically administered for healthcare” (Food for Specified Health Use, FOSHU) by recognizing their modified nutritional properties, after undergoing a profound bio-fermentation process. A further consensus meeting took place later on reaffirming the above concepts [2].

Such a categorization still has legal implication on improper marketing communication defining natural products, when they are misleading or loosely referring to data in the scientific literature, as a sort of abusive self-endorsement, but not specifically supporting the specific product itself [3]. As a matter of fact, the lack of a coherent terminology between countries has inevitably generated a large mass of publications, dealing with health claims with often incomparable end-points in clinical trials or food production process methodologies from the industries. As an expected counteraction, an overall mistrust on the meaning and real health benefit of “functional foods” is creeping now and then among government officials, public health professionals and consumers.

On the American side, the latest conference organized by the Functional Food Center held in San Diego in November 2014 ([4] and http://functionalfood scenter.net/17th-international-conference.html), gave rise to a revised and more grounded definition of functional food as:

“Foods that are natural or processed which contain known or unknown biologically-active ingredients, which in defined amounts, provide a clinically proven and documented health benefit for the prevention, management, or treatment of chronic disease”.

Novel biomarkers and Development Strategy leading to Nutrigenomics. A biochemistry and specific molecular biology research, coupled with advancing biotechnology tools have helped in shedding more light into the mechanisms, explaining how nutrients could indeed regulate relevant body functions involved in health issues, as well as in the risk reduction associated to life style. Such evaluations have to be compliant with reliable biomarker identification, either directly linked (functional factors) to the target biological function or indirectly

CHAPTER 5

Bioactive Properties of Sugar Fatty Acid Esters

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Abstract: Sugar fatty acid esters are biodegradable and biocompatible nonionic biobased surfactants or emulsifiers, obtained from abundant renewable resources. They have a dozen of applications in food, cosmetic and pharmaceutical industries. In this book chapter, we briefly reviewed the enzymatic synthesis of sugar fatty acid esters in solvent-free system and their bioactive characteristics, including antimicrobial activity, anti-tumor activity and anti-insect activity. In addition, we compared the antimicrobial and antitumor properties of sugar fatty acid esters synthesized from enzyme with commercial sugar fatty acid ester produced and purified from a chemical reaction.

Keywords: Anti-insect activity, Antimicrobial activity, Antitumor or anticancer activity, Sugar fatty acid esters.

BACKGROUND

Sugar-fatty acid esters, nonionic biobased surfactants, are synthesized from renewable resources such as saccharides (*e.g.*, fructose and sucrose) and fatty acids (*e.g.*, oleic, lauric and palmitic acids). Fig. (**1**) depicts the reaction scheme for sucrose oleate synthesis, catalyzed by the enzyme lipase. Sugar esters have a broad spectrum of applications in food, cosmetics and pharmaceutical industries because of their low toxicity and irritability, biodegradability, and biocompatibility. Their main use is as emulsifiers, due to their amphiphilicity.

As useful functional additives, emulsifiers are widely employed in food processing for the improvement of the stability of multiphase systems in food products. They enable two distinct and immiscible phases to form a stable quasi-homogeneous solution that remains stable for a significantly long time. In addition, emulsifiers modify and improve the physical properties of the continuous phase in food products, promoting their employment as dispersing

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M. Victorina Aguilar and Cristina Otero (Eds.)

agents, foamers, and stabilizers [1]. The relative proportion of hydrophilic and lipophilic behavior of surfactants and emulsifiers is often expressed as the hydrophilic-lipophilic balance, or HLB. The HLB value ranges from 0 to 20, with low numbers (<9) indicative of lipophilic behavior and high numbers (>11) representing hydrophilicity. The HLB of sugar esters can be tailored through controlling the number of fatty acyl groups per molecule and the length of the fatty acyl chain to cover almost the entire HLB range.

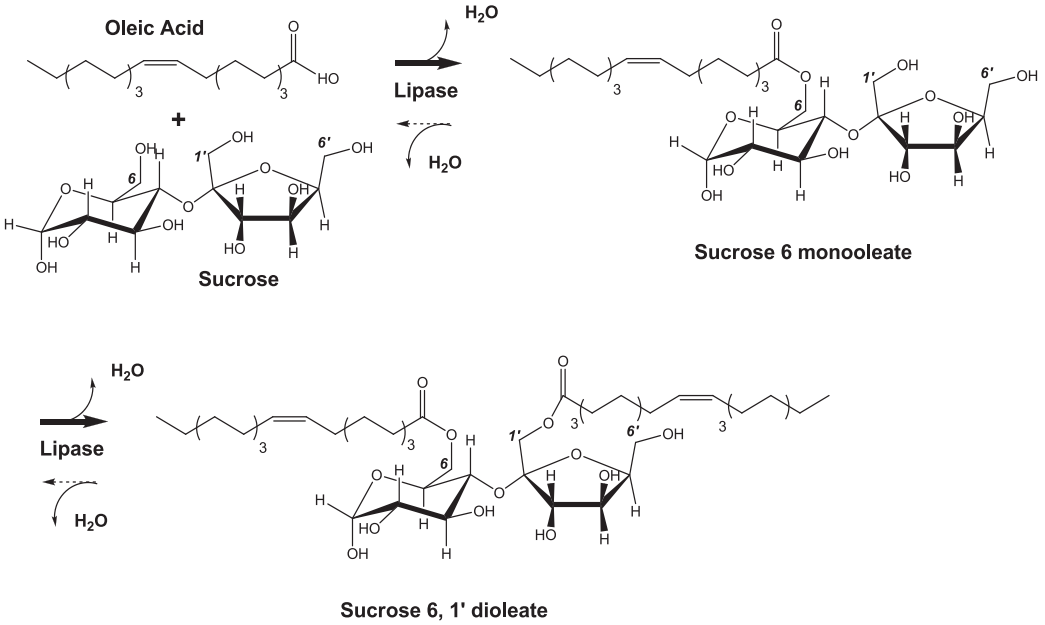

Fig. (1). Lipase catalyzed synthesis of sucrose oleate.

Food-based products that contain sugar esters include baked goods, fruit coatings, and confectionery foods [2, 3]. The effects of sugar esters on the nucleation, growth, and crystallization behavior of high-melting milk fat fraction-sunflower oil blends, have been evaluated for products such as chocolate and confectionaries [2]. Sugar esters are also used in coffee creamers, liqueurs, fruit drinks and whippable toppings [4 - 6]. The employment of sugar esters as additives for drug formulations and delivery has also been investigated. Sucrose stearate was employed as surfactant for nanoemulsions employed in transdermal drug delivery, to replace lecithin, which has a high tendency towards self-aggregation and is prone to chemical degradation [7]. In another study, sugar ester nano-vesicles were employed to encapsulate the antioxidant enzyme catalase for wound healing [8]. In addition, the effect of sucrose esters on transdermal permeation of lidocaine and ketoprofen was examined [9]. The investigators found that sugar

esters facilitate skin permeability and drug absorption [9]. The possession of antitumor properties and antimicrobial activity (discussed in the next section) has furthered the use of sugar esters in pharmaceutical products. Sucrose esters are also used in cosmetics and personal care products including oral and dental care [10, 11].

Chemical Synthesis of Sugar Esters

Sugar esters are commonly produced by chemical methods under extreme conditions, for instance high temperature and pressure, and often in the presence of alkaline or acid catalysts [12, 13], leading to safety issues, environmental concerns and undesired byproducts. For example, the synthesis of sorbitan-fatty acid esters using the conventional chemical method consists of a two-stage process, including dehydration of sorbitol in the presence of the acid catalyst (*e.g.* NaH_2PO_3) at 150-200°C, followed by alkali (*e.g.*, Na_2CO_3-)-catalyzed esterification with fatty acids at 200-250°C [1]. In addition, it was reported that base- (K_2CO_3-) catalyzed transesterification sucrose esters were performed in dimethyl formamide (DMF), an expensive and bioincompatible solvent, at 90 °C with fatty acid methyl ester, serving as acyl donor [14].

Enzymatic Synthesis of Sugar Esters

In contrast, biocatalytic synthesis (*e.g.* using lipases) has received great attention owing to improved sustainability of the reaction: near-ambient pressure and temperature (resulting in lower energy consumption and carbon dioxide emissions), the absence of alkaline or acidic catalysts (leading to lower amounts of waste products), and a narrow product distribution, because mono- and diesters are selectively synthesized *via* the primary hydroxyl groups of the saccharide acyl acceptor.

However, significant barriers hamper the application of immobilized thermophilic lipases for use in industrial production of sugar esters. The major issue is the poor miscibility of polar and non-polar substrates, leading to slow reaction rates. Several different methods have been used to overcome this hurdle. Among them, the utilization of polar organic solvent or their mixtures is considered as the most common approach. Common solvents employed for this purpose include *tert*-butyl or *tert*-amyl alcohol, methyl ethyl ketone, acetone, acetonitrile; or their mixtures with very polar solvents, such as dimethylsulfoxide (DMSO) [8, 15 - 19]. If such systems are operated correctly, partially solubilized acyl acceptor can be employed; and, the reaction medium's composition and temperature can be tuned to selectively precipitate out the monoester product [17 - 21]. In addition to

CHAPTER 6

Arabinoxylans: Bioactivities in Relation to Their Molecular Structure

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Abstract: Arabinoxylans are a group of compounds with a basic structure consisting of a xylose backbone with arabinose side chains. Variations in structure occur as a result of variations in the xylose chain length, the ratio of arabinose to xylose and the introduction of alternative side-chains. This allows for an enormous potential range of structures. Arabinoxylans are major components of the cell walls of cereals. They have been reported to have numerous health benefits. This chapter presents a systematic description of the molecular features of arabinoxylans and relates these to the different extraction technologies used to obtain them. The proposal, that their immune modulation activity is related to their molecular weight and structure, is presented. Results demonstrating the effects of various arabinoxylans in various *in vitro* immunological tests are discussed.

Keywords: Arabinoxylans, Extraction, Modification and Immune Modulation, Molecular structure.

INTRODUCTION

Arabinoxylans are major components of the dietary fibre derived from cereals. Dietary fibre consumption has been shown to have many health benefits such as prebiotic activity, immune stimulation activity, reducing post glycaemic response the risk of various diseases and improving lipid and fat metabolism [1 - 5]. The significance of arabinoxylans is focused on maintaining good health, and the increasing role of these materials in modulating and supporting the immune system is also emphasized.

There are several aspects of arabinoxylan chemistry, given their increasing

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M. Victorina Aguilar and Cristina Otero (Eds.)

importance in diet and health, are worthy of detailed examination. It is important to understand how this complex group of compounds produces these effects. The variation in structure is also considered to be of significance when extracting the arabinoxylans.

In this chapter, the structure and the variations in structure of arabinoxylans from different cereal sources will be discussed. The variations in structure may be the result of the extraction method used or may be a natural feature of the molecules, which influences their extraction. Examples of the extraction techniques will be presented and the associated structures identified with each technique reported. The different effects of the various structural types of arabinoxylans on cellular metabolism and the indicators of immune system function will be discussed.

Arabinoxylan Structures

Arabinoxylans are found in major dietary cereals, such as wheat, corn and rice. Arabinoxylans consist of a linear β-(1, 4) D-xylopyranose linked xylan backbone [3, 6]. Variations in structure occur as a result of modifications to the side-chains. Fig. (**1**) shows the basic backbone structure and possible substitutions.

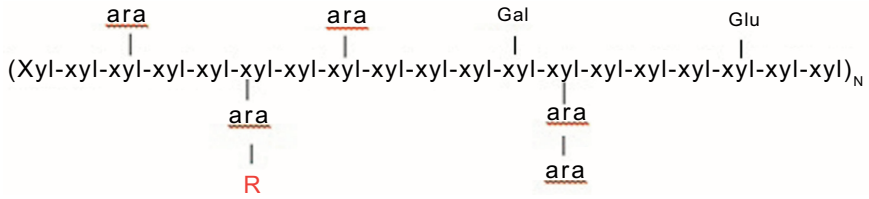

Fig. (1). General backbone structure and substitution variations for arabinoxylans. R= Ferulic acid or p-coumaric acid.

The chain consists of a xylose (xyl) backbone, N subunits in length, with arabinose side-chains at intervals along its length, forming monosubstituted xylose units. The main structure can be modified in a variety of ways (Fig. **1**); for example by replacing the arabinose groups with alternative sugar groups (Fig. **1**); by substituting 2 arabinose molecules on a single xylose (di-substituted Xyl); extending the side chain with additional arabinose subunits; extending the side chain with additional alternative sugar subunits; forming 2-O-acetyl ester side chains; addition to the arabinose side chain of ferulic acid or p-coumaric acid, which may permit either linkage to lignin, protein or glucan, or to ferulic acid side-chains on another arabinoxylan to form cross-links between chains as shown in Fig. (**2**).

The considerable variation in structure, which is possible, explains the complexity seen in function and in the variety of molecules obtained using different isolation techniques. It is clear from this brief outline of the general structures that there are a plethora of different molecules which might exist within the group currently known as arabinoxylans. Simply the chain length N can technically be infinite although in reality $N \neq \infty$. In practical terms arabinoxylans have been shown to range from 30 to 600 KDa [7]. In addition to chain length, the other simple variable which defines arabinoxylans is the number of arabinose side-chains occurring along the length of the xylose backbone. Generally, the number of arabinose units in an arabinoxylans molecule is reported as the Ara/Xyl A/X. Naturally, the A/X has been found to range from 0.15 to 0.89 [7]. Specifically, the xylose chain might have a single arabinose side chain unit or at the maximum some of xylose subunits in the chain can have 2 side chains, di-substituted xylose, both of which might consist of up to 2 arabinose molecules.

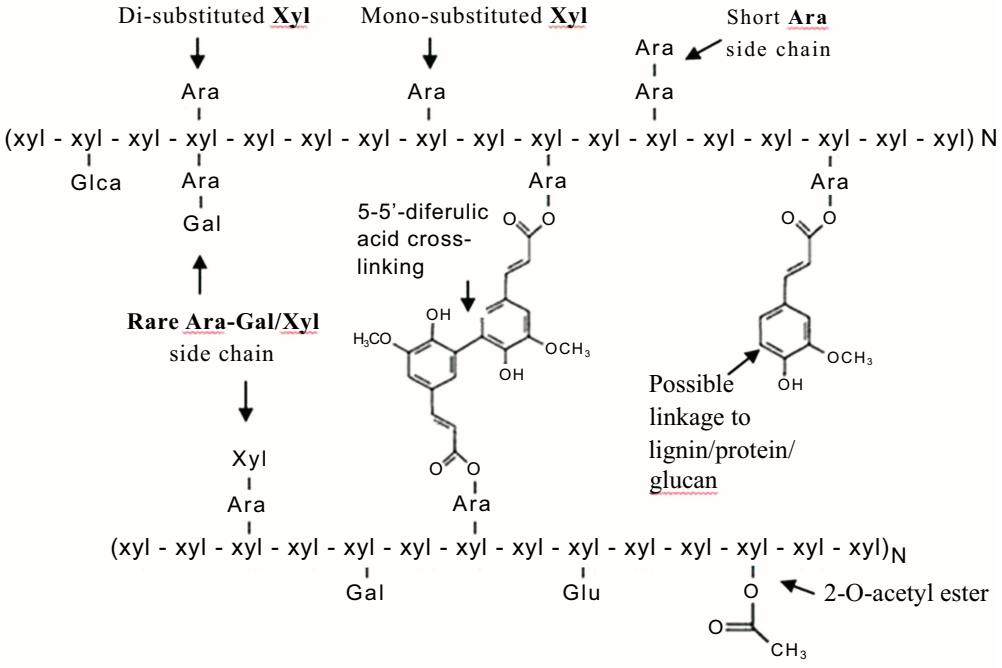

Fig. (2). Cluster structure for arabinoxylans.

The simple variation noted in Fig. (**1**) is the introduction of other side-chains consisting of different sugar moieties, such as glucose and galactose, either attached directly to the xylose backbone or *via* an arabinoxylose molecule. Alternatively, the side chain might be ferulic acid which presents a further range of options. The numbers and types of such side-chains are limited theoretically

CHAPTER 7

Yam (*Dioscorea* species): Future Functional Wild Food of Tribal Odisha, India

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Abstract: Food security and effective medicines continue to be challenging issues for the third world. Functional plants give us some bio-resources having food and medicinal values. Several species of the genus *Dioscorea* available in tribal Odisha in India are parochially used as food and medicines for various diseases and disorders. *Dioscorea* is a monocot having starchy tuberous root. Some tubers are sweet and some are bitter in taste, due to their high content of phenolic compounds and other anti-nutritional factors. These vines have diverse bioactive compounds including steroidal saponin, diosgenin *etc*. Keeping this in mind, the ethnobotanical values and contents of bioactive compounds of these vines were collected from field and literature survey. The analysis of the obtained data permits to justify its consideration as a future functional food. The present investigation also highlights the importance of wild edible plants as functional foods and their potentials for the formulation of new drugs.

Keywords: Bioactive compounds, *Dioscorea*, Ethnobotany, Functional food.

INTRODUCTION

To achieving Food and medicine are basic needs in order to achieve a healthy leaving. Executing food security and germane panacea in its totality continues to be challenges for this technically sound world. Both have equal lethal effect on the population of any country in all aspects [1 - 5]. Research evidences / published research articles and reports of various organizations in the recent years have emphasized on the urgent need for searching new options for food & medicine from natural resources, and to support the necessary scientific research to fight against the lethal diseases.

Several countries among the third world including India, suffer famines, food shortage, malnutrition, endemic lethal diseases, antibiotic resistance and other

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M. Victorina Aguilar and Cristina Otero (Eds.)

disorders, although they have the potential to produce adequate nutritional base and appropriate medicines for their vast increasing population [3, 6, 7]. Despite several policies and programs made on food and medicines for self sufficiency at state and national level, developing countries like India have not attained food and medicinal security at household level, particularly in rural and tribal areas [8].

A considerable proportion of the tribal population is still under-nourished. People living near to forest areas and mountain ranges cannot produce enough food grains to meet their requirement and have no proper medical facilities [9]. They cannot afford to purchase allopathic medicines. Therefore, a large share of such population meets its food and medicines requirement through non-conventional means, by using various wild plants and animal resources from the forest [10, 11]. The indigenous forest food and medicines are of great social significance to tribal population in developing countries. However, the forest food and medicinal plants are neglected and underutilized due to lack of awareness, little research attention, poor commercialization and deficient policy frameworks for harnessing their actual capabilities. The food and medicines from biodiversity and the traditional practices to utilize them, can provide basis for future optional food and medicines by which third world countries can fight against food scarcity and health care problems [2, 12, 13].

The appropriate effort should be given to find out food from biodiversity which have medicinal values, and this can fight many problems at a time. Such effort results in finding "Functional food". The main sources of functional food are animals and plants in nature. Plants are the major source for such food. There are about 1532 wild edible food species in India which have nutritional and medicinal values [14 - 16].

Among the indigenous functional food, the root and tuber are the most important wild food after grains. They have the highest rate of dry matter production per day, and are the major calorie contributors to rural and tribal populace. They play a vital role in supplementing staple foods with micronutrients to the remote people, through preserved and starchy stored food stuffs, during the time of food shortage and lean agricultural seasons. India harbours a rich genetic diversity of tropical roots and tuberous plants, such as Yams (Ban Aālu), aroids and several elements, like ginger, arrowroot, zedoary, ginger lily, wild turmeric and some orchids [17 - 20].

The hot spots of global biodiversity, such as Western Himalayas, North-East regions, humid parts of Western Ghats and Eastern Ghats, including East-Coastal region, are particularly rich in wild tubers and wild relatives of tropical root and

tuber. Odisha is the major part of Eastern Ghats having rich diversity of medicinal food plants along with various types of tribal communities with unique traditional skills [21]. Among the tuberous edible medicinal plants available in tribal Odisha, *Dioscorea* species or Yam are very common in all landscapes.

Dioscorea species belong to the Dioscoreaceae family; monocot climbers, very common and important, because they have adequate nutritional compositions as well as secondary metabolites, which make them excellent medicinal food. The starchy and other energy supplemented components of *Dioscorea* tubers showed its sound food values, while its anti-nutritional factors showed the background of ignorance [22]. They are rich in phenolic compounds and their derivatives, that have potential pharmacological activities such as antibacterial, anti-inflammatory, antifungal and anticancer. Therefore, *Dioscorea* provides an ideal source of biodiversity for plant breeders, and biotechnologists use it to produce new varieties and breeds having desired characters with effective energy components, as well as exertive bioactive compounds using various biotechnological tools, such as metabolite expression, gene silencing and other molecular and nano-technologies [23]. Keeping this in view, an attempt has been made to highlight the food and medicinal values of *Dioscorea* species collected from tribal Odisha and give emphasis on primary and secondary components of the *Dioscorea* species.

DIOSCOREA SPECIES & TRIBAL ODISHA: INDIGENOUS USES

The vegetation of Odisha gives immense opportunities to discover various types of functional food plants. With an ideal combination of rich forest resources, mountainous terrain, varied floristic composition, the home of aboriginal tribes and their indispensable dependence on forest for indigenous edible plants, Odisha offers a great scope for the study and assessment of *Dioscorea* species. The extensive and densely forested hilly tracts of tribal Odisha are the home of many aboriginals such as Ho, Kolha, Santhal, Bathudi, Bhumija, Mahali, Saunti, Munda, Gonda, Pauri Bhuiyan *etc.*, including primitive groups like Hill Kharias, Dongaria Kandha, Desia Kandha, Juang, Mankirdias *etc*. They have some unique skills, culture and rituals [19 - 27].

Their main occupation is food gathering, hunting, collection of forest products and traditional farming or agriculture. They mainly collect wild products for food and medicine. Their prime wild edible available in tribal Odisha are tubers (*Lasia spinosa* L. Thw., *Dioscorea* species, *Pueraria tuberosa* Willd. DC.), leaves (*Opilia amentacea* Roxb., *Amaranthus viridis* L., *Cordia obliqua* Willd.), flowers (*Indigofera cassioides* Rottl.ex DC, *Hibiscus sabdariffa* L., *Moringa oleifera* Lam.), vegetables (*Coccinia grandis* L. Voigt, *Solanum nigrum* L., *Abelmoschu*

CHAPTER 8

Biological and Medical Effects of Saponins of *Hedera helix* and Other Medicinal Plants

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Abstract: Saponins are biologically active components of many medicinal plants. From ancient times till our days the saponins containing medicinal plants are used in medicine with different purposes, in particular as anti-cancer agents in the folk (traditional) medicine. Therefore, many investigations are focused on the proapoptotic, toxic and proliferation suppressing properties of saponins. Publications from 2000 to 2014 dealing with medical applications of plant-derived saponins and saponin-containing herbs are reviewed.

Keywords: 18α-glycyrrhetinic acid, Alpha-hederin, Apoptosis, Astragalosides, *Astragalus* genus, *Bryonia* genus, Cell proliferation, Giganteosides, *Glycine* genus, *Glycyrrhíza* genus, Glycyrrhizin, *Hedera* genus, Necrosis, *Nigella* genus, Soyasaponins.

INTRODUCTION

The word “saponin” is originated from the Latin word “sapo”, which means soap, for their surface activity and ability to form foam in water solutions. Saponins are generally glycosides of steroids or polycyclic triterpenes and consist of a polycyclic aglycones attached to one or more sugar side chains. The non-sugar part of the molecule, or aglycone part, which is also called sapogenin, is either steroid (C27) or a triterpene (C30) [1]. Medicinal properties of saponin-containing medicinal plants are used in folk medicine from old ages till now, for instance, Licorice, the root of *Glycyrrhiza* spp. (Fabaceae), has been used since ancient Egyptian, Greek, and Roman times in the West and since the 2nd-3rd century B.C. in ancient China [2]. Seeds of *Nigella sativa* were found in the tomb of Egyptian Pharaoh Tutankhamen [3]. Recent investigations prove the medicinal importance

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M. Victorina Aguilar and Cristina Otero (Eds.)

of saponin-containing plants and show new areas of application of natural saponins.

MEDICAL APPLICATIONS OF EXTRACTS OF SAPONIN CONTAINING MEDICINAL PLANTS OF DIFFERENT SPECIES

Many plants containing saponins are applied with medical purposes. In this section we refer only to some characteristic examples of such plants of different taxonomic groups.

Hedera Genus (*Araliaceae*)

The ivy leaves extracts exhibit spasmolytic/antispasmodic, anti-inflammatory, antimicrobial, analgesic, anthelmintic, antitrypanosomial, antileishmanial, anti-tumor, antimutagenic, moluscocidal, antioxidant and antithrombin activities [4]. *Hedera helix* leaves extract and medical preparations of it are used in treatment of chronic inflammatory bronchitis [5], asthma [6], and also for cough treatment [7]. The *Hederae folium* (Ivy leaf) which consists of dried leaves of *Hedera helix* L. collected in spring or early summer contains as a minimum 3.0% of saponin hederacoside C. The biologically active compounds of *H. helix* are triterpene saponins (2.5–6%): bidesmosidic glycosides of hederagenin – hederacoside C (1.7-4.8%), hederacoside D (0.4-0.8%), hederacoside B (0.1-0.2%); and mono-desmoside α-hederin (0.1-0.3%) [4]. Structure formulas of two saponins from *Hedera helix* – alpha-hederin and hederacoside C are presented in Fig. (**1**).

Nigella Genus (*Ranunculaceae*)

Medicinal properties of *Nigella sativa* L. are described in reviews [8] and [9]. The seeds of *N. sativa* (Black seed) are widely used in the treatment of various diseases like bronchitis, asthma, diarrhea, rheumatism and skin disorders. *N. sativa* is popular among Muslims because it was mentioned in one of the Prophet Muhammad hadith stating that Black seed is the remedy for all diseases except death [9]. In the Bible, the Black seeds are described as the "curative black cumin" [3]. *N. sativa* has been extensively studied for its biological activities and therapeutic potential and has been shown to possess a wide spectrum of activities such as diuretic, antihypertensive, antidiabetic, anticancer and immunomodulatory, analgesic, antimicrobial, anthelmintic (especially anti-schistosomiasis activity), analgesic, anti-inflammatory, spasmolytic, bronchodilatory, gastroprotective, hepatoprotective, renal protective, and antioxidant properties. It is also used as a liver tonic; digestive, anti-diarrheal, appetite stimulant, and emmenagogue remedy [9]. Total saponins fraction from *N. glandulifera* seeds

reveals anti-inflammatory, analgesic, antitumor, and free radical scavenging activities [10]. Alpha-hederin was isolated from *N. sativa* seeds among different biologically active substances [8].

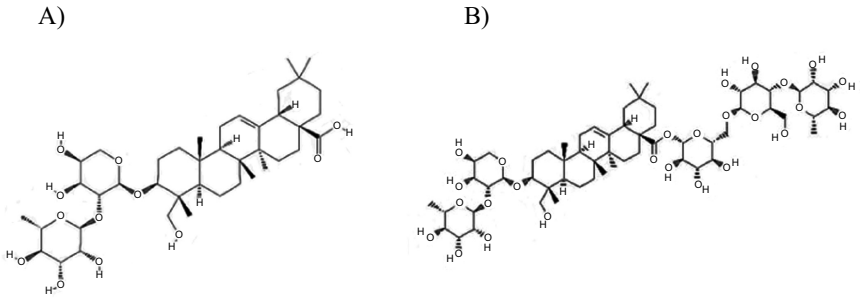

Fig. (1). Saponins from *Hedera helix* L.: **A)** alpha-hederin and **B)** hederacoside C.

Bryonia Genus *(Cucurbitaceae)*

The entire plant of *Bryonia laciniosa* L. is used as hepatoprotective, anti-pyretic, laxative, and correcting the metabolic abnormalities. In experiment in rats with streptozotocin-induced diabetes the alcohol extract of *B. laciniosa* seeds and its saponin-rich fraction alleviated hyperglycemia and hyperlipidemia. In addition, *B. laciniosa* could ameliorate the impaired renal function and inhibit liver damage associated with streptozotocin induced diabetes [11].

Glycyrrhiza Genus (*Fabaceae*)

The *Glycyrrhiza* spp. is widely used as an herb from ancient times. In traditional medicine the *Glycyrrhiza* roots are used for its demulcent and expectorant properties. It is useful in anemia, gout, sore throat, tonsillitis, flatulence, sexual debility, hyperdypsia, fever, coughs, skin diseases, swellings, acidity, leucorrhoea, bleeding, jaundice, hiccough, hoarseness, bronchitis, gastralgia *etc.*, the active component glycyrrhizin is utilized as a non-nutritional sweetener and flavoring agent in some candies and pharmaceuticals. Chemical analysis of saponins of Licorice root revealed high contents of triterpenoid saponins (4- 20%), mostly glycyrrhizin, a mixture of potassium and calcium salts of glycyrrhizic acid (also known as glycyrrhizic or glycyrrhizinic acid and a glycoside of glycyrrhetinic acid). Other triterpenes are liquiritic acid, glycyrretol, glabrolide, isoglaborlide and licorice acid [12]. The structural formulas of glycyrrhizinic acid, liquiritic acid, and glabrolide are presented in Fig. (**2**). Glycyrrhizin exerts an anti-inflammatory action. It stimulates production of hormones by adrenal glands and reduces the breakdown of steroids by the liver and kidneys. Glycyrrhizin is also effective in treatment of chronic hepatitis and liver cirrhosis [12].

CHAPTER 9

Bioactive Compounds and Diabetes

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Abstract: Diabetes mellitus (DM) is the most common endocrine disorder, and with a very high development rate. There are two main groups of DM: type 1 DM (basically due to an insulin deficiency), and type 2 DM (basically due to insulin resistance, *i.e.*: there is plenty of insulin, but actually the cells are resistant to its action). Both types lead to both abnormal glucose and lipids metabolism, sub-clinical inflammation and higher oxidative stress. We will speak mainly about type 2 DM. Its genesis is multifactorial, but we can ascertain that the diet is the principal modifiable factor. The benefits of a healthy diet are not limited to its nutrient content, must also provide other protective factors against oxidative stress, metabolic syndrome, diabetes and carcinogenesis content especially in plant foods, called compounds bioactive, serving in the body that can promote good health. A diet with high intake of phytochemicals and rich in antioxidant capacity with polyphenolic compounds (as the Mediterranean Diet), is related to a decreased risk of DM.

Keywords: Beta cells, Diabetes, Insulin resistance, Mediterranean Diet, Polyphenols, Prevention, Terpenes, Vitamins.

BACKGROUND

Type 2 diabetes mellitus (T2DM) is the most common endocrine disorder, and with a very high development rate. Basically it is due to insulin resistance: although there is plenty of insulin, the cells are resistant to its action, or in other words, are not able to use this insulin. The direct consequence is an increased

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glucose level and abnormal lipids metabolism, sub-clinical inflammation and higher oxidative stress. The final result of these processes, mediated through complex pathways, are both micro- and macrovascular complications, increased atherosclerosis, retinopathy, nephropathy, neuropathy... in the end complications that sooner or later will affect eyes, kidneys, heart, nerves and blood vessels [1, 2].

The prevalence of both T2DM and metabolic disease is increasing quickly all over the world [3]. Currently it is a major health problem, but in next years it will become an epidemic.

Many risk factors have been involved in the incidence and prevalence of T2DM. But the principal among all the modifiable risk factors is the diet. There are plenty of epidemiological papers published, demonstrating that if the diet is rich in phytochemicals (powerful antioxidants) and polyphenolic compounds, the risk of DM and its predisposing factors is diminished [4, 5].

According to current evidence about the pathways implicated in both insulin resistance and T2DM, multiple interventions (both pharmacologic and non-pharmacologic) have been developed. Their main objectives are double: normalizing glycemic levels and prevention of DM complications (angiopathy, neuropathy). An innovative approach to both prevention and treatment of DM and its complications, is the recent use of 1) functional foods, and 2) bioactive compounds [6, 7].

Bioactive compounds of 800 plants have been reported by Grover *et al.* [8] for DM. Their mechanism of action is multiple (inhibiting or stimulating the enzymatic activity and / or the protein expression). Marles and Farnsworth [9] listed 1200 species that have been used to treat diabetes worldwide, and after twenty years Ovalle-Magallanes *et al.* [10] affirmed that only in Mexico folk medicine, there are about 3500 different species; and for the treatment of T2DM, up to 383. According to Arumugam *et al.* [11], from about 250,000 higher plants, not even 1% of them have pharmacological studies and regarding DM this proportion is even lower.

Other plants, such as the one studied by Aragão *et al.* [12], have shown hypoglycemic and insulin-release effects [13, 14]. Trojan-Rodrigues *et al.* [15] also cited more than 81 species of the southern Brazil that have hypoglycemic effects, but no mechanisms of action were evaluated. Another review published by Mukherjee *et al.* [16] was based on the Indian medicinal plants with hypoglycemic potential showing their bioactive compounds (alkaloids,

imidazoline, polysaccharides, flavonoids, saponins and ferulic acid) and some strategies of mechanisms.

All of these plants, may operate through different mechanisms that affect blood sugar. Some of them may increase the insulin kinase, some of them may inhibit insulin's activity and others may increase reconstruction of pancreatic β cells. For example, according to Aslan *et al.* [17], several antioxidants (for example: tannins, vitamins C and E, flavonoids) may prevent pancreatic β cells destruction (probably by inhibition of the peroxidation chain reaction; in this way at least theoretically, protection from DM development could be provided). The antidiabetic property of tannins (mainly epicatechin and catechin derivatives) [18], coumarins, flavonoids, terpenoids, arginine and glutamic acid has been confirmed in some experimental animal models research [9, 17]. Moreover, fibers of plants may also interfere in the absorption of carbohydrates and thus have an effect on blood glucose [19]. However, we should not reach to the conclusion of any mechanism about the hypoglycemic effect of any plant or plant extract, because all of them usually contain plenty of different chemical compounds.

THE MEDITERRANEAN DIET

Much has been written about the Mediterranean Diet (MD), and not all is correct, or at least not the whole truth. It has been written that MD consists in a mix of dietary food ingredients, such as vegetables, fruits, whole grains, legumes and olive oil. The sources of protein are mainly fish and poultry. And red wine in small amounts is also a component. But the MD is not just a diet, actually it´s a lifestyle and could be perfectly be called "Mediterranean Lifestyle". It is not just an eating pattern (detailed in other chapter of the book), but actually the diet plus: cooking methods, healthy weight, no smoking and plus moderate daily physical exercise. That will make the almost perfect model for healthy living. The feeding behavior of man is influenced by numerous factors (geographic, climatic, socio-economic, *etc.*) of which will depend on the select of food; choice is an important determinant of nutritional status and health status.

In this sense, the ecological relationship of our ancestors with their environment configured peculiar eating habits that today constitute the so called MD, traditionally based on "Mediterranean trilogy" consisting of wheat, olive-tree and grapevine. Olive-tree and olive oil are the true symbol of culture and food of the Mediterranean.

Subsequently, many other foods were gradually incorporated into this diet: orange and lemons from the Far East; beans, tomatoes, corn, potatoes or eggplant were

CHAPTER 10

Evidence to the Vitamin E and Other Antioxidants Influencing Cardiovascular Diseases

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Abstract: Cardiovascular diseases are the main cause of death worldwide and therefore, the most important public health problem. Many factors play a role in the development of cardiovascular diseases, among others: obesity, hypertension, diabetes mellitus, hyperlipidemia, inadequate diet, sedentary life and smoking. Cardiovascular disease incidence can be improved increasing the intake of fruits and vegetables. Cohort studies and randomized controlled trials have shown that intake of dietary antioxidants (vitamins E, A, C, and poliphenols), antioxidant supplements or dietary patterns (Mediterranean diet) influence differently in the occurrence of cardiovascular events or death. This review examines relevant clinical reports on dietary compounds, supplements or dietary patterns to analyze what kind of patients (if any) with increased cardiovascular risk factors will get any benefit for these therapeutic options.

Keywords: Antioxidant dietary supplements, Antioxidant intake, Cardiovascular disease, Carotenoids, Cohort studies, Coronary heart disease, Evidence, Flavonoids, Mediterranean diet, Mortality, Poliphenols, Prevention, Randomized controlled trials, Stroke, Vitamin A, Vitamin C, Vitamin E.

INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of death worldwide and is therefore a major and growing health problem [1]. Plenty of factors had been involved in the origin of CVD. Some of these factors are fixed (gene, gender, age), but others factors are modifiable (diet, exercise, environment, smoking, and lifestyle conditions). In addition, other comorbidities play a role in the

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M. Victorina Aguilar and Cristina Otero (Eds.)

development of CVD, among others: obesity, hypertension, diabetes mellitus, hyperlipidemia, inadequate diet, sedentary life and smoking [2]. Atherosclerotic plaque formation is the main common feature of CVD. The first step for beginning this atherosclerotic plaque is an endothelium injury [3].

Both inflammation and oxidative stress are main pathways that result in endothelial dysfunction and consequent atherosclerosis. Although these classic cardiovascular (CV) risk factors and comorbidities have been profusely studied, there are also other less well known factors that can also play a role, for example, some factors in the diet. The diet is one of the most important modifiable factors related to CV risk. Consequently, it should be an important target in public health. Its role has been largely investigated for the benefit of the reduced feeding of specific components, such as sodium and saturated fat [4] or some cohort studies where the basal dietary intake showed an association with CV events [5].

The diet´s role in the formation of the atherosclerotic lesion can be helping directly in this process, *i.e.*: accumulating lipids in plasma (triglycerides, phospholipids, LDL-cholesterol), hyperglycemia, free radical production, increasing blood pressure and platelet aggregation, among others.

An adequate nutrition might have a role in the treatment and prevention of CVD [6, 7]. Because it is well known that many CV liver and kidney diseases are a consequence of an inadequate lifestyle and impaired diet (either qualitatively and/or quantitatively), a well-balanced nutrition should be crucial in their prevention and treatment. Certain epidemiological studies had shown a relationship among some dietary patterns with antioxidant power and CV health. Cardio-protective potential of bioactive components in the diet have been widely studied, and suggest that some nutraceuticals and functional foods, including antioxidants (like vitamin E, vitamin C, polyphenols, carotenoids, and minerals) might play a beneficial role.

ANTIOXIDANT INTAKE AND CVD

A reduced risk in CVD (and other chronic diseases with oxidative stress), had been associated with chronic intake of fruit and vegetables [8]. First studies pointed out to the possibility that dietary antioxidants (mainly vitamin E, polyphenols and carotenoids) could be the actors of this CV protection. This was supposedly mediated by lowering the oxidative stress, as suggested in several epidemiological and preclinical studies. Nonetheless, human randomized studies failed to demonstrate the hypothesis. We still do not know exactly the explanation for this paradox, sometimes it is extremely difficult to match all the different

peculiarities of the populations. To obtain some tracks through comparative studies, some tables show in scheme the evidence of some controlled prospective studies and randomized clinical trials [8].

Different studies show that the atherosclerotic process (damage and posterior endothelial proliferation, with foam cells production) are related to the state of peroxidation of LDL-cholesterol. Antioxidant vitamins are usually considered as the principal defensive pathways of non-enzymatic antioxidant systems of the body. Alpha-tocopherol (the main constituent of vitamin E), vitamin C, pro-vitamin A (beta-carotene) and selenium are known natural antioxidants. Some trials including a large number of patients and long follow-up, have been performed to ascertain if the supplementation with antioxidant vitamins reduces CVD. Some studies (both epidemiological and experimental) suggest favourable effects of administration with supplements of antioxidant vitamins in the progression of atherosclerotic lesions, reducing CVD. Nonetheless, other end-points published of well-defined prevention and treatment, failed to demonstrate this hypothesis [9].

VITAMIN E INTAKE AND CVD

Vitamin E is a crucial antioxidant, which plays a very important protective role against free radicals. Vitamin E consists basically of two close molecules: tocotrienols and tocopherols, each with different isomeric forms: alpha (α), beta (β), gamma (γ) and delta (δ). The first one α–tocopherol is responsible for the majority of this activity (about 90%) in tissues [7].

Vitamin E daily nutritional requirements (usually 10 mg/day) is variable, depending on the intake of polyunsaturated fatty acids. Vitamin E is also a main component of cell membranes and also of circulating lipoproteins. Therefore, vitamin E is one of the most important antagonists of lipid peroxidation. As a membrane protector, vitamin E also enhances normal endothelium function, reducing the proliferative stimulus of myocytes in middle tunic and reduces monocyte chemotactic factors [10]. The protective role of vitamin E to the endothelium is supported by a stimulating action on the synthesis of PGI2, a vasodilator and antiplatelet agent [11]. As vitamin E is also one of the most fat-soluble vitamins, it persists during a long time in membrane lipoproteins; in this way, its daily dietary supplementation may not be required [12]. Vitamin E acts as a strong anti-inflammatory (more when in high doses) [13].

Plenty of evidence available supports an important inverse association not only of plasmatic vitamin E and CVD, but also between the intake of vitamin E and

CHAPTER 11

Metabolic Syndrome and Bioactive Compounds

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Abstract: Metabolic syndrome is defined as a set of coexisting metabolic disorders that increase an individual's likelihood of developing type 2 diabetes, cardiovascular disease and stroke, among other chronic diseases.

In Chapter 9 of this same book, we have already explained in detail, the main concepts about Mediterranean diet and bioactive compounds and diabetes.

In this chapter, we update the evidence about dietary bioactive compounds with potential in preventing / treating the Metabolic Syndrome. We will only focus on the studies where the evidence is relevant, not on the multiple studies *in vivo*, very interesting indeed, but with no current clinical relevance.

Bioactive compounds should be of the greatest interest because of their multiple positive effects on health, prevention of Metabolic Syndrome related diseases or lowering their complications.

Keywords: Bioactive compounds, Metabolic syndrome.

BACKGROUND

Inappropriate intake of food, no matter if in quantity, quality or both, or inadequate metabolism of a person, could lead to malnutrition because of deficit or overweight, or obesity due to excessive intake [1].

Overweight and obesity can be defined as the excessive amount of fat that can be risky for health and predispose to cardiovascular diseases.

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The body mass index (BMI) or Quetelet index, is used for recognising overweight and obesity in adults. It is a measure of relative size based on the mass and height of an individual, and it is defined as their body mass (in kg) divided by the square of their height (in meters) (kg/m^2). According to WHO [1], a BMI equal or over 25, defines overweight, and equal or over 30 defines obesity. Of course, excess weight and obesity are highly associated with type 2 diabetes mellitus, insulin resistance and many other chronic diseases, including cardiovascular diseases [2].

Individuals with excess of intra-abdominal fat have greater risk for type 2 diabetes mellitus and cardiovascular diseases.

According to WHO, overweight and obesity are the fifth risk factor of death in the world. The so called "abdominal obesity" and "intra-abdominal adiposity", are the preliminary signs for cardio-metabolic complications, such as increased cholesterol and triglycerides levels, glucose intolerance and insulin resistance, also known as "Metabolic Syndrome" (=MetSyn) [1].

Vascular risk is increased [3] by 3.8% in hyperlipidaemia, 3% in diabetes, 2.9% in smokers, 2.4% in hypertension and 2.3% in abdominal obesity. Hypertriglyceridemic waist (waist with abdominal fat plus fasting plasma triglyceride concentrations) increases the risk of myocardial infarction death in next 2.5 years.

Visceral fat goes directly from the liver to vena porta. The immediate consequence is the release of fatty acids by adipose tissue. They damage the liver (increasing triglycerides) or the pancreas (hyperinsulinism), with long term damage to pancreas, exhausting the pancreas and promoting diabetes, and increasing the risk of cardiovascular events.

There are plenty of definitions for the MetSyn. For practical purposes we will take the definition of the International Diabetes Federation [4]. For a person to be defined as having the MetSyn, they must have central obesity plus any two of four additional factors (Table **1**).

Table 1. Metabolic Syndrome definition (IDF 2005).

1) Raised TG level: ≥ 1.7 mmol/l (150 mg/dl).
2) Reduced HDL-cholesterol: < 1.03 mmol/l (40mg/dl) in males and < 1.29 mmol/l (50 mg / dl) in females (or specific treatment for these lipid abnormalities).
3) Raised blood pressure (systolic BP ≥ 130 or diastolic BP ≥ 85 mmHg) (or treatment of previously diagnosed hypertension).
4) Raised fasting plasma glucose [FPG ≥ 5.6 mmol/l (100 mg/dl)] (or previously diagnosed type 2 diabetes).

Other definitions for MetSyn are from Reaven [5], OMS 1999 [6], NCEP-ATP 2001 [7], EGIR 2002 [8] and AAEC 2003 [9].

In abstract, the MetSyn defines a state of high cardiovascular risk. Non-diabetic individuals with the MetSyn have a fivefold increased risk of developing diabetes. Those patients already diagnosed with diabetes have a two to four times greater risk of cardiovascular disease and stroke [10].

Preventing MetSyn will prevent from a lot of complications derived from diabetes mellitus and in the end, from cardiovascular diseases also.

As told before, the prevalence of MetSyn, like type 2 diabetes mellitus is quickly increasing all over the world, even in developing countries, and it is an outstanding health problem [2] that will turn in a big epidemic (if it is not already established).

We suggest our reader to take a look on Chapter 9: "Bioactive compounds and diabetes", where we outline the main characteristics about bioactive compounds, and the outstanding concepts about Mediterranean diet and bioactive compounds (classification, characteristics and principal effects) have already been explained in detail.

In recent years, in Western society, where the lack of food is not a problem, people are beginning to aware about the close relationship between diet and health. As a result, functional foods, beneficial to health, are having a great impact on the food sector. Research carried out to produce these foods has focused to the study of the physiological role played by dietary proteins. There are certain fragments within the sequence of food proteins that may show biological activity once released by hydrolysis. These fragments, known as "bioactive peptides", can be produced *in vivo* by the action of gastrointestinal enzymes and can also be obtained *in vitro* using specific enzymes, or during the preparation of certain foods.

Since their discovery in 1979, bioactive peptides with different biological activities have been described. Some of them have the ability to improve lipid profile, others reduce arterial tone and control hypertension, other have different or combined effects to improve the MetSyn.

In this chapter, we will update the evidence about dietary bioactive compounds with potential in preventing the MetSyn. We will only focus on studies where the evidence is well documented, because if not, this chapter will be endless. And also the approach will be a practical one, not only explaining what peptides work

CHAPTER 12

Urso-, Glycoursodeoxycholic and Tauroursodeoxycholic Acids: From Basic Research to Clinical Applications in CNS Disorders

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Abstract: Tauroursodeoxycholic acid and glycoursodeoxycholic acid are the conjugates of ursodeoxycholic acid, which is largely used in the treatment of hepatobiliary diseases due to its detergent properties. These bile acids exist only in minor quantities in the normal human body. Ursodeoxycholic acid was approved by U.S. Food and Drug Administration (FDA) for cholesterol gallstone dissolution, and as a cytoprotective agent in primary biliary cirrhosis. Orally administered ursodeoxycholic acid is later conjugated with taurine and glycine in the liver and originates the conjugated species that have fewer side effects than the counterpart free species. Because glycoursodeoxycholic acid may represent more than 50% of total bile acids, and tauroursodeoxycholic usually less than 10%, we consider glycoursodeoxycholic acid as the one having the highest clinical relevance in people taking ursodeoxycholic acid. However, its mechanisms of action have been less explored than the free and the taurine conjugated species. In this overview, the biological properties of glycoursodeoxycholic acid are highlighted and their mechanisms of action compared with ursodeoxycholic acid and tauroursodeoxycholic acid. Recent studies have demonstrated that such bile acids have unexpected efficacy in the treatment of certain neurodegenerative diseases, opening up the range of opportunities for their therapeutic use. Therefore, we additionally summarize current knowledge on the potential applications of such bile acids in the prevention and recovery of diseases associated to central nervous system (CNS) dysfunction and pathology. Forthcoming studies will hopefully better elucidate the benefits of glycoursodeoxycholic acid over those of ursodeoxycholic acid and tauroursodeoxycholic acid for the treatment of age-associated neurodegenerative diseases, a major public health issue and a challenge to the health care system.

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Keywords: Bile acids, Bile acid physiology, Blood-brain barrier, Cytoprotection, Endothelial cells, Hepatobiliary disorders, Neurodegenerative diseases, Neurogenesis.

INTRODUCTION

Bile acids (BAs) are physiological detergents that derive from cholesterol (Fig. **1**) and are synthesized in human liver at a rate of 0.2-0.6 g per day (averaging 0.5 g). BAs are implicated in generating bile flow to facilitate intestinal absorption and transport of nutrients, fats and vitamins [1]. Once BA biosynthesis is completed, with the generation of the primary BAs, cholic acid (CA, 3α,7α,12α-trihydroxy-5β-cholan-24-oic acid) and chenodeoxycholic acid (CDCA, 3α,7α-dihydroxy- 5β-cholan-24-oic acid), there is conjugation with the amino acids taurine (tauroconjugates) and glycine (glycoconjugates) in the liver. The BAs are then excreted into the bile canaliculi through the canalicular protein bile-salt export pump (BSEP) [2, 3] (First metabolic pump system, step 1 in Fig. (**2**)). Bile enters the gallbladder where it is concentrated and stored, before its release into the intestine after meals, to participate in fat digestion and absorption (reviewed in [4]). The bacterial flora converts the primary BAs into the secondary BAs deoxycholic acid (DCA) from CA, and lithocholic acid (LCA) from CDCA, by the action of the enzyme 7-α-dehydroxylase. These BAs are efficiently reabsorbed entering in the enterohepatic circulation (Second metabolic pump system, step 2 in Fig. (**2**)) and being transported to the liver (step 3) by portal blood, where they join the primary BAs, before being conjugated and re-excreted into the bile [5]. This recycling is known as the enterohepatic circulation of BAs and can occur several times per day [3]. In addition, BAs that arrive into the colon can be deconjugated by bacterial enzymes and originate the free BAs species that are excreted in the faeces (5% of total BAs).

In humans, the bile acid pool is formed by the primary and secondary BAs. In human serum, the total BA concentration is usually between 2 and 10 μmol/L [6 - 8] and bile representation of CA, CDCA and DCA is approximately 40:40:20, respectively [5]. However, there are variations by ageing since the BA synthesis and bile flow decreases markedly in the elderly [8].

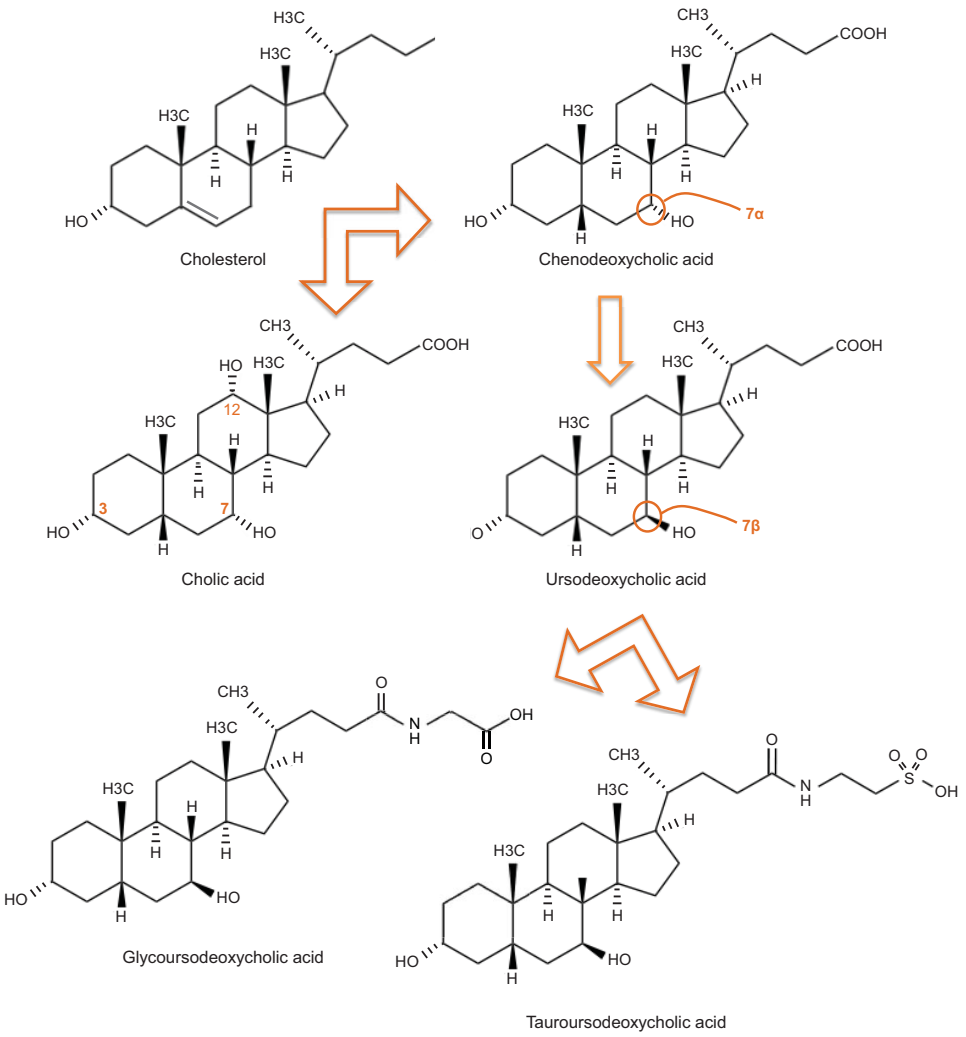

Fig. (1). Chemical structures of the primary bile acids (cholic and chenodeoxycholic acids) synthesized from the cholesterol in the liver. The formation of ursodeoxycholic acid, the 7 beta-hydroxy epimer of chenodeoxycholic acid, is produced in the colon by bacterial transformation. The conjugation of bile acids with glycine and taurine occurs in the liver, as the formation of glycoursodeoxycholic acid and tauroursodeoxycholic acid from the ursodeoxycholic acid.

Cholestasis is an impairment of bile formation/flow at the level of the hepatocyte and/or cholangiocyte. One of the most used drugs in the treatment of cholestasis is the ursodeoxycholic acid (UDCA), with the systematic (IUPAC) name of 3α,7β-dihydroxy-5β-cholan-24-oic acid (Fig. **1**). It is a secondary BA with hydrophilic properties, formed by 7β-epimerization of the CDCA in the gut by intestinal bacteria [9]. UDCA exists in low quantity in human bile (about 3% of the bile acid pool) [10], but in high amount in the Chinese black bear [11]. It has been

CHAPTER 13

Bioactive Compounds and Prostate Cancer Therapy

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Abstract: Prostate cancer (PCa) is the most common solid neoplasm and is one of the major causes of cancer mortality among men. Recent studies have shown the beneficial effect of some nutrients in PCa management. Natural phytochemicals often have pleiotropic effects, targeting virtually every molecular signaling pathway, which makes them promising agents for multi-target anti-cancer therapy. Furthermore, they are usually well-tolerated, easily available and cost-efficient. This chapter describes the variety of naturally occurring bioactive compounds used for the treatment and prevention of prostate cancer, especially focusing on dietary polyphenols and their analogs, including green tea polyphenols, resveratrol and curcuminoids, vitamins, organosulfur compounds from onions and garlic (predominantly allyl derivatives) and phytochemicals from cruciferous vegetables. While some of these compounds (*e.g.* curcumin and sulforaphane) have established anticarcinogenic properties, the others provide contradictory results in preclinical and clinical studies. Selenium and vitamin E supplementation is the most prominent case of such controversy. In this chapter, we cover molecular mechanisms that lead to PCa progression, as well as key mechanisms of antitumor action. Impact of different nutrients on cancer-associated epigenetic modifications is analyzed. Results of epidemiological studies and dietary interventions conducted to date are also provided. Main challenges associated with design and interpretation of such interventions are discussed, together with major issues of developing naturally occurring compounds into clinically used agents. While a promising scientific area, further research is required to completely elucidate the relationship between nutrients and PCa.

Keywords: Bioactive compounds, Dietary phytochemical, Minerals, Polyphenols, Prostate cancer, Therapy, Vitamins.

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M. Victorina Aguilar and Cristina Otero (Eds.)

INTRODUCTION

Epidemiology of Prostate Cancer

Prostate cancer, usually referred to as PCa, is the most common solid neoplasm and is also one of the major causes of cancer mortality among men. Although PCa is widespread, its incidence is the highest in Europe, North America and Oceania and the lowest in South-East Asia [1, 2]. Different factors may affect one's probability of developing PCa, including age, race, ethnicity, level of sun exposure, *etc*. We strongly encourage the reader to take a look at detailed assessment of prostate cancer epidemiology and risk factors in [3].

Molecular Foundations of Prostate Cancer

Cancer is a very complex disease, which is usually associated with an array of deregulated molecular signaling pathways, including PI3K/Akt/mTOR, NFκB, Notch, Wnt, Ras/Raf, cAMP, *etc*. Moreover, the population of cancer cells within one tumor is heterogeneous, which means that targeting one single pathway may be insufficient for effective therapy. In addition, the recent research shows the ability of cancer cells to switch from their main survival pathway to an alternative when the main pathway is inhibited. Besides, the more closely these pathways are related, the easier it is for a cell to switch from one to another [4]. All this information leads us to a conclusion that a multi-targeted therapy inhibiting several closely related molecular pathways is needed to achieve optimal results. This can be attained by administering either several single-targeted drugs or one multi-targeted. Current therapies usually consist of a combination of single-targeted drugs that is one of the reasons for severe adverse effects and toxicity. That is why dietary agents, which are naturally multi-targeted and non-toxic, are promising drug candidates and supplements.

Growing evidence suggests that the occurrence and progression of cancer is strongly dependent not only on genome, but also on epigenome and on their interaction. While genetic alterations are non-modifiable, some of the epigenetic modifications can be reversed. These modifications include DNA methylation and modifications of histones and small non-coding RNAs (Table **1**).

DNA methylation is an addition of a methyl group to pyrimidine ring of cytosine in CG pair. Normally, CpG sites are sparsely located in intergenic regions and repetitive sequences and are often methylated. Hypomethylation of such sites makes chromatin less densely packaged and so transcription of the DNA may occur. It can also result in the loss of imprinting control or demethylation of

normally silenced promoters. Hypomethylation of the repetitive sequences and transposons may lead to the DNA instability and breakage. Unlike intergenic regions, promoter sites of the genes often have CpG dense regions (CpG islands), and their cytosine moieties are usually unmethylated to assure transcription. Hypermethylation of CpG islands located in promoter regions of tumor suppressor genes (TSGs) and DNA repair genes can lead to the gene silencing and thereby account for numerous cancers. Inhibiting DNA methyl transferases (DNMTs), particularly DNMT3b, during cancer therapy may help demethylate TSG promoters and therefore reactivate TSGs.

Table 1. Modifiable and non-modifiable factors influencing epigenetic modifications.

Modifiable	**Non-modifiable**
Nutrition	Ethnicity
Smoking	Inheritance
Alcohol intake	Age
Physical activity	Gender
Exposure to toxins/radiation	

Histone tails can undergo post-transcriptional modifications, such as mono-, di-, tri-methylation, acetylation, ubiquitylation, phosphorylation and ribosylation, that activate or inactivate corresponding chromatin region, therefore altering expression level of the genes within that region, sometimes leading to cancer. For instance, in cancerous prostate tissues increased H3K4diMe modification has been associated with activation of genes involved in cell proliferation and therefore with risk of tumor recurrence. However, it is rarely one alteration that affects gene expression, usually it is a combination of specific modifications. Normally, histone methylation leads to the chromatin condensation and gene suppression, while histone acetylation usually result in open chromatin structure and gene expression. Histone deacetylases (HDACs) remove acetyl groups from histones and other proteins, notably transcription factors and DNA repair enzymes.

Small non-coding RNAs consist of microRNA (miRNA), piwi-interacting RNA, small-interfering RNA and small nucleolar RNA. Together they regulate gene expression in at least 30% of human genes by several mechanisms, notably inhibition of translation and heterochromatin formation. Genes encoding miRNAs can be subjected to both DNA methylation and histone modifications and so miRNAs can be silenced. Alterations in miRNA expression are associated with different types of cancer. For instance, in prostate cancer cells promoters of

CHAPTER 14

Health Effects and Risks of Caffeine, Theobromine and Theophylline

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Abstract: Methylxanthines, namely caffeine, theobromine and theophylline are found in several beverages and food products such as coffee, cocoa, tea and cola drinks. These substances can provide benefits to humans but also prove harmful mainly when consumed in high amounts. However, the beneficial and deleterious effects of methylxanthines are controversial in research findings, largely because of inconsistencies within research conducted and factors associated within studies. For instance, some authors have reported methylxanthines could have potential antioxidant activity under physiological conditions but opposing studies do not support these claims. Factors that influence the effects of methylxanthines include age, gender and health status of the individual. In addition to these, other factors such as doses, consumption of drugs or pharmaceuticals, alcohol and tobacco habits and diet also play a role in methylxanthine effects and metabolism. Therefore, our review is to provide a clearer understanding of the beneficial/adverse effects of methylxanthines in humans. A description of the physiological and toxicological effects of these methylxanthines is provided.

Keywords: Bioactive compounds, Caffeine, Human health and risks, Methylxanthines, Theobromine, Theophylline.

INTRODUCTION

Xanthine and its derivatives belong to the purine compound family and include numerous compounds that are naturally found in plants and animals. They are intermediates in the production of the guanosines in cells: guanosine monophosphate (GMP); guanosine diphosphate (GDP) and guanosine triphosphate (GTP); and they play a role in the catabolism of nucleotides and nucleic acids. Coffee, cocoa, tea and cola drinks can include biologically active derivatives of xanthines known as methylxanthines: caffeine (1,3,7-trimethyl-

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M. Victorina Aguilar and Cristina Otero (Eds.)

xanthine); theophylline (1,3-dimethylxanthine) and theobromine (3,7-dimethylxanthine). Methylxanthines are present in drinks and food products in different concentrations. Coffee only contains caffeine, the main methylxanthine in cocoa is theobromine, and tea presents decreasing concentrations of caffeine, theobromine, and theophylline [1 - 5]. Tables **1** and **2** provide a description of the methylxanthine content in different beverages and food products.

Table 1. Content of caffeine in several beverages (Deshpande, 2002) [4].

Beverage	Caffeine (mg/cup)
Prepared coffee	90-500
Instant coffee	60-100
Decaffeinated coffee	1-4
Tea	60-75
Cola drinks	40-60

Methylxanthines could also be classified as purine alkaloids and are widely consumed by every segment of the population. However, there is a lack of current knowledge in the total daily methylxanthine intake in countries in the European Union, such as in Spain and in the UK. The introduction of these substances in general food composition databases for nutritional and clinical purposes will fill this void and will also enhance and promote the health of the population through the possibility of the implementation of intervention and epidemiological studies as suggested by Fitt *et al.* [6].

Caffeine, theobromine and theophylline present numerous health benefits for humans but also adverse effects. Pharmacological effects of these methylxanthines include enhancing mood and concentration levels by stimulation of the central nervous system, diuresis, cardiovascular and metabolic effects, as well as bronchial relaxation to name a few. Methylxanthines do not produce chemical dependence in humans [2]. Generally, the toxicity of methylxanthines is very low if consumed from natural sources, although caffeine may be toxic when it is consumed in dietary supplements enriched in caffeine or when combined with drugs of abuse such as alcohol [7]. However, humans may also be exposed exposed to high amounts of methylxanthines as these compounds can be chemically synthesised and used in a variety of food products and beverages as well as for the treatment of serious illnesses. For example, the use of theophylline as a pharmaceutical drug for the treatment of asthma has shown toxicity in several studies which is discussed below.

Moreover, there are new trends and habits in our societies that are enhancing the intake of methylxanthines, such as the consumption of caffeine-containing "energy drinks". These products have become popular as it is considered that these caffeine-enriched products may "improve the performance" or "stimulate the metabolism" in the consumer. However, as it is comprehensively discussed in this chapter, the consumption of high doses of caffeine could have deleterious effects on human health. The content of caffeine in several energy and soft drinks is also provided in Table **3**.

Table 2. Content of methylxanthines in different cocoa products (Franco *et al.* 2013).

Product	Caffeine (mg/kg)	Theobromine (mg/kg)	Theophylline (mg/kg)
Raw ground paste	5600	33,000	200
Roasted ground paste	330	36,000	Below limit of detection
Baking chocolate	1580	10,040	Below limit of detection
Milk chocolate	56	1004	Below limit of detection
Dark chocolate	625-875	500-7500	Not determined

The main purpose of this chapter is to provide an overview of physiological and toxicological effects of caffeine, theobromine and theophylline on humans.

Physiological Effects of Methylxanthines

Methylxanthines have been demonstrated to have multifunctional physiological effects. However, in normal doses they will mainly affect the adenylyl cyclase pathway mediated by adenosine. In general, methylxanthines can improve lung function and produce bronchodilatation. These substances, particularly theophylline, are being used for the treatment of asthma and the treatment of premature infants with apnea of prematurity [2, 8].

The blood-brain barrier is permeable to methyxanthines. Thus, caffeine has psycho-stimulant properties and affects sleep. These substances do not affect blood pressure in healthy individuals but present different effects in the circulatory system. For example, it has been reported that the ingestion of theobromine through cocoa can have beneficial effects on the heart and the vessels [9].

In the kidney, natural methylxanthines can act as a diuretic increasing urine production, with theophylline the most active of all of them. The diuretic effect of these substances depends on several factors including age and health status of the individual. About 4 to 5 cups of coffee can cause diuresis, which is about 300 mg

CHAPTER 15

Effect of Cooking on the Bioactive Compounds

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Abstract: Consumption of vegetables, cereals and fish products are highly recommended by nutritionist pyramidal outliners since they are important for the proper intake of all food nutrients needed for a healthy diet. However, despite the already well known compounds such as vitamins, minerals, essential amino acids *etc.* which are essential because human body cannot synthetize them by itself, these food also contains bioactive compounds (carotenoids, glucosinolates, flavonoids and other phenolic compounds, dietary fibers, ω3-fatty acids, *etc.*) showing specific functionalities far more important than the classical nutrition because they reduce the risk of several diseases by showing health beneficial properties such as *i.e.* antioxidant, immunomodulatory, anticancerogenic, hypocholesterolemic, antimicrobial activities. However, the culinary methods utilized, during the domestic processing of those foods, modify not only their nutritional composition but also the concentration and availability of the bioactive compounds, being cooking one of the most influencing because of the high temperatures utilized. The media and the technology used might also affect positively or negatively, *i.e.*, the lower temperatures used during aqueous cooking are beneficial for carotenoids, dietary fibers and other compounds not soluble in water, but methods such as boiling induce leaching of water soluble compounds such as glucosinolates, phenols *etc*. Steam-pressure is detrimental for dietary fibers but reduces cooking times and osmotic processes making them suitable for the maintenance of water soluble compounds. Maillard reactions induced during dry heating or cooking using fats or oils as medium protect some compounds from leaching but destroy others because of the high temperatures generated. They also induce cross-linking impairing the proper digestion and absorption of formed complexes. The use of lipid medium during frying also modify the ω3/ ω6 ratio of products containing ω3-polyunsaturared fatty acids.

Keywords: Bioactive compound, Cooking technology, Nutritional value.

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M. Victorina Aguilar and Cristina Otero (Eds.)

INTRODUCTION

The use of fire to cook hunted animals and collected plants was a turning point not only for the development of culinary technologies but because it triggered human evolution and cultural development. Since then, culinary methodologies have widely evolved, but domestic processing still maintain its original basic structure. Domestic cooking was usually carried out using aqueous or lipid fluids as media or without any liquid but just dry heat. Later, combinations of these three methods and discovering of new processing devices broader the effect of cooking on the major food constituents modifying the content and bioavailability of many nutrient and biological compounds.

Vegetables and fish can be consumed raw but usually they are cooked. This thermal treatment induces beneficial effects on the food properties, such as improved palatability and nutrients bioavailability or shelf life extension. But processing also results in several changes modifying the content of health promoting phytochemicals like glucosinolates, flavonoids, carotenoids, dietary fibers, ω3-PUFAs *etc*. The relevance of these compounds for human consumption has been associated with a protective effect against oxidative processes in relation to cardiovascular, central nervous system and neurodegenerative diseases and with a reduced risk of cancers in tissues such as gastrointestinal tract, lung, colon, bladder, pancreas, skin, breast and prostate [1]. The scientific and social interest to enrich cereals, vegetables *etc.*, in these physiologically actives compounds to support a healthy diet, may provide a health benefit beyond basic nutrition (Regulation /EC/ No 1924/2006 [2];). Optimizing the composition of food and food products, would be a very cost-effective method for improving nutrition and disease prevention, since diet-induced health improvements would not carry any added costs for the health sector, even more it might help to reduce these costs [3].

COOKING TECHNOLOGIES USING WATER AND AQUEOUS FLUIDS

Domestic cooking involving an aqueous fluid as heat-transferring medium is conditioned by the physical properties of water *i.e.* it implies cooking temperatures close to its boiling point (100ºC at sea level), exclusive addition of water-soluble condiments (salts and other ions, sugar, spices *etc.*) or pH changes if acid or alkaline solutions are added. Moreover, if pressurized steam is used, it will modify its boiling temperature, cooking times, amount of energy transferred to the food, osmotic processes *etc*. Thus, depending of all possible variations within these parameters, the cooking methodologies will have a different name

such as blanching, boiling, steaming, pressure cooking *etc.*, and they will differently influence the extractability, the degradation level and the biological properties of many of the bioactive compounds present in foods. Moreover, the food matrix in which the compounds are integrated will also influence differently their degree of transformation since they will not be similarly affected if the food matrix is rich in proteins, carbohydrates or lipids because heat also differently modifies the structure of these other molecules.

Moist heat will also individually influence each type of compound within a class depending on their specific radical groups, presence of double bounds, structural conformation *etc.*, for instance carotenoids are yellow to red pigments with a symmetrical tetraterpene skeleton as basic structure that might suffer hydrogenation, cyclation, oxidation *etc.* yielding a wide range of derivatives [4]. Carotenoids are considered as bioactive compounds mainly because of their antioxidant properties. They are excellent lipid antioxidants because they can act following two mechanisms. On the one hand, they can interrupt the reactions chain occurring during lipid autoxidation acting as radical trapping antioxidants. Even more, they can extinguish active singlet O_2 before causing damages. Carotenoids are also involved in the skin photoprotection by reducing the immunosuppression caused by UV, activate natural killer cells and enhance intercellular communication through the gap junctions. They also protect from degenerative diseases such as AMD (age-related macular degeneration) and cancer. The all-*trans*-β-carotene is the most biologically active form of β-carotene and blanching or short boiling procedures induce isomerization to *cis*-isoforms (Fig. **1**) a transformation that can be visually detected by the color change from orange to a more yellowish discolouration but, *cis*-configuration is more stable to heat temperature. Since they are lipid compounds, they are less affected by aqueous media than other more water-soluble compounds *i.e.* phenols or ascorbic acid because almost no osmotic influence or leaching processes might take place [5]. Although, further increase in the cooking temperature induce epoxide formation and further degradation. However, the low cooking temperatures utilized during boiling (compared to those used for frying or baking) usually preserve most of carotenoids from vegetables [6, 7] and increase their extractability. That is the reason for the increasing levels of carotenoids noticed in several vegetables after boiling or steaming such as broccoli and cauliflower. Cryptoxanthin concentration was found 26% higher after boiling than in the raw vegetable. After steaming and boiling respect. 26% and 24% more zeaxanthin was noticed as well as increasing levels of lutein, α- and β-carotene compared to raw broccoli or cauliflower [8].

SUBJECT INDEX

A

B

C

D

E

F

G

H

I

K

L

M

N

O

P

Q

R

S

T

U

V

X

It is really an interesting comprehensive book on bioactive compounds. It covers different aspects of the bioactive compounds from the extraction, identification and quantization of different types of structures to the study of bioavailability, including the effect of cooking on the bioactive compounds. In addition it includes chapters on the relationship between bioactive compounds and health disorders such as diabetes, metabolic syndrome and cardiovascular diseases among others. It is, therefore a book worth to be recommended.

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She organizes/teaches courses of high specialization about synthesis and characterization of bioactive products, and biodiesel; characterization of materials; and applied biocatalysis. She is inventor of several patents, author of several hundred of scientific publications in scientific international journals and books, supervisor of Doctoral Thesis and Masters, and undergraduated and postdoctoral scientists. She does consulting services for private companies and laboratories.