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FRONTIERS IN BIOACTIVE COMPOUNDS

Volume 1

NATURAL SOURCES, PHYSICOCHEMICAL CHARACTERIZATION AND APPLICATIONS

Editor:
Constantin Apetrei

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Frontiers in Bioactive Compounds

(Vol. 1)

Natural Sources, Physicochemical Characterization and Applications

Edited by:

Constantin Apetrei

Department of Chemistry, Physics and Environment
Faculty of Sciences and Environment
"Dunarea de Jos" University of Galati
47 Domneasca Street
800008 Galati, Romania

Frontiers in Bioactive compounds

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FOREWORD

The field of bioactive compounds is very important for both scientific and applicative point of view. This field is interdisciplinary and in this book the authors propose several themes related to chemical composition, sensors, biosensors, electronic sensory systems, interaction modeling and protection methods applied on bioactive compounds. Therefore, the authors propose an integrated approach regarding bioactive compounds including sources, physicochemical detection, interactions with membranes and the protection of bioactive components against environmental factors.

This integrated approach is not common in books but I believe that is very useful for specialists and researchers working in inter-, multi- and trans-disciplinary fields related to bioactive compounds. The book could be of interest for scientists specialized on chemistry, biochemistry, pharmacy as well as for persons working in food industry, biotechnology, nanotechnology etc. This book will be helpful for a wide range of readers - from university students to researchers.

I think that the authors can be confident that there will be many grateful readers who will have gained a broader perspective of the fields of bioactive compounds as a result of their efforts.

Dr. Eleonora-Mihaela UNGUREANU
Department of Inorganic Chemistry
Physical Chemistry and Electrochemistry
Faculty of Applied Chemistry and Materials Science
Polytechnic University of Bucharest
Romania

PREFACE

This book presents the composition and health benefices of bioactive compounds from natural products, and the principal methods used for separation, detection, quantification and protection of bioactive compounds. A special attention is devoted to membrane model interactions as well as to sensors and biosensors development.

The book is organized in the following way:

Chapter 1 deals with the description of chemical composition of olive oil, highlighting the chemical structure and health benefices of biologic active compounds present in this food.

Chapter 2 describes the composition, chemical structures and benefices of bioactive compounds from red wine. A special attention is devoted to polyphenolic compounds, especially to resveratrol, a compound with multiple benefices and without toxic effects.

Chapter 3 presents the recent advances in the analysis of bioactive compounds based on molecular recognition. Separation techniques (solid phase extraction, chromatography, electrophoresis), and sensing (electrochemical, optical, *etc.*) are revised.

Chapter 4 describes the electrochemical sensors, especially voltammetric sensors, dedicated to the analysis of antioxidants. The principal methods used to improve voltammetric sensors selectivity and sensitivity by modifying the surface using chemical or biological materials are described, also.

Chapter 5 presents the most representative and novel biosensors for the detection of polyphenols, biogenic amines, mycotoxins and allergens in wine, emphasizing the wealth of biosensing strategies and the particularities of wine as a complex analytical matrix.

Chapter 6 provides a review of the most recent works in the field of electronic sensory system for characterization of bioactive compounds, from electronic noses to sensors and biosensors.

Chapter 7 describes the use of Langmuir monolayers as cell membrane models made to interact with bioactive compounds. Trends in the field are discussed as motivation for innovative research to bridge the gap between biology (and medicine) and the physicochemical framework used in cell membrane modeling.

The book closes with Chapter 8 presenting the newest information about the bioactive compounds encapsulation, which ensures the stability of the volatile compounds during thermal processing, the protection of bioactive components against environmental factors, the increase of the solubility of bioactive compounds, and the controlled release of bioactive components.

I appreciatively acknowledge the distinguished specialists who contributed in this book. I consider myself privileged to have had the occasion to work together with colleagues of universities from Spain, Italy, Brazil and Romania. Finally, I want to thank Bentham Science Publishers for the opportunity to edit this book.

Dr. Constantin APETREI
Department of Chemistry, Physics and Environment
Faculty of Sciences and Environment
"Dunarea de Jos" University of Galati
47 Domneasca Street
800008 Galati, Romania
Phone: +40-0236460328
Fax: +40-0236461353
E-mail: apetreic@ugal.ro

List of Contributors

Alina Vasilescu	International Centre of Biodynamics, 1B Intrarea Portocalelor, sector 6, 060101, Bucharest, Romania
Bernardo C. de Gennaro	Dipartimento di Scienze Agro Ambientali e Territoriali, Università di Bari, *via* Orabona 4, Bari, Italy
Bogdan Bucur	Centre of Bioanalysis, National Institute of Research and Development for Biological Sciences, 296, Splaiul Independentei, Bucharest, Romania
Carlo Franchini	Dipartimento di Farmacia - Scienze del Farmaco, Università di Bari, *via* Orabona 4, Bari, Italy
Cecilia Cristea	Analytical Chemistry Department, "Iuliu Hațieganu", University of Medicine and Pharmacy, Cluj-Napoca, Romania
Celia García-Hernández	Department of Inorganic Chemistry, Engineers School, Universidad de Valladolid, Valladolid, Spain
Constantin Apetrei	Department of Chemistry, Physics, and Environment, "Dunarea de Jos", University of Galati, 47 Domneasca Street, Galati, Romania
Cristian Dima	Faculty of Food Science and Engineering, "Dunarea de Jos", University of Galati, Romania
Cristina García-Cabezón	Department of Materials Science, Engineers School, Universidad de Valladolid, Valladolid, Spain
Cristina Medina-Plaza	Department of Inorganic Chemistry, Engineers School, Universidad de Valladolid, Valladolid, Spain
Daniel Matatagui	Centro de Ciencias Aplicadas y Desarrollo Tecnológico - UNAM, México D.F
David Paniagua-Albillos	Department of Inorganic Chemistry, Engineers School, Universidad de Valladolid, Valladolid, Spain
Ede Bodoki	Analytical Chemistry Department, "Iuliu Hațieganu", University of Medicine and Pharmacy, Cluj-Napoca, Romania
Felippe J. Pavinatto	São Carlos Institute of Physics, University of São Paulo, São Carlos, São Paulo, Brasil
Filomena Corbo	Dipartimento di Farmacia - Scienze del Farmaco, Università di Bari, *via* Orabona 4, Bari, Italy
Ioana Silvia Hosu	National Institute for R&D in Chemistry and Petrochemistry, Splaiul. Independentei 202, Bucharest, Romania Institute of Electronics, Microelectronics and Nanotechnology (IEMN), UMR-CNRS 8520, Université Lille 1, Avenue Poincaré—BP 60069, Villeneuve d'Ascq, France

Jesús Lozano	Escuela de Ingenierías Industriales, Universidad de Extremadura, Badajoz, Spain
José Antonio de Saja-Sáez	Department of Condensed Matter Physics, Faculty of Sciences, Universidad de Valladolid, Valladolid, Spain
José Pedro Santos	GRIDSEN, Instituto de Tecnologías Físicas y de la Información (ITEFI-CSIC), Madrid, Spain
Luciano Caseli	Institute of Environmental, Chemical, and Pharmaceutical Sciences, Federal University of São Paulo, Diadema, São Paulo, Brasil
Ma Carmen Horrillo	GRIDSEN, Instituto de Tecnologías Físicas y de la Información (ITEFI-CSIC), Madrid, Spain
Maria Lisa Clodoveo	Dipartimento di Scienze Agro Ambientali e Territoriali, Università di Bari, *via* Orabona 4, Bari, Italy
María Luz Rodríguez-Méndez	Department of Inorganic Chemistry, Engineers School, Universidad de Valladolid, Valladolid, Spain
Osvaldo N. Oliveira Jr	São Carlos Institute of Physics, University of São Paulo, São Carlos, São Paulo, Brasil
Pasquale Crupi	Dipartimento di Scienze Agro Ambientali e Territoriali, Università di Bari, *via* Orabona 4, Bari, Italy
Radu Oprean	Analytical Chemistry Department, “Iuliu Haţieganu”, University of Medicine and Pharmacy, Cluj-Napoca, Romania
Robert Săndulescu	Analytical Chemistry Department, “Iuliu Haţieganu”, University of Medicine and Pharmacy, Cluj-Napoca, Romania
Silvia Rodríguez-Sánchez	Department of Inorganic Chemistry, Engineers School, Universidad de Valladolid, Valladolid, Spain
Stefan Dima	Department of Chemistry, Physics and Environment, Faculty of Sciences and Environment, “Dunarea de Jos”, University of Galati, Romania
Thatyane M. Nobre	São Carlos Institute of Physics, University of São Paulo, São Carlos, São Paulo, Brasil
Tiziana Dipalmo	Dipartimento di Scienze Agro Ambientali e Territoriali, Università di Bari, *via* Orabona 4, Bari, Italy

CHAPTER 1

Extra Virgin Olive Oils: Bioactive Compounds and Health Benefits

Maria Lisa Clodoveo[1,*], Tiziana Dipalmo[1], Pasquale Crupi[1], Bernardo C. de Gennaro[1], Carlo Franchini[2], Filomena Corbo[2] and **Constantin Apetrei[3]**

[1] *Dipartimento di Scienze Agro Ambientali e Territoriali, Università di Bari, via Orabona 4, 70126 Bari-Italy*

[2] *Dipartimento di Farmacia, Scienze del Farmaco- Università di Bari, via Orabona 4, 70126 Bari-Italy*

[3] *Department of Chemistry, Physics, and Environment, "Dunarea de Jos" University of Galati, 47 Domneasca Street, 800008 Galati-Romania*

Abstract: Due to the unique extraction process, extra virgin olive oils conserve numerous substances, which are often excluded from other vegetable oils during the extraction and refining steps. Previous works showed that fatty acids profile of lipids are important for the dietary. In the last five decades many researchers demonstrated that monounsaturated fatty acids are involved in prevention of diseases such as the metabolic syndrome and cardiovascular ones. Moreover, vitamin E is active against weakened immunity, aging, environmental toxic substances, diabetic syndrome symptoms, enhancing the health status from different points of view, such as cardiovascular and neoplastic ones. Hydroxytyrosol has many effects; it is an anti-inflammatory compound, with antibacterial, antioxidant and cardioprotective properties. However, not all extra virgin olive oils are equal. Inside the market category "extra virgin" there is a wide number of products ranging from the highest quality to the standard quality ones. In 2012 the European Commission approved three health claim applicable to the extra virgin olive oil but they are still scarcely used by the producers.

Keywords: Bioactive compounds, Extra virgin olive oils, Health claim.

[*] **Corresponding author Maria Lisa Clodoveo:** Department of Agricultural and Environmental Science, University of Bari, Italy; E-mail: marialisa.clodoveo@uniba.it

Constantin Apetrei (Ed.)

INTRODUCTION

The Mediterranean Diet is inscribed in 2010 on the "Representative List of the Intangible Cultural Heritage of Humanity". This is a lifestyle, which has its roots in antiquity. It is a legacy of very ancient traditions having been part of the life of those countries bordering the Mediterranean Sea. According to many authors, in fact, people who follow the principles of this diet get various health benefits, preventing heart attacks and strokes. Actually this regimen embraces natural and healthy food in balance with each other, such as cereals and their by-products, vegetables, fresh and dried fruit, wine, meats and low-fat cheese, and of course the "Prince" of all: Extra Virgin Olive Oil (EVOO). As reported by the European Regulation, EVOOs are "the oils obtained from the fruit of the olive tree (*Olea europaea* L.) (Fig. **1**) solely by mechanical or other physical means under conditions, particularly thermal conditions, that do not lead to alterations in the oil, and which have not undergone any treatment other than washing, decantation, centrifugation and filtration".

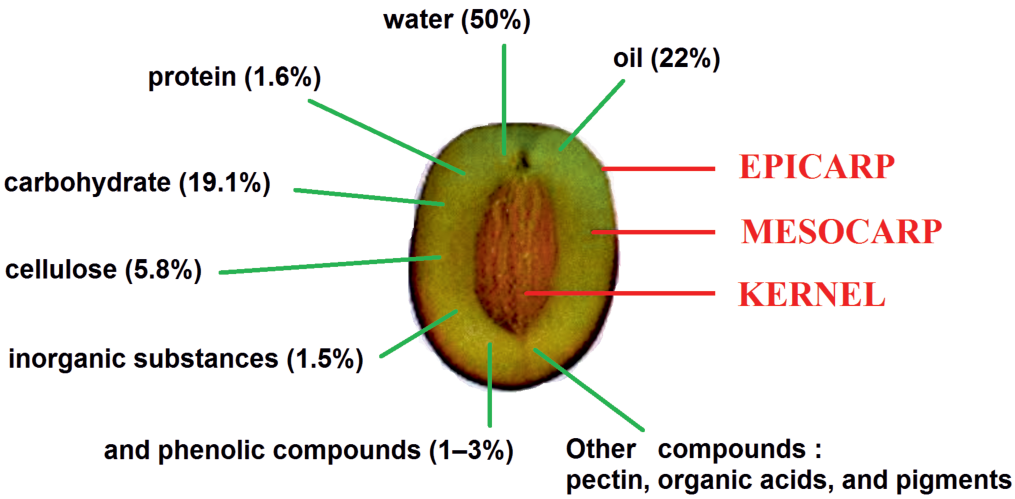

Fig. (1). The fruit of olive component (the epicarp or epidermis, the mesocarp or flesh and the endocarp or kernel, composed by a woody shell enclosing a seed) and average composition.

The Olive Fruit

The olive fruit, an oval-shaped drupe, is composed by epicarp, mesocarp and endocarp; the epicarp changes the color from green to black during the maturation stage, the mesocarp represents about 87% of the whole fruit and the hard endocarp or stone contains the seed or kernel. It represents about the 23% of fruit mass.

The seed contains 2–4 g oil /100 g. Olive fruit weight may range from 2–12 g, although some varieties may weigh as much as 20 g [1]. The chemical composition of resulting olive oil depends on parameters including (i) cultivar, (ii) agronomic procedures, (iii) geographical area, and the (iv) maturity index. Variety, territorial areas and agronomic practices have a heavy effect on the fruit development. The extraction methods and storage conditions can modify oil composition [2].

The Olive Oil

Due to the unique extraction process, extra virgin olive oils conserve numerous substances, which are often excluded from other vegetable oils during the extraction and refining steps. The "unsaponifiable fraction" is composed by more than 200 minor components and represents about 2% of the total mass [3]. This fractions is composed by chemical substances that show a high value from the nutritional and healthy point of view. Moreover, they contribute to enhance the shelf life and the sensory properties of olive oil, useful to identify adulteration. This fraction includes:

- tocopherols,
- sterols,
- pigments (carotenoids and chlorophylls),
- phenols,
- hydrocarbons,
- aromatic and aliphatic alcohols,
- triterpene acids,
- waxes and minor constituents.

The composition of "unsaponifiable fraction" depends from many factors, such as: (i) the variety, (ii) the maturity index, (iii) the crop year (alternate bearing: "on year" and "off year"), (iv) olive harvesting methods, (v) the storage time of fruits,

CHAPTER 2

Wine: Biologic Active Compounds and Health Benefits

Constantin Apetrei*

Department of Chemistry, Physics and Environment, Faculty of Sciences and Environment, "Dunarea de Jos" University of Galati, Romania

Abstract: Wine contains several hundreds of compounds, most of them with impact on quality attributes and health benefices. Among these compounds, phenolic compounds play a significant role in sensory properties of wines as well as in antioxidant properties. Phenolic compounds are highly efficient compounds displaying antioxidant properties and preventing human cardiovascular diseases, also. Furthermore, the polyphenolic compounds are capable of quenching free radicals, thereby inhibiting the oxidation process of low density lipids (LDL). This effect about LDL could reduce the risk of atherosclerosis, cardiovascular diseases, heart attack, cancer, DNA degradation *etc*. Moderate drinking of red wine is beneficial against coronary disease and several forms of cancer. The phenolic compounds thought to be responsible are catechins and tannins. Catechins are powerful antioxidants, preventing cellular damage. Resveratrol and quercetin may improve the immune system, prevent towards heart diseases, inhibit cancer formation, and even prolong life. The amino acids are essential nutrients and have various benefits on human body such as muscle maintenance, tissue repair, and immunity. Amino acids yield biogenic amines that have allergenic or toxic effects on humans. Beneficial nitrogen compounds such as melatonin and serotonin are also found in red wines. Among vitamins, only vitamins B_1, B_2 and B_{12} are present in wines.

Keywords: Antioxidant, Catechin, Free radical, Polyphenols, Resveratrol, Wine.

* **Corresponding author Constantin Apetrei:** Department of Chemistry, Physics and Environment, Faculty of Sciences and Environment, "Dunarea de Jos" University of Galati, 47 Domneasca Street, 800008 Galati, Romania; E-mail: apetreic@ugal.ro

INTRODUCTION

A definition of “wine” is “the drink resulting from the fermentation by the yeast-cells, and also in certain cases by the cells of lactic bacteria, of the juice from the crushing or maceration of grape-cells” [1].

Wine is a natural multicomponent alcoholic beverage, which includes various bioactive compounds. The chemical composition is related to different factors, among them could be highlighted quality of grape, winemaking process, storage and ageing of wine, transport and storage of bottles *etc* [2 - 5].

Wine compounds include water, alcohols, acids, sugars, phenolic compounds, nitrogen containing compounds, vitamins and different aroma compounds [2 - 5]. The phenolic compounds from grape and oak barrels are a huge group of biologic active compounds with vital functions in winemaking [2 - 8]. Sensory characteristics of wine, for example the taste (sweet, sour, salty, bitter and *umami*), astringency, and colour are related to several compounds, the phenolic compounds being the most important [9 - 12]. Excellent antioxidant properties of wine is often correlated with principal health benefices [13 - 16]. A reasonable drinking of red wine is recommended and it is part of Mediterranean diet [17, 18].

PHENOLIC COMPOUNDS

Phenolic compounds are secondary metabolites present in the vegetable kingdom [19 - 22]. Phenolics have a similar structure involving a benzene ring substituted with one or more hydroxyl groups [23, 24].

Phenolics are produced from one amino acid phenylalanine. The phenylpropanoid biosynthetic pathway is recognized as the principal one. In plants, it have been identified more than 4,000 different phenolic compounds. Their function in plants is related to numerous functions: defence from UV radiation, defence against some pathogens, pigmentation, attraction of pollinators, and spreading of seeds [25 - 27].

White wines contains basically phenolic compounds from the flesh of the grapes, for instance gallic acid, hydroxynammic acid esters, catechin, epicatechin, gallocatechin gallate, procyanidin and catechin gallate. The phenolics amounts in white wines varies and usually they are in the range of few mg/L [28, 29]. In addition, red wines contains some special classes of phenolic compounds such as: flavonol-3 group (catechin), flavane (3,4)-diol group (leucocyanidin), flavonol-3 (quercetin), anthocyanins and tannins, in addition to compounds present in white wines [30 - 32].

Chemical structures of phenolic compounds comprise from aromatic based compounds with low molecular mass to polymeric, high molecular mass compounds such as tannins. There are two groups of phenolic compounds: flavonoids (having a common C6-C3-C6 skeleton) and non-flavonoids [33 - 35].

Classification and principal phenolic compounds found in grape and wine is summarized in Table **1**.

Table 1. Classification of phenolic compounds

Phenolic compounds	
Non- Flavonoids	Flavonoids
Phenolic acids - Hydroxybenzoic acids - Hydroxycinnamic acids Stilbenes	Flavonols Flavononols and flavones Flavanols - Catechins - Condensed Tannins - Procyanidins - Prodelphinidins Anthocyanins

Phenolic acids from wines include hydroxybenzoic acids and hydroxycinnamic acids [36 - 38]. Hydroxybenzoic acids have as principal characteristics a C6-C1 skeleton. The most frequent hydroxybenzoic acids (Fig. **1**), from wine are gallic acid, gentisic acid, p-hydroxybenzoic acid, protocatechuic acid, syringic acid, salicylic acid, and vanillic acid [39, 40].

Gallic acid is the hydroxybenzoic acid present in greater amounts in wine. It yields from the grape and from the hydrolysis of tannins [41, 42].

Hydroxycinnamic acids have as identity characteristics a C6-C3 skeleton. The different compounds present in wine are caffeic acid, caftaric acid, p-coumaric acid, p-coutaric acid, ferulic acid, fertaric acid and sinapic acid [43, 44].

CHAPTER 3

Recent Advances in the Analysis of Bioactive Compounds Based on Molecular Recognition

Robert Săndulescu*, **Cecilia Cristea, Ede Bodoki** and **Radu Oprean**

Analytical Chemistry Department, "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania

Abstract: Most of the biological processes, either normal or pathological, are based on molecular recognition. Molecular recognition, defined as the ability of one molecule (host) to "recognize" another molecule (guest), which presents complementarity, mainly through bonding interactions and molecular geometry, may be exploited in the analytical sciences, as long as the specific interaction produces a detectable change that can be measured by an instrument. The most impactful and acknowledged analytical use of molecular recognition has been recorded in separation sciences, namely in solid phase extraction and affinity chromatographic applications, but also in optical and electrochemical chemo- and biosensing. In living organisms the molecular recognition system is represented by natural receptors, characterized by the highest affinity and specificity, being the natural targets for toxins and mediators of physiological processes. By consequence, a large variety of bioelements, such enzymes, antibodies, nucleic acids, and cells were investigated in the last decades, being integrated in various separation and sensing devices which were successfully applied in biomedical and environmental analyses or monitoring. However, their isolation, purification, stability and cost are critical issues. Therefore, the development of artificial biomimetic receptors, such as cyclodextrins and derivatives, calixarenes, ligands (valinomycine, crown-ethers, *etc*.), aptamers, and molecularly imprinted polymers focused the attention of many research groups. The most recent achievements in the field of analytical sciences based on molecular recognition, such as separation techniques (solid phase extraction, chromatography, electrophoresis), and sensing (electrochemical, optical, *etc*.) are revised.

* **Corresponding author Robert Săndulescu**: Analytical Chemistry Department, Faculty of Pharmacy, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania; Email: rsandulescu@umfcluj.ro

Constantin Apetrei (Ed.)

Keywords: Antibodies, Aptamers, Biomimetic receptors, Calixarenes, Chiral discrimination, Cyclodextrins, DNA, Electrochemical sensors and biosensors, Guest-host interaction, Molecular recognition, Molecularly imprinted polymers, Separation methods.

MOLECULAR RECOGNITION

Most of the biological processes (normal and pathological), such as: enzyme metabolism, DNA-protein, RNA-ribosome, protein synthesis, active and passive ion transport across membranes (ion channels, ion pumps, ionophores) and receptor-substrate (antigen-antibody, sugar-lectin) binding are based on molecular recognition.

Molecular recognition is generally defined as the ability of one molecule (host) to "recognize" another molecule (guest), which presents complementarity, mainly through bonding interactions and molecular geometry. The effects of the molecular recognition, consisting in the specific interaction between host and guest molecules (such as, DNA hybridization, specific interactions between biotin and avidin or between protein A/G and antibody), through covalent (disulfide bridges between cysteine side chains) and non-covalent binding of various strength such as: hydrogen bonding, metal coordination, hydrophobic forces, van der Waals forces, π-π interactions, halogen bonding, electrostatic and electromagnetic effects, may be exploited in the analytical sciences, as long as the specific interaction produces a detectable change that can be measured by an instrument.

Some authors [1] distinguish between *static molecular recognition*, consisting in a complexation reaction between the specific recognition sites of the host molecule and a guest molecule to form a host-guest complex, and *dynamic molecular recognition*, which allow the control of the binding processes in biological systems. Thus, the binding of the first guest to the first binding site of the host affects the association constant of a second guest with a second binding site.

Perhaps the most impactful and acknowledged analytical use of molecular recognition has been recorded in separation sciences, namely in solid phase extraction based purifications of biomacromolecules and affinity chromatographic applications [2]. The advances of affinity chromatography have also stimulated immobilized ligand based methods to characterize and exploit molecular recognition, such as chromatographic separations but also optical and electrochemical chemo- and biosensing.

In spite of their outstanding sensitivity (in the range of 10^{-9}-10^{-12} M), the electrochemical methods are not selective, meaning that all the electroactive species from a given sample or matrix are non-specifically detected. Therefore, the design of specific or at least very selective interfaces between the analyte or the sample and the measuring systems was and still is the "hot aim" of a great number of research teams around the world. A great and various number of examples of such sensing entities is given by nature itself, the development and functioning of all biological systems being based on a multitude of "signal molecules" and the corresponding "natural receptors". Thus, many efforts were made in order to modify the electrode surface with an immobilized ligand in such a manner, to be able to distinguish a specific analyte in a multicomponent matrix. Due to the fact that "natural receptors" present the greatest selectivity or even specificity, a great number of papers about various sensors and biosensors were published in the last decades.

Molecular Recognition in Chiral Analysis

Differentiation between enantiomers is a fundamental phenomenon as chiral compounds interact in a stereospecific way with each other. Chiral recognition and differentiation in living organisms represents one of the most intriguing natural phenomena. This reflects the regulatory systems' (*i.e.* transporters, enzymes, receptors) high degree of selectivity, which assures in the organism a high fidelity transfer of the molecular information.

Chiral molecular recognition and enantio-differentiation can be considered as the highest form of molecular recognition, exploiting very small energetic differences between the forming transient complexes, therefore a great effort has been directed in the last decade in the understanding of the underlying fundamental mechanisms. The current state of knowledge will be briefly outlined in the following, however additional details on the mechanism of chiral separations can

CHAPTER 4

Electrochemical Sensors for the Detection of Antioxidants

María Luz Rodríguez-Méndez[1,*], **Celia García-Hernández**[1], **Cristina Medina-Plaza**[1], **Cristina García-Cabezón**[2], **David Paniagua-Albillos**[1], **Silvia Rodríguez-Sánchez**[1] and **José Antonio de Saja-Sáez**[3]

[1] *Department of Inorganic Chemistry, Engineers School, Universidad de Valladolid, 47011 Valladolid, Spain.*

[2] *Department of Materials Science, Engineers School, Universidad de Valladolid, 47011 Valladolid, Spain.*

[3] *Department of Condensed Matter Physics, Faculty of Sciences, Universidad de Valladolid, 47011 Valladolid, Spain*

Abstract: The Mediterranean diet has largely demonstrated its health benefits which are related to the intake of antioxidants. Besides the health benefits, antioxidants influence in the organoleptic characteristics, the quality and the preservation of foods and beverages.

Although a variety of methods have been described to detect antioxidants and to evaluate the antioxidant capacity of foods, field experiments still represent a challenge. In this context, electrochemical techniques can represent an advantage thanks to higher sensitivity, short experimental times and inherent portability. In this chapter, the electrochemical sensors dedicated to the analysis of antioxidants will be presented. Particular attention will be paid to voltammetric sensors. The methods used to improve their selectivity and sensitivity by modifying the surface using chemical or biological materials will be described. The new developments obtained thanks to new nanomaterials and nanostructured films will be evidenced. Finally, the advantages of multisensor systems based on electrochemical sensors will be described.

[*] **Corresponding author María Luz Rodríguez-Méndez:** Department of Inorganic Chemistry, Industrial Engineers School, Paseo del Cauce, 59, 47011, Valladolid, Spain; Tel: +34 983423540; Fax: +34 983423310; E-mail: mluz@eii.uva.es

Constantin Apetrei (Ed.)

Keywords: Antioxidant, Biosensor, Food, Langmuir-Blodgett, Nanoparticle, Phthalocyanine, Sensor.

INTRODUCTION

Over the last years the action of free radicals in living organisms has been extensively studied. Their destructive action can be reduced by antioxidants, a broad group of active biological substances which are widespread in the nature [1, 2]. An antioxidant is defined as a substance that, in contact with an oxidizable substrate, protects that substrate from oxidation, protecting then the organism from harmful effects of oxidative stress. It has been demonstrated that the consumption of antioxidants decreases the risk of cardiovascular diseases [2 - 4]. The main antioxidants in food include substances such as ascorbic acid (vitamin C), vitamin E and urate. In addition, other antioxidants are present in lower concentration such as beta-carotene, glutathione and a variety of polyphenols [5]. The chemical structure of polyphenols is specially adapted to their antioxidant action (hydrogen or electron donor, or capture of free radicals). Many polyphenols are better antioxidants than vitamin E or C.

The habitual and moderate consumption of fruits and vegetables such as oranges or grapes and beverages such as fruit juices or wines, can produce beneficial health effects due to their content of phenolic compounds, particularly quercetin and resveratrol. Epidemic studies have related the intake of polyphenols to a decrease of the coronary cardiopathy or the cancer risk in humans [6]. Polyphenols also affect the quality and organoleptic characteristic of foods and their contribution to the flavor is extremely important. They also contribute to the antioxidant capacity of foods. The contribution of each component to the global antioxidant capacity depends on its antioxidant power, its concentration and the interaction with other components [7].

Different methods have been developed to assess the antioxidant capacity of foods [8]. However, up to now, a single method has not been recognized as the most adequate, and the results obtained depend on the method used. The antioxidant capability of a complex mixture, is not only given by the addition of the antioxidant capacities of each one of its components; it also depends on the synergic and inhibitory effects of each compound with the rest. The methods to assess the antioxidant activity are usually based on the evaluation of the capability of an antioxidant or of a mixture of antioxidants to inhibit the oxidative damage caused by an oxidizing agent to a substrate. Any test for the evaluation of the antioxidant capacity needs an appropriate substrate to monitor the inhibition of the oxidation, an initiator of the oxidation (free radical) and an appropriate measure of the endpoint of the oxidation. This measure can be carried out by chemical, instrumental or sensorial methods.

When approaching the study of the antioxidant activity of foods and beverages, it has been recommended to use more than one method. The reason is that each method gives different information: certain antioxidants do not react with certain oxidizing species, but they do react with others. As a consequence different values can be obtained with different methods. The antioxidant activity can be evaluated by means of the measure of the absorbance capacity of the radical oxygen (ORAC) [9] and by means of the trolox equivalent antioxidant capacity (TEAC) (Trolox is a soluble analogue of vitamin E) [10]. On the other hand, the measure of phenols using the Folin-Ciocalteu method, based on a redox reaction, is also an accepted method to measure the antioxidant activity of foods in function of their Polyphenolic content [11, 12]. ORAC and TEAC are used to measure the antioxidant capacity, while Folin is an indicator of the antioxidant capacity in function of the global content of polyphenols in foods. Taking into account that the antioxidant activity of many foods and beverages is mainly related to the Polyphenolic content, this method is preferred by some authors. The evaluation of the total polyphenol index (TPI) measuring the absorbance at 280 nm is also recommended, because this method evaluates the reducing power of wines [13]. The knowledge of the phenolic profile of foods and beverages and their relation with the antioxidant capacity are of great interest to evaluate the modifications that foods can undergo. All these techniques have been developed for the analysis of foods at the laboratory level. They are not suitable for routine, on line industrial or field analysis because they require long periods of sample preparation, analysis and interpretation.

Natural antioxidants are usually electroactive compounds. This electroactivity can be exploited for electrochemical detection. Similarly, the antioxidant capacity can

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CHAPTER 5

Biosensors for Characterisation of Bioactive Compounds from Wines

Bogdan Bucur[a], **Ioana Silvia Hosu**[b,c] and **Alina Vasilescu**[d,*]

[a] *Centre of Bioanalysis, National Institute of Research and Development for Biological Sciences, 296, Splaiul Independentei, Bucharest 060031, Romania*

[b] *National Institute for R&D in Chemistry and Petrochemistry, Splaiul. Independentei 202, 060021, Bucharest, Romania.*

[c] *Institute of Electronics, Microelectronics and Nanotechnology (IEMN), UMR-CNRS 8520, Université Lille 1, Avenue Poincaré—BP 60069, 59655 Villeneuve d'Ascq, France*

[d] *International Centre of Biodynamics, 1B Intrarea Portocalelor, sector 6, 060101, Bucharest, Romania*

Abstract: Biosensors are promising analytical tools for the determination and characterisation of bioactive compounds in wines, providing fast, sensitive and cost-effective detection, assisting in the assessment of quality, sensory and safety attributes of wines. In this chapter, some of the most representative, recent examples of biosensors for the detection of polyphenols, biogenic amines, mycotoxins and allergens in wine are discussed, emphasizing the wealth of biosensing strategies and the particularities of wine as a complex analytical matrix.

Keywords: Allergens, Biogenic amines, Biosensor, Mycotoxins, Polyphenols, Wine.

INTRODUCTION

Bioactive compounds are generally perceived as those compounds that can exert a beneficial effect on living cells or organisms, as per a widely accepted definition [1].

[*] **Corresponding author Alina Vasilescu:** International Centre of Biodynamics, 1B Intrarea Portocalelor, sector 6, 060101, Bucharest, Romania; E-mail: avasilescu@biodyn.ro

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There is however, a second line of thinking among researchers who adopted a broader definition of "bioactive" to include any type of effect on living cells, being that beneficial or toxic. In line with this later definition naming bioactive "a compound which has the capability and the ability to interact with one or more component(s) of the living tissue by presenting a wide range of probable effects" [2], this chapter will summarize the current information regarding the use of biosensors for the detection and characterisation of four groups of bioactive compounds in wine: (poly)phenolic compounds, biogenic amines, mycotoxins and allergens.

Biosensors are analytical tools which combine the specificity of biorecognition using an enzyme, antibody, DNA sequence, whole cell, peptide *etc.* with a sensitive physical transducer (electrochemical, optical, mass-sensitive, *etc.*) in order to detect a target molecule. The biorecognition element is immobilized on the transducer by adsorption, entrapment, covalent binding, cross linking, *etc.* and the biorecognition event is translated into an electrical signal, as illustrated in Fig. (**1**).

Biosensors have been intensively researched in the last decades as potential alternatives to conventional methods in the food, biomedical and environmental fields based on their promise for fast, sensitive, specific, portable, low-cost analysis and compatibility with miniaturization.

Wine analysis was among the practical applications explored with biosensors, which were used either for monitoring the progress of fermentation processes, to verify relevant quality attributes, to assess the sensory characteristics or for classifying wine samples. Due to the complexity of wine matrix, the development of biosensors for wine poses specific analytical challenges, particularly related to the presence of possible interfering compounds. A few examples of biosensors for the detection of bioactive compounds in wine are given in Table **1** and some illustrative biosensors are discussed in more detail below, for each group of bioactive compounds.

Detection of Phenolic Compounds

Wines are a rich source of polyphenolic antioxidants that could act as powerful scavengers of free radicals species formed during metabolic processes in human cells. High levels of free radicals are triggers of oxidative stress, associated with various diseases [16]. Natural antioxidants have a huge potential for alleviating a number of medical conditions linked to oxidative stress induced by free radicals, as indicated by several ongoing clinical trials.

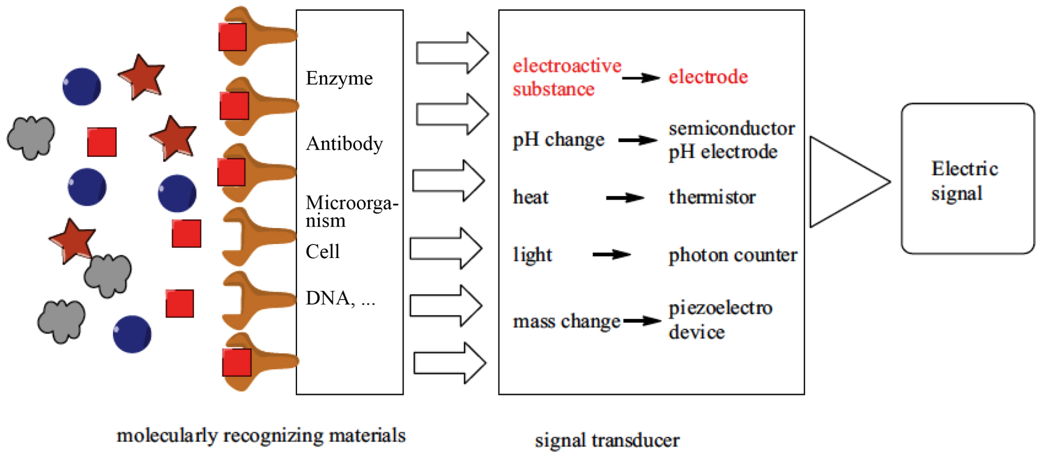

Fig. (1). Schematic diagram for the principal of the biosensors. Mechanism of the biosensor and the detection method. Figure reproduced from [109] by permission of INTECHOPEN (www.intechopen.com) and under the terms and conditions of the Creative Commons Attribution license (http://creativecommons.org/licenses/by-nc-sa/3.0/).

Table 1. Examples of recent biosensors for (poly)phenolic compounds in wine.

Bioactive compound/ matrix	Biological and chemical elements/transduction system	Specificity/Mode of action	Figures of merit	Reference
Phenol	Microbial cells/ Electrochemical Transducer	*Pseudomonas putida* immobilised on gold electrodes.	Linear range: $0.5x10^{-6}$ to $6x10^{-6}$ M Sensitivity: $5.03x10^{-3}$ A M^{-1}	[3]
Phenol	QD (CdS or Cu_2S) -MWCNTs and bacteria/ Electrochemical transducer	*Acaligense* spp. catalyses the conversion of phenol to catechol and o-quinone, and the QD-MWCNTs act as electron mediator of these redox reactions.	LR: $0.5x10^{-3}$ – $5x10^{-3}$ M (CdS) $0.7x10^{-3}$ - $10x10^{-3}$ M (Cu_2S)	[5]

CHAPTER 6

Electronic Sensory Systems for Characterization of Bioactive Compounds

Jesús Lozano[1], **Daniel Matatagui**[2], **José Pedro Santos**[3] and **M. Carmen Horrillo**[3,*]

[1] *Escuela de Ingenierías Industriales, Universidad de Extremadura, Badajoz, Spain*

[2] *Centro de Ciencias Aplicadas y Desarrollo Tecnológico, UNAM, México D.F.*

[3] *GRIDSEN, Instituto de Tecnologías Físicas y de la Información (ITEFI-CSIC), Madrid, Spain*

Abstract: This chapter provides a review of the most recent works in electronic sensory system for characterization of Bioactive Compounds. Focus is placed on the food applications and those compounds that are good for health. This chapter reviews the main electronic sensory techniques, from electronic noses to sensors and biosensors devices used for Bioactive Compound Characterization. The chapter demonstrates that although Bioactive Compounds are difficult to detect with sensor systems due to their high molecular weight and low volatility of the compounds and therefore few references are found in the literature, it is important to stand out that these systems could be a promising field for research and developing of industrial instruments applied to real contexts.

Keywords: Bioactive compound characterization, Biosensors, Electronic noses, Electronic sensory systems, Nanoparticles, Optical sensors, Sensors, Surface-enhanced raman spectroscopy, Surface plasmon resonance.

INTRODUCTION

Nutrition research has shown repeatedly and consistently that diets, the best adapted to the goals of healthy diet, are those based mainly on food consumption

* **Corresponding author M Carmen Horrillo:** GRIDSEN: Instituto de Tecnologías Físicas y de la Información (ITEFI-CSIC). C/ Serrano, 144. 28006 Madrid, Spain; E-mail: carmen.horrillo.guemes@csic.es

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of plant sources (fruits, vegetables, whole grains, nuts and legumes), using sparingly foods of animal origin. In fact, low intake of fruits and vegetables, along with hypertension, hypercholesterolemia, smoking, physical inactivity and overweight and obesity are among the main risk factors of total mortality in the European population. Today we know that the benefits of a prudent diet are not limited to content of nutrients, must also provide other protective factors against stress, carcinogenesis and oxidative contents especially in plant and foods, called "bioactive components".

Today, nutritional currents are influencing on the concept that we have above health. New dietary strategies are being carried out to see the effect of diet on regulation of the genetic and molecular levels and therefore improving the human health. Hereby, the bioactive compounds (extracts of plants or single compounds therefore) need to be identified and developed to complement a balanced diet. Therefore it is very important to evaluate the efficacy and safety of these compounds. The development of characterization techniques presents a great interest for the scientist community, as well as the nanotechnology, which is also being directed to the development of delivery systems for bioactive compounds.

What are Bioactive Substances?

Bioactive substances are food components that influence the cellular activity and the physiological mechanisms with beneficial effects on the health [1]. These substances have an important role [2 - 4] in the prevention of many diseases (CVD, coronary heart disease, stroke, hypertension, various cancers [GI, prostate, breast, *etc.*], neurodegenerative, inflammatory diseases, ocular ones [macular degeneration, -DMAE- cataracts], obesity, diabetes, osteoporosis, longevity [lifespan essential], *etc.*).

Besides their important antioxidant action (through mechanisms such as sequestration of free radicals, inhibition of the production of hydrogen peroxide, activation of endogenous defense mechanisms [catalase, superoxide dismutase], metal chelation, *etc.*), many other biologically plausible mechanisms may be responsible for their protective effect.

Bioactive Compound Classification

The main bioactive components and the foods where they can be found are listed in Table **1** [5]. They can be classified into three groups: terpenoids (carotenoids and sterols); phenolic compounds (such as phytoestrogens and flavonoids quercetin, the most common dietary flavonoid); and sulfur compounds.

SENSORS AND ELECTRONIC SENSORY SYSTEMS FOR CHARACTERIZATION OF BIOACTIVE COMPOUNDS

There are several electronic sensory systems, including optical, nanotechnology and gas sensors based systems for the characterization of bioactive compounds and some experiments have been described in the bibliography. Table **2** summarizes the sensors and sensory systems applications for detection and characterization of different bioactive compounds, mainly in the food industry.

As a general rule, the sensing of Bioactive Compounds is not an easy task due to the molecular weight of these substances. Their low volatility makes it difficult to detect them with gas detection techniques. However, the sensing of these compounds is very important because of high value of these compounds for the health. Main compounds and their sensing techniques can be found in Table **2** and some of them described in the following subsections. Electrochemical methods for wine bioactive compounds have been widely discussed elsewhere in the book thus few references to these methods will be given in this chapter.

Table 1. Bioactive compounds classification.

Bioactive compound	Food
Terpenoids (several thousand): - **Carotenoids**: • Carotene: - α-carotene, beta-carotene (precursor of vitamin A). - Lycopene. • Xanthophylls: - ß-cryptoxanthin (provitamin A). - Lutein, zeaxanthin.	ß-carotene vegetables and orange fruit (*e.g.*, carrots, mango, apricot, melon, peach, passion fruit, plum); dark green leafy vegetables (such as spinach); tomato and derivatives. Lycopene: tomatoes, watermelon, red pepper, pink grapefruit. ß-cryptoxanthin: oranges, papaya. Lutein and zeaxanthin: green leafy vegetables (*e.g.*, beets, spinach, lettuce, celery), oranges, potatoes, tomatoes, red peppers, corn, avocado, melon.

CHAPTER 7

Bioactive Biomolecules Interacting with Cellular Membranes: Modelling with Langmuir Monolayers

Felippe J. Pavinatto[1,*], **Thatyane M. Nobre**[1], **Luciano Caseli**[2] and **Osvaldo N. Oliveira, Jr.**[1]

[1] *University of Washington, Clean Energy Institute, Seattle - WA, United States*

[2] *Institute of Environmental, Chemical, and Pharmaceutical Sciences, Federal University of São Paulo, Diadema, São Paulo, Brasil*

Abstract: Cell membranes are essential for life, since they compartmentalize cells, the basic unit of living matter, and mediate their symbiosis with the environment. In many events in which cells participate, interaction with biomolecules such as polysaccharides, proteins and peptides, is crucial. In spite of the developments in physics, chemistry and biology in the last century, the study of cells and bioactive biomolecules interacting "*in vivo*" is still elusive. Therefore, simplified models are employed to obtain fundamental knowledge from these systems. In this chapter, the use of Langmuir monolayers as cell membrane models made to interact with biomolecules is described. Firstly, a brief introduction on the nature of nanostructured Langmuir monolayers is provided. In particular, we describe technical aspects related to the experimental setup and procedures to guide novice researchers in the field. We also describe the fundamentals and possibilities of the most useful techniques applied to film characterization. In the final section, examples are given of Langmuir monolayers successfully employed to obtain relevant molecular-level information from systems involving biomolecules. Trends in the field are discussed as motivation for innovative research to bridge the gap between biology (and medicine) and the physicochemical framework used in cell membrane modeling.

[*] **Corresponding author Felippe J. Pavinatto:** University of Washington, Clean Energy Institute, Seattle - WA, United States; E-mail: felippe@uw.edu

Constantin Apetrei (Ed.)

Keywords: Biomembrane Models, Biomolecules, Cell Membrane Interactions, Langmuir Monolayers, Peptides and Polysaccharides, Proteins.

1. INTRODUCTION

Cells are the basic unit of living matter, and in every living system (*e.g.* human body) they function to perform specific tasks and keep the balance of life. This very specialized duty takes place in constant interaction with the environment, and is subject to the action of external agents, naturally present or artificially administered to the medium. In this context, the membrane "represents" the cell in the outside environment, for it faces interacting molecules and mediates their action on the cell. The cell membrane also controls the influx and efflux of molecules, thus determining some intracellular processes and ultimately cell composition. Needless to say, it is virtually impossible to characterize this complex, dynamic system "on the fly", even using state-of-the-art techniques. The characterization of molecular-level interactions, in particular, is hard to attain for such elaborated structure. Hence, simplified models are used to mimic the membrane and study its interaction with bioactive compounds in a controlled fashion. In this chapter, we will describe and exemplify the use of Langmuir films for this goal.

The topic covered here has been reviewed and discussed a few times, and most of the relevant literature in the field is listed in the references. We conceived this chapter with a different focus, to contribute with something unique to the literature. Approximately half of the chapter is devoted to discussing systems in which Langmuir films are used as models to study interaction of cell membranes with biomolecules (section 4). In this part, our chapter distinguishes itself by highlighting studies where (in our opinion) more compelling and close-to-application cases are studied, and contributions from the models are really relevant. In addition, in section 2 we describe Langmuir films and why they are useful as membrane models. In section 3, we introduce the many characterization techniques used for these films. We opted to concentrate on practical aspects rather than on elaborated theoretical description, since we aim at offering "shortcuts" for the effective, precise learning of the methods by scientists interested in the field and in its possibilities. In other words, we aim at providing an accurate and robust material for a textbook.

2. LANGMUIR MONOLAYERS AND THEIR USE AS MEMBRANE MODELS

Langmuir films are named after Irving Langmuir, a prominent American scientist that studied liquid surfaces in the first decades of the 20th century at the General Electric Laboratories, New York [1]. In his seminal paper, Langmuir described with great detail the action of surface forces as well as the nature of interfacial ultrathin (monomolecular) floating films formed on several liquid surfaces. Among other contributions, this work rendered him the Nobel Prize in Chemistry in 1932. Since then, knowledge has evolved and the (nano)structure of the films is nowadays known in detail. If deposited in the right amount on the water surface, amphiphilic molecules can spread as a monolayer, with their hydrophilic portion facing the water and the hydrophobic part oriented towards the air. A scheme of the Langmuir film architecture is shown in Fig. (**1**).

The resemblance of the film structure with one of the leaflets of a cell membrane is clear. Although in some cases, as in gram-negative bacteria, the cell membrane has a multilayered structure, in the vast majority of the cells of either prokaryotes or eukaryotes the membrane is a bilayer of lipids in which proteins are inserted. The drawing in Fig. (**1**) illustrates a film of a single component (*e.g.* phospholipid) with two tails attached to a single head. However, the Langmuir film can be made of more than one component, have transmembrane or peripheral proteins incorporated to it, or even be prepared from a membrane extract with a composition very similar to the one found in the actual membrane.

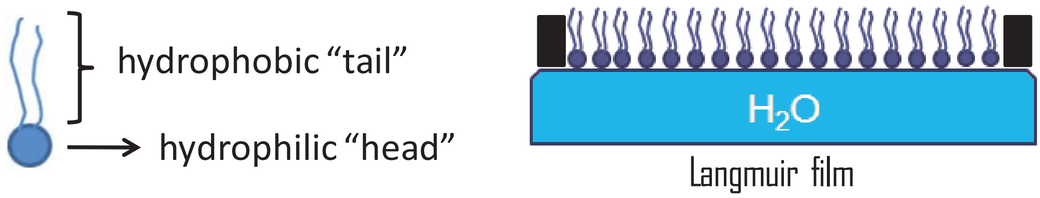

Fig. (1). Left: representation of an amphiphilic molecule with its hydrophilic and hydrophobic parts. Right: schematics of a Langmuir film, where amphiphilic molecules are assembled as a monolayer at the air-water interface. [Adapted with permission from [2]. Copyright 2010, American Chemical Society].

CHAPTER 8

Protection of Bioactive Compounds

Stefan Dima[1,*] and **Cristian Dima**[2]

[1] *Department of Chemistry, Physics and Environment, Faculty of Sciences and Environment, "Dunarea de Jos" University of Galati, Romania*

[2] *Faculty of Food Science and Engineering, "Dunarea de Jos" University of Galati, Romania*

Abstract: The vegetal extracts are complex blends of chemical compounds from various classes: polyphenols, carotenoids, vitamins, essential oils, *etc.* It is known that these components are highly sensitive to the action of oxygen, light radiations and high temperature. These factors contribute to the degradation of bioactive compounds and implicitly to the decrease of their biological potential. Therefore, the encapsulation of bioactive compounds in different matrices is required in order to prevent the aforementioned shortcomings.

The purpose of this review is to synthesize the newest information about the biocomponents' encapsulation. The encapsulation of bioactive compounds, ensures the stability of the volatile compounds during thermic processing, the protection of bioactive components against environmental factors, the increase of the solubility of bioactive compounds, the controlled release of bioactive components, *etc.* Various techniques are used for the encapsulation of bioactive compounds, some of them being: emulsification, coacervation, gelation, extrusion, spray drying, spray cooling, *etc.*

Keywords: Biopolymers,Coacervation, Emulsions, Encapsulation, Gelation, Microparticles, Nanoparticles.

INTRODUCTION

Scientific history provides numerous examples of man's creativity manifesting itself by reproducing certain naturally occurring phenomena and processes.

* **Corresponding author Stefan Dima:** Department of Chemistry, Physics and Environment, Faculty of Sciences and Environment, "Dunarea de Jos" University of Galati, 47 Domneasca Street, 800008 Galati, Romania; E-mail: Stefan.Dima@ugal.ro

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Encapsulation is a case in point. The encapsulation process is found in the primary stages of the universe, when the chemical elements and molecules got together into structural units wrapped in a semipermeable membrane able to protect them against external factors [1]. These structures, called coacervates, have evolved towards what we call today biological cell. The biological membrane and the membrane surrounding the microcapsule share the following characteristics:

- It delimitates the space within the cell or capsule from the outer environment;
- It protects the components of the cell or capsule against the aggression of the external factors;
- It communicates with the outer environment by transfer of mass, energy and information.

In order to better understand the encapsulation process, the present paper will attempt to briefly answer the following questions: What is encapsulation? Why encapsulate bioactive components? How to do the encapsulation of components? What are the advantages and disadvantages of encapsulation? The answers to these questions are found in the preoccupations of the scientists in the entire world, whose results are published in the thousands of papers issued annually in various specialised journals.

WHAT IS ENCAPSULATION?

Encapsulation is an innovative technology aimed at increasing the functionality of materials with applications in a wide range of areas: medicine, pharmacy, food industry, agriculture, *etc.*

This technology achieves the entrapping of a solid, liquid or gaseous substance, called encapsulate, active, core, payload, fill or internal phase into another substance, called shell material, wall material, carrier material, coating material, external phase or support phase, under the form of particles of various sizes [2]. These particles are a delivery system providing the embedding, transport, and release of component in various parts of the human body, such as skin, nose, lungs, mouth, stomach, the small intestine, or colon [3].

Certain particles obtained by encapsulation have a homogeneous internal morphology and are called core-shell particles or capsules. In these particles the active substance exists under the form of one or more nuclei surrounded by one or more layers of encapsulating material (Fig. **1a, b, c**). Other particles have a heterogeneous internal morphology and are called matrices or microspheres [4].

In these particles the active substance is diffused into a matrix, which may be a polymer hydrogel or a porous material (Fig. **1d**). Sometimes these matrices may be covered with one or several layers of material, thus improving the particle qualities (Fig. **1e**). A special group of particles in which bioactive compounds may be encapsulated are mixed micelles obtained by the self-assembling of amphiphile molecules, emulsions, nanoemulsions, microemulsions and liposomes.

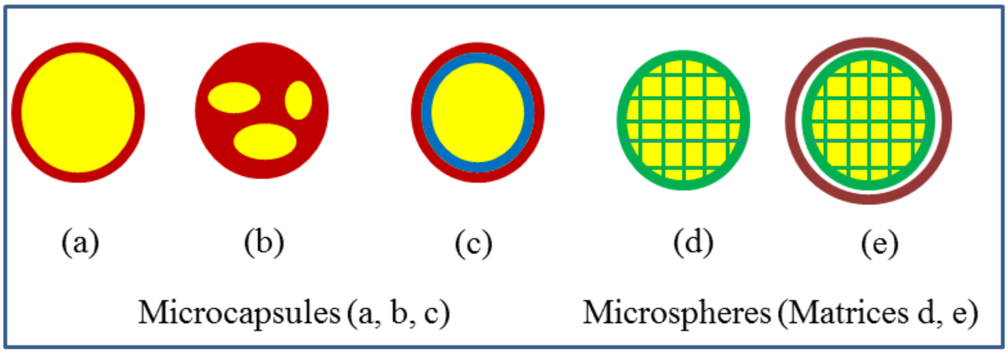

Fig. (1). Microcapsule (a, b, and c) and microspheres (d and e) morphology.

The classification of particles according to size is still a topic of debate among researchers [5 - 8]. Nevertheless, in most studies, the size of 100 nm is acknowledged as the upper limit for nanoparticles and the lower limit for microparticles. Thus, particles with sizes between 1nm and 100 nm are considered nanocapsules, and those with sizes between 100 nm and 1000 nm are considered microcapsules [4, 5, 7, 9, 10]. This threshold dimension (100 nm) is said to be the point where a sudden fall of the bulk material properties occurs. In fact, the threshold size of altering the bulk material properties depends on the nature of the material. That is why various scientific papers provide different size ranges for nano and microparticles.

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Constantin Apetrei

Constantin Apetrei was born in Falticeni (Romania), in 1975. He received BSc in Chemistry in 1997 from the University "Al. I. Cuza" of Iasi (Romania) and the PhD in 2007 from the "Dunarea de Jos" University of Galati (Romania). He is presently Full Professor at the "Dunarea de Jos" University of Galati (Romania) in the Department of Chemistry, Physics and Environment, Faculty of Sciences and Environment. His current field of research interests is related to the development of electrochemical sensors and biosensors for the analysis of foods and beverages. He is co-author of over 50 papers in peer-reviewed journals and 8 book chapters. He was the editor of one monograph.