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BIOTECHNOLOGICAL PRODUCTION OF NATURAL INGREDIENTS FOR FOOD INDUSTRY

FIRST EDITION

Editors:
Juliano Lemos Bicas
Mário Roberto Maróstica Jr.
Glaucia Maria Pastore

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FOREWORD

Hence, depending to whom the question is addressed, the assessment about the necessity of additives in food will turn out extremely different. Industry, on one hand, aims to produce qualitatively high ranking food with minimal costs. Beside palatability (aroma, taste, texture and color) the food should offer extended shelf life and high nutritional impact. The consumers, on the other hand, want to have all thesebenefits without the addition of any food additive. In terms of food the “all without mania” (flavors, taste enhancers, colorants, preservatives, antioxidants, thickeners, gelling and stabilizing agents *etc.*) is prevalent in developed countries. However, consumer’s empathy of preparing their daily meal on the basis of fresh ingredients is more and more decreasing nowadays.

Therefore processed food, especially convenient food, purchased in the supermarket and not on the farmers market represents the dominating basis of modern nutrition. Hence, quality, nutritional value and shelf life must be guaranteed for longer and longer storage times. Moreover, nowadays food should offer additional benefits regarding lifestyle and health. To comply with all of these requests, food additives are indispensable in industrial food processing. For a long time no economical alternative to chemical synthesis of food additives existed. Not at the least because of the depletion of the fossil fuels reserves together with several concerns about climate changeand the all over chemophobia, the production of natural food additives seems to cut this *Gordianknot* of different interests. Natural additives serve consumers wish on natural nutrition and approve food industry the application of indispensable additives. Whether recovered from natural resources or produced biotechnologicallyroutes natural additives are more accepted in public mind. However, natural resources are often limited which calls for biotechnological alternatives.

The aim of the book is to provide coverage of natural food additives and their production. Within this textbook, comprising 13 chapters, international well accepted experts in the field give a prevailing and comprehensive overview on food additives such as flavors colors, sweeteners, amino and organic acids, vitamins, prebiotics, edible oils, antimicrobial compounds, biosurfactantsand enzymes. This book secures the experts in the field as well as interested consumers to inform themselves about the current state of the art of biotechnological processes for the production of natural food additives. Furthermore legal, economic and ecological aspects are also addressed. It is demonstrated that biotechnology alongside the attribute “natural” can compete against chemical production processes because of improved production strains, the use of stable and often immobilized used enzymes, disposition of cheap waste streams of food producing processes as precursors and/or as the nutrient medium for producing microorganisms. Last but not least, it is more and more evident that a liable application of genetic engineering is an indispensable part of white and green biotechnology as it is already the case for red biotechnology. The conclusion of all chapters is that biotechnology, particularly genetic engineering, is a powerful tool which will help to cope at least with some of humankinds future challenges in the light of limited resources and a fast growing world population.

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PREFACE

After the advent of Organic Chemistry (1800's), the supply for organic chemicals achieved industrial scale. This was particularly important for Food Industry, which soon incorporated synthetic substances for the preparation of food products: saccharin, benzoate and indigotine, for instance, were already in use at the beginning of 20th century and are still applied today as food additives. However, public awareness involving the possible toxic effects of artificial ingredients has increased the demand for new alternative sources of their natural counterparts. Moreover, the impact of human activity on environment has been considered a major issue to be overcome, leading to intense search for sustainable or alternative production processes.Therefore, biotechnology has emerged as an important tool to supply natural ingredients for food industry, since they occur under controlled conditions, may use renewable sources and are recognized as an environmentally friendly technique.

Although such approach has been used empirically for the production of fermented food (bread, wine, beer, cheeses *etc.*) aiming at either preservation or modification of their sensory attributes, it was only recently that science begun to able to understand and explain the phenomenology behind these biotechnological processes, which reflected in an increased number of R&D projects for the production of food ingredients by microorganisms, enzymes of cell cultures.The fact is that, nowadays, the so-called White Biotechnology is considered an inextinguishable resource of natural food ingredients. Additionally, food biotechnology remains a vigorousresearch field and many fundamental studieson this subject are still needed. This may be evidenced by the intense growth, during the last decades, of articles and patents covering all aspects of biotechnological production of food ingredients. Most of these processes are already found in commercial scale, but others are still waiting for further developments.

This e-Book aims to be a fundamental reference for people who want to deepen into this field, particularly those students, scientists, researchers and professionals working with Food Science and Technology, Food Chemistry, Food Biotechnology, Food Engineering, Bioprocess Engineering, Biotechnology, Applied Microbiology, Nutrition and others. It is divided in 12 chapters. The first one presents a brief overview of food biotechnology, particularly those aspects involving and historical perspective and some examples of commercially relevant products and processes currently available. All other chapters are devoted each one to a particular class of products with potential interest for food or feed industries: sweeteners (Ch. 2), aminoacids and nucleotides (including flavor enhancers) (Ch. 3), organic acids (Ch. 4), vitamins and nutraceuticals (Ch. 5), aroma compounds (Ch. 6), colorants (Ch. 7), edible oils (Ch. 8), hydrocolloids (Ch. 9), antimicrobial compounds (Ch.

10), biosurfactants (Ch. 11) and enzymes (Ch. 12).

We hope you enjoy it!

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CHAPTER 1

Introductory Overview of Biotechnological Additives

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Abstract: The use of biotechnology in the manufacture of food and beverages has been practiced for many years. Because of this important developments over the years, biotechnology can be considered as a significant part of human life and industrial development, enabling the creation of breakthrough products and technologies to combat diseases, protect the environment, increase crop yields and to produce feed, fuels, renewable energy, industrial additives and several other useful products.

Keywords: Enzymes, Food ingredients, Fuels, Genetic engineering, Microbial fermentation.

INTRODUCTION

Biotechnology can be briefly defined as "any technological application that uses biological systems, living organisms, or derivatives, to make or modify products or processes for specific use" [1]. In this sense, biotechnology involves the application of tools based on biotechnology in traditional industrial processes ("bioprocess") and the manufacturing of biobased products (such as fuels, chemi-

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cals and plastics) from renewable feedstock [2].

The biocatalysts used in these processes, such as bacteria, yeast and fungi or microalgae, are considered as an inexhaustible source of a diverse range of important compounds and industrial additives. Currently, microbial biotechnology plays an important role in the production of food additives, fine and bulk chemicals, solvents, enzymes, agrochemicals and biopharmaceuticals, and many others [3, 4].

Biotechnology presents several advantages compared to conventional chemical production models and also to the direct extraction of a desired additive from nature [5]. This happens mainly because microbial biocatalysts display desirable chirality and are biodegradable and the reactions are conducted in mild conditions, with lower energy consumption and lower environmental impact [4].

Furthermore, the industrial roles of biotechnology have been considerably expanded in the current scenario to produce renewable chemicals for industrial and economical purposes, also aim at reducing the use of petrochemical derivatives and the depletion of fossil fuels, in this way producing biofuels and bioenergy as a primary product through a ‘biorefinery’ concept [2].

Thus, biotechnology presents unique opportunities to produce natural food ingredients with industrial and economical interest. In this sense, the aim of this chapter is to present the historical development of biotechnology and also to illustrate some of the major products from this important industrial sector.

HISTORICAL ASPECTS OF BIOTECHNOLOGY FOR MODERN DEVELOPMENTS

First, it is important to understand the difference between the “traditional” and the so-called “modern biotechnology” [6].

The traditional biotechnology can be considered as the fermentation process used to produce beer, wine, cheese, soy sauce and others [7], and the biotechnology process in agriculture started with the history of agriculture itself. With the emerging development of agriculture, humankind began to select the plants with the best yields and resistance according to its needs [8]. Therefore, the biotechnological techniques are not new, considering that the manufacture of food and beverages, for example, has been practiced for more than 14,000 years with vinegar, alcoholic beverages, sourdough and cheese [9]. In fact, Food Biotechnology has been developed empirically since Ancient History and, ever since, the fermentation technology has been applied as the main tool to preserve food products or improve aroma, flavor and texture. Despite its long history, food science and technology has only recently understood the phenomenon involved in such biotechnological processes, and, today, the use of microbial and enzymatic processes for the production of food ingredients is highly developed [10].

Modern biotechnology, in turn, is based on recombinant DNA techniques, which started with the creation of the first recombinant gene, a couple of decades ago, and is currently helping to improve food, beverages, medicine and fuels [11]. The major examples of application of this technique are genetic modified organisms (GMO), metabolic engineered microorganisms and several breakthrough for the creation of crucial products for human use, such as new drugs, healthier foods and so on [11, 12]. Two major case studies will be presented in sequence to illustrate the development of biotechnology over the years.

Enzymes

For several years, enzymes have played an important role in many industries (food/feed, detergent, biofuels, textile and others). Currently, most food products have at least one ingredient produced with enzyme technology. Some examples produced with the enzymatic process include: sweeteners, syrups, bakery products, alcoholic beverages, precooked cereals, baby food, fish meal, cheese and dairy products, egg products, fruit juice, soft drinks, vegetable oil and puree, candy, spice and flavor extracts, liquid coffee, flavors and tenderized meat [13].

The industrial production of enzymes for use in food processing started in 1874, when Christian Hansen extracted chymosin (or rennin) from calf stomachs for the clotting of milk for cheese production [14, 15]. However, the mechanism of enzymes was unknown until 1877, when Moritz Traube proposed a “protein-like material that catalyzes fermentation and other chemical reactions”.

The first patent for the industrial use of enzymes was named Taka-Diastase, an

CHAPTER 2

Alternative Sweeteners: Current Scenario and Future Innovations for Value Addition

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Abstract: Sustainable growth and consistent demand for zero or low-calorie alternative sweeteners by the global market are mainly attributable to public consciousness about health impact of artificial sugar substitutes. Despite limited market for natural sweeteners, a spurt in preference to plant derived-alternative sweeteners is known. Sugar substitutes, such as non-nutritional artificial sweeteners, low calorie or zero calorie natural sweeteners that include sugar alcohols and plant derived non-saccharide sweeteners find use in making various types of foods and beverages. From an industry point of view, approval for usage of sugar substitutes in food products by the regulatory agencies can initiate major trends. These trends can contribute to the safety and health consciousness of consumers and also to food and beverage industries to get better market and price. There is a need to further refine the available technologies for the production of alternative sweeteners, especially natural sweeteners through a plant-derived or microbial cell based production platform with the intervention of metabolic engineering to produce novel sweeteners.

Keywords: Acesulfame, Alitame, Artificial sweeteners, Aspartame, Erythritol, Isomalt, Lactitol, Low-Calorie sweeteners, Maltitol, Mannitol, Natural sweeteners, Neotame, Non-saccharide sweeteners, Polyols, Rebaudioside, Reduced-Calorie Sweeteners, Sorbitol, Stevioside, Sucralose, Xylitol.

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INTRODUCTION

A wide range of food products containing natural sweeteners with an emphasis to bring down number of calories has gained momentum, especially to address obesity and diabetes *etc.*, which are prevalent in consumers and attributable to changing dietary habits and sedentary lifestyle. Moreover these alternative sweeteners are helpful to manufacturers of food products and also to consumers, in a high sugar price environment that prevails today. Sweetener can be defined as any substance added to food or beverage to make it taste sweeter. Sugar substitutes as a food additive mimic the effect of sugars in taste usually with fewer calories. Sugar substitutes can be classified as natural, synthetic or artificial on the basis of by their production [1]. The first category (synthetic) includes some of the important artificial sweeteners, such as aspartame, neotame, acesulfame, saccharin, sucralose *etc.* (Fig. **1**). Many of them are known as high-intensity sweeteners which are much sweeter and have a minimal energy contribution in food compared to sucrose (Fig. **2**). Food containing high-intensity sweeteners prevent excessive calorie intake and are claimed to be helpful in weight loss and in diabetes management [2]. The second category of sugar substitutes is natural sweeteners that occur naturally in certain fruits and vegetables, but can also be manufactured artificially. These natural sweeteners can be grouped in two major categories, comprising saccharides and non-saccharides. Saccharides based on natural sweeteners, also known as nutritive sweeteners or carbohydrates, contain polyhydroxy aldehydes or ketones, such as sucrose, glucose, trehalose *etc.* Non-saccharides based on natural sweeteners can be grouped in five major classes, such as terpenoids, proteins, flavonoids, steroidal saponins and polyols. Polyols or Sugar alcohols are compounds with multiple hydroxyl functional groups and commonly added to foods because polyols have lower calorie than sugars. Maltitol, lactitol, sorbitol, xylitol, erythritol, and isomalt are some of the more common examples of polyols (Fig. **3**). Another major class of non-saccharide sweeteners includes flavonoids and their derivatives (Fig. **4**), such as neohesperidin, phyllodulcin, naringin *etc.* Steroidal saponins are another class of non-saccharide sweeteners composed of rhamnopyranosyl units such as osladin (Fig. **5**). Though the above mentioned categorization is acceptable for demarcation of some sugar substitutes, it is also important to have knowledge about their alternative source whether natural or a derivative, processed or refined, or chemically derived from herbs or sugar. All these alternative sweeteners include non-nutritive, low calorie, low glycemic or saccharide-derived and non-saccharide sweeteners.

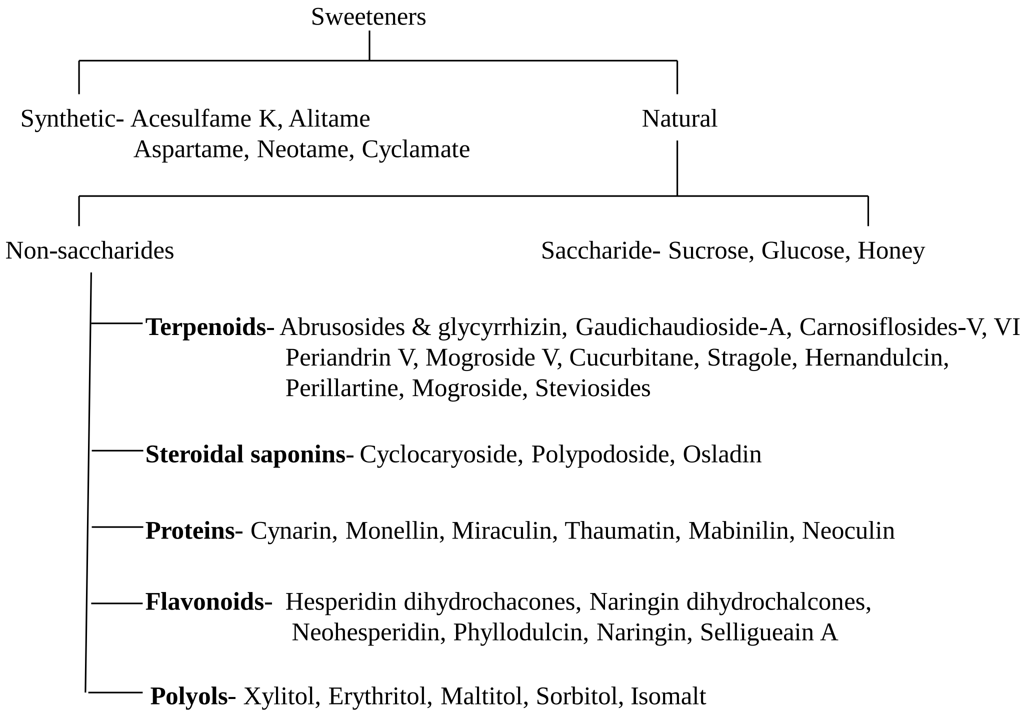

Fig. (1). Classification of sweeteners.

In the USA, the Food and Drug Administration regulates artificial sweeteners as food additives that must be approved as GRAS (Generally Recognized as Safe). Most of the sugar substitutes approved for food use belong to artificial sweeteners category such as acesulfame, aspartame, saccharin, neotame, and sucralose. Food Safety and Standards Authority of India (FSSAI) also approved four artificial sweeteners, including aspartame, acesulfame K2, saccharin and sucralose to be used in food industry. The food and beverage industries are replacing sugar with low calorie sweeteners in a variety of food products. Low calorie sweeteners increase a fraction of the cost in food production. Many reviews on sweeteners focused mainly on chemistry, biosynthesis, production, characterization and application in foods [3 - 9]. In view of the ever-increasing demand for alternative

CHAPTER 3

Biotechnological Production of Amino Acids and Nucleotides

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Abstract: L-amino acids and nucleotides find various applications in food biotechnology. L-glutamic acid and its salts as well as 5'-nucleotides are used as flavor enhancers. Other L-amino acids are used as food or feed additives, in parenteral nutrition or as synthons for the chemical and pharmaceutical industries. L-amino acids and nucleotides are synthesized from precursors of central carbon metabolism. Based on the knowledge of the biochemical pathways microbial fermentation processes of food, feed and pharma amino acids and of nucleotides have been developed. Production strains of *Corynebacterium glutamicum,* which has been used safely for more than 50 years in food biotechnology, and *Escherichia coli* are constantly improved using metabolic engineering approaches. Research towards new processes is ongoing. Fermentative production of L-amino acids in the million-ton-scale has shaped modern biotechnology and its markets continue to grow steadily.

Keywords: *Corynebacterium glutamicum*, *Escherichia coli*, Essential amino acids, Feed additives, *Flavor enhancers*, Food additives, GMP, IMP, L-pyrrolysine fermentation, L-selenocysteine, Metabolic engineering, MSG, Nucleotides, Production strain development, Proteinogenic amino acids, XMP.

INTRODUCTION

As the building blocks of proteins, amino acids play an important role in human and animal nutrition. Essential amino acids cannot be synthesized by humans and animals and need to be taken up with the food or feed. Addition of essential amino

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acids improves feed quality and reduces manure. In the feed industry L-lysine is produced by fermentation in the million-ton scale while D,L-methionine is produced chemically. The amino acids L-glutamic acid and its salts finds application as flavor enhancer and annually about three million tons are obtained by fermentation. Since nucleotides provide the “umami” taste as glutamate, they are used as flavor enhancers and produced mainly by fermentation. This review focuses on microbial fermentation processes of food, feed and pharma amino acids and of nucleotides. Metabolic engineering of producing strains mainly *Corynebacterium glutamicum* and *Escherichia coli* is emphasized.

GLUTAMIC ACID, ITS SALTS AND 5’-NUCLEOTIDES AS FLAVOR ENHANCERS

Professor K. Ikeda discovered and isolated monosodium glutamate (MSG) in the early beginning of the 20th century in protein hydrolysates of “konbu”, seaweed (*Laminaria japonica*) [1], while investigating the relationship between the chemical structure of a substance and its smell and taste [1]. “Konbu” is responsible for the dominant and distinct taste of the traditional Japanese soup base “dashi” [2]. Ikeda used methods and steps of classical chemistry and finally low-pressure evaporation resulted in the crystallization of a single substance, which could be identified as glutamic acid [1]. This substance, which elicits the specific taste of “umami”, is the fifth taste quality besides salty, sweet, bitter and sour upon which the human taste is based [1]. Glutamate became more and more important after World War II, where especially protein-rich meat was very rare in Japan and MSG was used for seasoning tasteless food [2]. But for this purpose traditional production of L-glutamate by decomposition of soybean and wheat proteins followed by extraction in an industrial scale, like it was performed by Ajinomoto at this time [3], was too expensive and research started to aim at fermentative production of glutamic acid [4].

In the first approaches the synthesis of L-glutamate was achieved by fermentative production of 2-oxoglutaric acid with subsequent chemical conversion to glutamate. High yields for 2-oxoglutaric acid of about 50% on sugar basis were reported with the use of some bacteria of the genus *Pseudomonas*. Although several methods for the conversion of 2-oxoglutaric acid to L-glutamate had been developed, a one-step synthesis of L-glutamate from glucose and ammonia would have been superior [4]. In the 1950s, Japanese researchers of Kyowa Hakko Kogyo isolated a coryneform soil bacterium, which first was named *Micrococcus glutamicus* No. 534 [5] and later *Corynebacterium glutamicum* [6]. Studies showed that this bacterium was able to secrete glutamic acid under biotin limiting conditions, which finally led to a fermentation process with considerably reduced costs for the direct production of glutamate from cheap sugar and ammonia [6]. This was the starting point of the large-scale fermentative glutamate production with a steadily increasing market up to ~2.93 million tons in 2012 [7].

The umami taste can also be provided by the purine nucleotides disodium 5'-monoinosinate (IMP) and disodium 5'-monoguanylate (GMP) [8 - 10]. Shintaro Kodama isolated the nucleotide IMP from dried bonito tuna in 1913. GMP was isolated from shiitake broth in 1960 by Akira Kuninaka who also described synergy between glutamate, IMP, and GMP. The umami taste quality of IMP and GMP differs with IMP providing a strong initial taste enhancement and GMP proving a delayed, but about twofold stronger taste enhancement. Foods rich in IMP *e.g.* dried bonito tuna or in GMP *e.g.* shiitake broth are known, however, pure or enriched preparations of these nucleotides are available commercially. Their market volume increased steadily over decades and the global annual nucleotide production has reached ~34,000 tons in 2012 [7]. The 5′-nucleotides IMP and GMP are now produced by enzymatic degradation of ribonucleic acid or by chemical phosphorylation of the nucleosides inosine and guanine that are obtained by fermentation [11]. Since yeast is rich in ribonucleic acid (RNA), the first commercial yeast extract containing GMP was produced in 1974 and nowadays yeast extracts with various contents of IMP and GMP are available. Direct fermentation for the production of IMP and xanthosine 5'-monophosphate (XMP) has also been reported [12]. The highest titers for fermentative IMP and GMP production have been obtained with *Corynebacterium ammoniagenes* mutants (150 g/L; [13]).

While the worldwide glutamate consumption has increased dramatically over the recent decades, health and safety concerns have become more and more important [14, 15], especially since glutamate was associated with the so-called Chinese Restaurant Syndrome [16]. Moreover glutamate was also suspected to cause

CHAPTER 4

Biotechnological Production of Organic Acids

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Abstract: Organic acids are the intermediates or products of many metabolic pathways, such as the Krebs cycle and lactic and acetic fermentations, and are important contributors to the taste and flavor of many fruits, vegetables and also fermented foods. Organic acids are widely applied in the food, pharmaceutical and chemical industries and can be produced by microbial fermentation, chemical synthesis or obtained using enzymes. The demand for organic acids is growing continuously, and numerous efforts and research investments have been made to increase the yield and productivity through the selection of new strains, by obtaining genetically modified microorganisms, optimizing fermentation processes and improving the recovery and purification processes. This chapter focuses on the production and application of citric, acetic, lactic, fumaric, malic, gluconic and ascorbic acids.

Keywords: Acetic acid, *Acetobacter sp*, Acidulants, Ascorbic acid, *Aspergillus niger*, *Aureobasidium pullulans*, *Brevibacterium* sp , Citric acid, Fumaric acid, Gluconic acid, *Gluconobacter* sp, *Ketogulonigenium vulgare*, Lactic acid, *Lactobacillus* sp, Malic acid, Organic acids, *Penicillium* sp, *Rhizopus* sp, *Saccharomyces cerevisiae*, *Yarrowia lipolytica*.

INTRODUCTION

The market for organic acids increased from US $2.5 billion in 2008 to US $4.0 billion in 2013 [1]. The primary organic acids used in food applications are citric, acetic, lactic, malic, fumaric and gluconic acids. Citric, malic and fumaric acids

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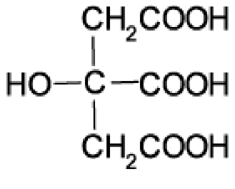
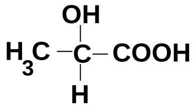
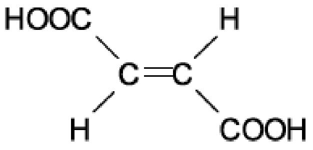
are naturally found in fruits and vegetables and have been used as acidulants in beverages, canned fruit, juices, powdered drinks, gelatin powder, and others. Some fruits are known for the presence of high concentrations of certain organic acids. With some exceptions, citrus fruit juices contain high concentrations of citric acid and smaller amounts of malic acid. Malic acid is the main organic acid of apples, while grapes have a high concentration of tartaric acid, and the star fruit (*Averroha carambola*) has a high oxalic acid content.

Organic acids can be applied in foods, pharmaceuticals, detergents, cosmetics and other products, such as acidulants, chelating agents, polymers and chemical compound precursors (Table **1)** [2]. Organic acids are added to foods to lower the pH and adjust the acid taste of products such as fruit juices, jellies, candies, gelatin powders, carbonated drinks, powdered drinks, yogurts, desserts, fruits, canned vegetables, pickles, olives, cheeses and other foods, as well as to control microbial growth. Acidulants and their salts aid in food preservation, enabling a reduction in the time used in the sterilization processes and preventing the germination of microbial spores at low pH values. Organic acids can act in synergy with antioxidants, preventing rancidity and other reactions by forming complexes with copper and iron ions. They are also used as buffer systems in foods to modify the consistency and melting point of candies and cheeses. Trisodium citrate is used as a stabilizer in cheese, ice cream and milk, and citric acid, in combination with ascorbic acid and other antioxidants, for the inhibition of enzymatic browning in juices and fruit pulps [3].

Citric acid is the most important acidulant for the food and beverage industries and is widely used due to its easy assimilation, palatability, high solubility, low toxicity and low cost. Malic acid is also found in fruits and vegetables and is mainly used in beverages, candies and foods. Fumaric acid is used in the food industry and is an intermediate in the production of malic acid and L-aspartic acid, the latter being used to obtain aspartame. The use of organic acids depends on their ease of handling and storage, degree of hygroscopicity and solubility, on their properties, such as flavor modification, compatibility with other acids and food components, and their ability to act as Ca^{2+} chelators.

Cost is the most important factor in the production of organic acids due to the competition between industries. Currently citric acid is primarily produced by the submerged fermentation of media containing sucrose, molasses, glucose or hydrolyzed starch using *Aspergillus niger* strains [7, 11]. The fermentation process should be optimized by selecting a microbial strain showing a high production of the metabolites of interest by using optimized medium cultivation and optimal conditions of temperature, pH, agitation and aeration to obtain high yields (g acid/g glucose), productivity (g acid/L/h) and final acid concen-trations (g/L).

Table 1. Production and applications of organic acids.

Organic Acid	**Structural formula/ Estimated price**	**Annual production metric tons/year**	**Production method**	**Applications**
Citric Acid $C_6H_8O_7$	 $HO-C(CH_2COOH)_2-COOH$ US $1.0 - 10.0/Kg	1, 600, 000 [2]	Fermentation	Food; beverages; pharmaceutical industry; pH stabilizer in cosmetics; chemical and metallurgical industries; metal cleaning [4, 5, 6]
Acetic Acid $C_2H_4O_2$	**H_3C — COOH** **US $0.5 - 0.6/Kg**	190, 000 [6]	Fermentation	Food industry (vinegar).
		7, 000, 000 [6]	Chemical synthesis	Vinylacetate for polymers, ethyl acetate [6]
Lactic Acid $C_3H_6O_3$	 $H_3C-CH(OH)-COOH$ US $1.0 - 1.3/Kg	250, 000 [7]	Fermentation	Food; beverages; biodegradable plastic PLA [6, 8]
Fumaric acid $C_4H_4O_4$	 US $1.1 - 1.6/Kg	90, 000 [2]	Chemical synthesis	Food acidulant; medicine; raw material for polyester resins [2, 6]

CHAPTER 5

Vitamins and Nutraceuticals

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Abstract: Vitamins are a class of molecules that play an essential role in metabolism. In order to enhance or to complete the nutritional value of a food, it is sometimes, necessary to add vitamins during processing. Most vitamins may be produced by chemical synthesis, but the natural sources are both more appealing to the consumer and, sometimes, more bioavailable. Natural and genetically modified microorganisms are a possible alternative to produce vitamins. Another broad class of molecules, nutraceuticals, includes substances which are not essential in diets, but which may have beneficial roles besides that of supplying building blocks, energy, coenzymes and minerals for the body. This chapter presents both the classes of compounds, vitamins and nutraceuticals, their use in processed food, and focuses on the classical and microbial sources of vitamins and then role in the human body, and some representative nutraceuticals. Aminoacids, organic acids, prebiotics, polyunsaturated oils, hydrocolloids, enzymes and other molecules which are bioactive or nutritionally important, but pertinent to other classes of additives, are not discussed in this chapter.

Keywords: Antioxidant, Coenzymes, Fermentation, Metabolism.

INTRODUCTION

During the last two decades, food industries have developed new products in order to meet customer's demand: practical, attractive and tasty products that also provide functional and nutraceutical components, are even seen by consumers as beneficial for health. This demand for healthier products, together with the inno-

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vation-driven marketing of processed foods, created a tendency of new product development where not only flavor, stability, or nutritional value, but also health issues drive the development of new products [1].

The development of a recommended "dietary reference intake" (DRI) for several classes of nutrients sheds light on the use of specific functional and nutraceutical components of food. The average levels of vitamin intake may be below the DRI in foods without supplementation [2, 3], because of segregation of parts of the raw food (*e.g.* separation of starch from cereals, or thermal degradation).

The importance of certain foods in maintaining healthy life is long known. Ancient cultures such as Greek and Egyptian reported that definite diseases appeared to be related to nutritional factors. However, it was only in the 20th century that vitamins were discovered and described as essential micronutrients for living organisms [4]. Casimir Funk has coined the term "vitamine" (from vital amine), later changed to "vitamin", in 1912. These substances may be defined as "organic compounds in food, needed in very small amounts for growth and maintaining good health". Vitamins are fundamental for animal survival because of their specific role in the physiology and metabolism [5, 6]. Several essential vitamins are not synthesized by the human body, and these have to be directly added to foods, as defined by regulating agencies [7]. Each vitamin has a specific set of roles on metabolism: as cofactors for the energy metabolism, or precursors to hormones, antioxidants, cofactors for several enzymes, and also play an important role in the oxidation-reduction reactions [5].

Given this importance for nutrition and health, which is distinctive from other functions of food components (such as sources of energy and biomolecule building blocks), vitamins may be seen as *nutraceutical components* of foods. Several foods or food components may be claimed to be "nutraceuticals", a term coined by DeFelice in 1989 to describe food or food components which provide health care improving the human immunological functions, and so on [8].

The term nutraceutical may encompass a wide range of dietetic supplements, functional foods, herbs and cereals – all that is needed to apply the term is that the food, substance or extract is not specifically a drug, and that it can be claimed to have activity beyond that of sustenance, containing beneficial vitamins, proteins, dietary fibers, *etc*. Moreover, a nutraceutical component could be present on beverages, medicines, supplements and foods [9, 10].

Nutraceutical supplements (*e.g.* concentrated carrot oil, rich in β-carotene) are still subjected to regulations as any other food, but not to specific pharma regulations. Because of this, claims regarding the bioactivity of a food or supplement are poorly regulated. Nutraceutical regulation is, however, being actively discussed [9 - 11] and a common ground will probably soon be reached.

Since there is a broad group of molecules that may be deemed "nutraceuticals", some of these are inevitably discussed in other chapters throughout this book: aminoacids (Chapter 2) may have an anabolic stimulating activity [12]; several colorants (Chapter 7) are also antioxidants or antimicrobials [13, 14]; microbial oils (Chapter 8), being rich in polyunsaturated fatty acids, are antioxidant and some are essential precursors of prostaglandins and cerebrosides [15, 16]; and hydrocolloids (Chapter 9) may be beneficial for the digestion and regulation of fat and glucose absorption [17, 18]; This chapter focuses on the activity and bioproduction of vitamins and selected nutraceuticals.

Vitamins and Nutraceuticals in Foods

Nutraceutical foods contain dietetic fibers, polyunsaturated fatty acids, proteins, peptides, aminoacids, minerals, vitamins and antioxidants such as glutathione or selenium [19]. Although nutraceuticals may be divided into *dietary supplements* and *functional foods,* when considered for their chemical composition or activity, these groups may be divided into more segments. Table **1** summarizes the most important nutraceuticals that are naturally present in foods – which makes their use easily defensible.

Regulation of Vitamins and Nutraceuticals in Foods

Regulatory or advisory agencies such as FDA (the USA Food and Drug Administration), AMA (American Medical Association), IFT (Institute of Food Technologists), FNB (Food and Nutrition Board) and FAO (Food and Agriculture Organization of the United Nations) have recommendations regarding the addition

CHAPTER 6

Biotechnological Aroma Compounds

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Abstract: Aroma is one of the main attributes of foodstuff and thousands of volatile compounds are known, contributing to an innumerable number of aroma characters. Aroma compounds may be recovered from natural sources, chemically synthesized or produced by biotechnological means using microorganism and enzymes. This chapter is devoted to the use of biotechnological tools for aroma production, focusing on examples of commercially relevant aroma compounds which are currently produced biotechnologically and on emerging bioprocess approaches used for the microbial production of aroma compounds. Some case studies will be presented to illustrate some aspects of aroma production.

Keywords: Acetoin, Aroma, Bacteria, Bioaroma, Bioflavor, Bioreactor, Biotransformation, γ-decalactone, *De novo* synthesis, Diacetyl, Esters, Genetic engineering, Flavor, Fungi, Lactones, Natural, 2-phenylethanol, Vanillin, Volatile compounds, *Yarrowia lipolytica*.

INTRODUCTION

The sensory evaluation of foodstuff involves the combination of the main human

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senses. The first impression of a product is, in general, given by sight (color, appearance), which is usually followed by its odor perception (olfaction). During chewing and swallowing, gustation (taste), olfaction (aroma), touch (texture, mouth feel) and hearing (sound of crispness/crunchiness) are also involved. Complementarily, flavor is a perception given by a complex combination of olfactory, gustatory and trigeminal sensations perceived during tasting [1], also simplistically considered as the interaction of aroma and taste [2] (For the multisensory interactions underlying the flavor perception, it is recommended the reading of Auvray & Spence [3]). It is believed that aroma is more important than taste on determining the overall flavor [4]. The distinction of both aroma and taste is evident, at least in terms of complexity. Differently from the taste molecules, which result in a limited number of sensations (sweet, salty, sour, bitter and umami), volatile molecules perceived by olfactory system have numerous aroma descriptors. Therefore, considering that at least 8,000 volatile compounds have already been identified (Table **1**) there are countless possible combinations and intensities (*i.e.* aroma perceptions), resulting in a unique aroma description for each food product. In this sense, aroma may be considered the main element defining the characteristic flavor and the acceptance of food products. Thus, aroma compounds are frequently employed to reinforce or improve the sensory perception of food and beverages.

Table 1. List of all volatile compounds in food in the VCF database [12].

Compounds	**Number of identified compounds**
Hydrocarbons	985
Alcohols	834
Aldehydes	431
Ketones	742
Acids	438
Esters	1551
Lactones	134
Bases	675
Sulfur compounds	798
Acetals	118
Ethers	99

(Table 1) contd.....

Compounds	**Number of identified compounds**
Halogens	112
Nitriles and amides	112
Phenols	260
Furans	370
(Ep)oxides, pyrans, coumarins	302
Oxazol(in)es	90
Anhydrides and phtalides	42
Compounds from sources other than VCF Database	2814

Aroma compounds are volatile molecules (with a molecular weight rarely higher than 300 Da) which are able to sensitize specific cells located at human's olfactory cavity [5], where they arrive from inhaled air (nasal route) or from mouth (orthonasal route), resulting in perceptions called "odor" or "aroma", respectively [6]. These compounds are very potent, with detection thresholds generally in the range of parts per billion (ppb) [7]. As a result, the aroma molecules are usually present as minor components (about 50 ppm), although they may represent up to 50% of the cost of the final product depending on their quality and application (*e.g.* some beverages) [8]. Additionally, these substances do not contribute to the nutritional value of foods, although several biological activities (antimicrobial, antioxidant, somatic fat reducing, blood pressure regulating and anti-inflammatory properties) have been attributed to different aroma compounds [9].

In terms of chemical classification, aroma molecules may present different functional groups: hydrocarbons, alcohols, aldehydes, ketones, acids, esters/lactones and others (Fig. **1**). Among them, esters (and lactones) and carbonyl compounds (aldehydes plus ketones), followed by hydrocarbons and alcohols, are quantitatively the most important ones (Table **1**). The important contribution of volatile terpene hydrocarbons [$(C_5H_8)_n$], *i.e.* mono (n = 2) and sesquiterpenes (n = 3) and their oxygenated counterparts (see some examples in Fig. (**1**)) is worth mentioning, which are usually the main constituents of essential oils [10].

CHAPTER 7

Natural Colorants from Microorganisms

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Abstract: Color additives are a necessity in the modern food industry. These additives are colored because of their peculiar molecular structure, which also frequently imparts other properties such as antioxidant activity. The natural diversity of microorganisms offers several opportunities for the development of "biopigments", which may be produced with high productivity and without seasonality concerns. While the number of artificial colors used foods is inescapably being reduced, that of permitted natural pigments is slowly growing. It is not possible to simply isolate a colored microorganism strain and use it as an additive: the color must be proven safe, and that is why only a dozen pigments from fungi, yeast, bacteria and microalgae are already permitted and are commercially produced. And yet, these few biopigments are paving the way for new developments, where the knowledge that involves microorganism isolation, bioactivity assays, biomass production and fractionation is used for the study of new alternatives. This chapter gives a general view of permitted natural colors, focusing on commercially relevant microbial biopigments and their production processes.

Keywords: *Arthrospira (Spirulina)*, *Ashbya gossypii*, Astaxanthin, Biopigments, *Blakeslea trispora*, β-carotene, *Chlorella*, *Dunaliella*, *Haematococcus*, Microalgae, Microorganisms, *Monascus*, Natural color, Phycobilin, *Porphyridium*, Riboflavin.

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INTRODUCTION

Color is the first sensation perceived by us when we come in contact with food. It is used for consumers to infer product properties: freshness, maturity, composition, and quality, for example. The consumer associates, by experience, cultural heritage and instinct, specific ranges of colors with specific classes of foods. In fact, certain matches such as green bananas or dark meat are immediately associated with unpleasant taste; and unnatural matches such as blue foods are usually repulsive.

As the first quality assessment of a food, color has impact in the other senses as well, influencing, for example, the perception of flavor identity [1]. In fact, color has a subjective effect so strong as to dominate even label information read by the consumer - as tested for drinks by [2] and [3].

Colorants must be added to most processed foods for several reasons, such as to standardize batches with natural variation, to compensate for color fading during processing or storage, to impart expected colors to products (*e.g.* red to an artificially strawberry-flavored candy), to intensify the colors naturally found in food and to protect other food components. However, it shall never be used to mask flaws due to bad processing or manipulation of food products [4].

To be used as food additives, these colorants must be chemically stable and nontoxic. The explosion of synthetic organic chemistry at the end of the 19th century led to the poorly regulated use of dozens of artificial color additives. These were gradually banned during the 20th century. Nowadays, there is intensification towards the use of natural color additives, which are perceived as safer and healthier than its artificial counterparts, as shown by market research [5].

In order to be used as a natural color additive, a colored molecule such as chlorophyll or a carotenoid must usually be extracted and concentrated from the raw material and processed into an additive, perhaps in solution or mixed with bulking agents. Several raw materials – vegetables, fruits, a few animals, and microorganisms - may be processed into color additives.

After a brief general discussion about natural color additives, this chapter emphasizes those produced by microorganisms, its production and uses.

DEFINITIONS, HISTORY AND REGULATION

Any substance may selectively absorb certain wavelengths of the electromagnetic spectrum; when this absorption occurs in the visible light range (wavelengths from 400 to 700nm), we observe color. The absorption of one color leads to the perception of its complementary color: a carotenoid which absorbs blue light, for example, will be seen as a yellow substance (Fig. **1**). Absorption over multiple wavelengths, which is usual for natural substances, creates different tones and shades.

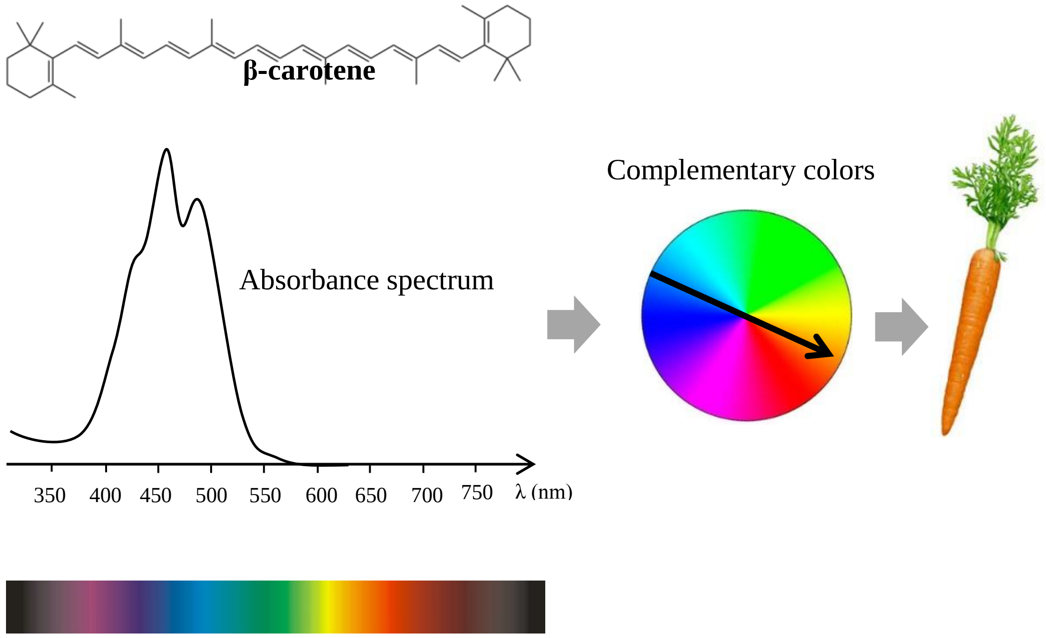

Fig. (1). Colored substance spectrum and color complementarity.

The absorption of radiant energy is central to life, as it is the basis for photosynthesis and vision. Nature offers a large variety of colors on the "warm" side of the spectrum, and substances responsible for the color may be extracted and used as "colorants" or "color additives". The main natural colorants present in foods may be grouped in different classes, based on their chemical structure:

CHAPTER 8

Microbial Single-Cell Oils: Precursors of Biofuels and Dietary Supplements

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Abstract: For centuries, men have used microorganisms for their activities and abilities to produce metabolites of interest such as antibiotics or pigments. Lipids are now under the spotlight as applications can be found in several domains. With the growing awareness of climate change and the depletion of petroleum resources, microbial lipids, which share similar fatty acids profiles with those of vegetable oils currently used in biofuels, compete as potential candidates for the development of green biodiesel. Oleaginous microorganisms can assure this production with substantial productivity, using various low-cost types of substrates. Lipids formed by microorganisms can also be interesting from a dietary point of view, as some microorganisms are able to produce polyunsaturated fatty acids. These PUFAs, such as those belonging to the omega-3 and omega-6 series, are known for their benefits to human health. The use of microorganisms represents a promising way to produce PUFAs at lower cost and with a higher yield. This chapter discusses various potent microorganisms, especially bacteria and fungi, for single-cell oils production designed either for the energy field or the dietary domain, the metabolic ways involved, the culture conditions and the downstream processes of manufacturing.

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Keywords: Alpha-linolenic acid, Arachidonic acid, Biodiesel, Biofuel, Dietary supplement, Docosahexaenoic acid, Edible oil, Eicosapentaenoic acid, Essential fatty acid, Fatty acid, Fungi, Gamma-linolenic acid, Linoleic acid, Lipid, Oleaginous yeast, Omega-3 series, Omega-6 series, Polyunsaturated Fatty Acid, Transesterification, Vegetable oil.

INTRODUCTION

With an ongoing growth of the world's population, planned to reach 9.6 billion in 2050 according to experts, numerous challenges concerning food and energetic demands are expected. Currently, the daily world consumption of petrol reaches 84 millions of barrels, which amounts to 139,000 litres used per second. More than half of this quantity is used to feed vehicle motors.

One tenth is consumed in thermal power stations producing electricity whereas one twentieth is used for heating. However, the heavy exploitation of fossil fuels has led to a decrease in the world reserves. Consequently, a constant increase in the price of oil is observed reaching $108 per barrel in September 2013.

Along with the drop of the fossil fuels reserves come several concerns about climate change. Combustion of fossil fuels causes the production and the release of greenhouse gases (GHGs) into the environment. Carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) are the major GHGs produced. These gases absorb part of the solar rays and redistribute them as radiations in the atmosphere, leading to a rise in the global temperature. That is why the question of petrol replacement by a green substitute is more than ever a burning issue. One of the possible alternatives to traditional fuels is the use of biofuels. Biodiesel is defined as a mixture of fatty acid alkyl esters which can be produced from renewable sources and can be solid or liquid [1]. The technical properties of biodiesel must be the availability [2], lack of negative effect upon the environment, biodegradability [3], low potential risk (no explosive vapour) [4] and easy transportability to existent storage places [3, 5, 6]. With characteristics similar to petrodiesel [7], biodiesel is an attractive alternative in the long run for its biodegradable, nontoxic and clean renewable qualities. So far, the raw materials tested for the production of biodiesel are vegetable and animal oils or fats and waste from cooking oils [8] but industrial biodiesel is mainly produced from vegetable oils (such as wheat, soy, rapeseed). Despite its efficiency, this type of biodiesel cannot be used on a global scale. The use of oleaginous plants to produce biodiesel is a threat to the human population because it diverts edible elements from their food role, creating a food security problem. The SCO (single cell oils) produced by oleaginous microorganisms and similar to vegetable oils appear as a response. They share a similar fatty acids profile as that of vegetable oils and can thus be substituted for petrodiesel after a transesterification step [9].

The other emerging problem will be to provide enough food resources to an increasing population, while these resources are diminishing due to an over-exploitation of the planet: modern agriculture causes the impoverishment of soils, water exhaustion and chemical pollution, putting a pressure on the environment and the ecosystems. Searches for new food sources should particularly insist on finding alternatives to essential nutrients (carbohydrates, lipids, proteins, mineral salts, vitamins) present in "traditional" foodstuff (cereals, meat, milk, fish, vegetables and fruit). Among these fundamental elements, the essential fatty acids are of major importance. The essential fatty acids are polyunsatured fatty acids (PUFAs) which cannot be synthesized by mammals and must be therefore brought by food [10]. Two families of PUFAs exist: the omega-3 family and the omega-6 family. Their health benefits have been described which make them indispensable to a proper development [11]. The fatty acids composing the omega-3 family can be obtained by consuming oily fish such as salmon, white tuna and sardine. The omega-6 family is present in borage oil, evening primrose oil, blackcurrant seeds and also in mother's milk. However, while mammals cannot produce these fatty acids by themselves, some microorganisms possess the ability to synthesize them. This competency creates a new prospect in the domain of food supplements, as we can therefore contemplate the option of producing and administering PUFAs to a larger number of people.

This chapter will discuss the single-cell oils produced by oleaginous microorganisms in particular fungi and bacteria which can be used either in the food industry or in the energy domain. It will present the different types of microorganisms involved in the production of the lipids of interest, their culture conditions and the production strategies of these oils. The downstream process of

CHAPTER 9

Biotechnological Production of Hydrocolloids

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Abstract: Hydrocolloids are an heterogeneous group of long chain polymers which have a variety of structures. Hydrocolloids are used in several industrial sectors. In foods, they are used to control and regulate a colloidal state and help in modifying the food sensory properties. Hydrocolloids are obtained from various natural sources such as seaweeds, plant seeds, tubers, plants, microorganisms, and animals. Due to their importance primarily as food additives, the annual use of hydrocolloids is constantly increasing. In this regard, studies on the use of cheaper sources and production processes are always relevant – this includes the production of hydrocolloids by microorganism cultures. This chapter presents the main hydrocolloids, both natural and biotechnologically produced, their main characteristics, and legal aspects of their application in food.

Keywords: Food additives, Functions of hydrocolloids, Hydrocolloids, Hydrocolloids production.

INTRODUCTION

As a definition, 'hydrocolloid' means particles of 10 to 1000 nm in diameter dispersed in water as a continuous phase [1]. The term can be used to group of polysaccharides and proteins characterized by forming viscous dispersions and/or gels when dispersed in water [2]. Hydrocolloids have different structures with influenced in their behavior in solutions; that are related to differences in relation

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to flexibility, branchings, molecular weight and ionic charge [3]. Besides, the nature of the functions and interactions between protein and polysaccharide may have great variations which affects the interactions in food systems. [4, 5].

Hydrocolloids are used in a variety of industrial sectors. In foods are used to control and regulate a colloidal state, as thickening and gelling, to improve texture and/or viscosity, and stability of food system [6, 7].

Most food additives as hydrocolloids are sold in powdered form and must be dissolved in a liquid before use. Their solubility and dissolution kinetics are thus important factors in the development, formulation and quality control of food hydrocolloids [8]. The characterization of these additives may involve rheological, structural, microscopic and molecular aspects. The rheological aspect is the most important characterization being used as a determinant analysis of the product texture that is directly connected to sensory characteristics and acceptability of the consumer market [2].

One of the main functions of hydrocolloids in food is in the composition and stability of emulsions. Several factors such as temperature, pH, ionic strength and salts, can alter the viscosity of hydrocolloids solutions [9]. Most hydrocolloids can act as stabilizers but only a few can act as emulsifying as this requires an ability to facilitate the formation and stabilization of fine droplets during and after emulsification [10, 11].

Hydrocolloids are obtained from various natural sources such as agar, carrageenan and alginate are from seaweeds, guar gum, locust bean gum, tara gum and tamarind gum from plant seeds, konjac mannan from tubers, starch, pectin and cellulose from plants, xanthan gum, gellan gum, curdlan, dextran and cellulose from microorganisms, gelatin, caseinate, whey protein, egg white protein and chitin and chitosan from animals [12]. Hydrocolloids such as guar gum, locust bean gum, and tara gum are not pure polysaccharides [3]. Commercial carrageenan preparations are often mixed to provide characteristics for specific applications. This occurs because the structures of all naturally occurring polysaccharides in plants vary according to the growth conditions of these plants [13].

The most significant hydrocolloids used in food are pectins, xanthan and alginates [14]. So, this chapter deals with the main hydrocolloids used in food and its principals characteristics.

FUNCTIONS OF HYDROCOLLOIDS IN FOOD PRODUCTS

Food additives are utilized in food formulations for various purposes to enhance properties like flavor, color, shelf life and rheological properties. Hydrocolloids modify the food sensory properties, and are used to improve specific purposes [2]. These functional ingredients are present in dairy and bakery products, canned foods, salad dressings, beverages, sauces, soups and other processed foodstuffs [15].

At relatively low concentrations, hydrocolloids provides viscosity enhancement and/or prevention of sedimentation of dispersed particles. Proteins and hydrocolloids contribute to the microstructural properties of foods, because they can confer structure to the continuous phase of the medium [16, 17].

The functional properties of hydrocolloids include thickening, gelling, emulsifying, stabilization and controlling of the crystal growth of ice and sugar. For thickening, the hydrocolloids commonly are starch, xanthan, guar, locust bean, karaya, tragacanth, arabic gum and cellulose derivatives. The gelling are alginate, pectin, carrageenan, gelatine, gellan and agar [2].

The most important hydrocolloids for foods are starch and its derivatives, pectin, galactomannans, carrageenan, alginate, agar, arabic gum, cellulose and its derivatives [18], gelatin and xanthan gum. For each system, the selection of suitable hydrocolloid depends on functions and desirable properties in foods [19].

In many dairy products, hydrocolloids are adding to control texture interacting with the casein [20, 21]. For applications fermented dairy products, the choice of the proper type and level of hydrocolloid are important factors. In yogurt, suitable hydrocolloids often include carboxymethyl cellulose, pectin, alginate and xanthan gum, the hydrocolloids do not mask the natural flavor of the product [22].

Arozarena *et al.* [23] studied, in yellow cakes, the substitution of egg proteins with use of optimum leavening agent, emulsifiers and xanthan gum. Miller and

CHAPTER 10

Natural Antimicrobial Compounds

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Abstract: Processed foods depend on conservation methods to ensure biological stability of the product until consumption. Foods in general are a rich source of nutrients, and therefore may support the proliferation of opportunistic microorganisms. Most of these microorganisms only reduce the nutritional value and sensorial quality of the product; but in some cases, pathogenic contaminants may also grow. Traditional antimicrobial additives are decreasing in use due to some disadvantages related to physicochemical and sensory aspects, such as undesirable interactions with the food matrix, accumulation into consumer organism and even possible allergic reactions. Besides food traditional methods for preservation such as acidification or reduction of water activity, a possible strategy for increasing food shelf life is the use of natural antimicrobial compounds. Nowadays, the great challenge of the food industry is to make better use of these additives ensuring product integrity and, at the same time, generate minimal residual effects, avoiding undesirable physicochemical and sensorial modifications. In this context, there is an increasing preference of antimicrobial compounds from natural sources (microbial, animal or plant) targeting a wide use in the production of technologically advanced foods which, besides high quality and healthy, must be biologically friendly, meeting the demands of 21st century consumers.

Keywords: Additives, Bacteria, Bacteriocin, Biotechnology, Chelators, Microbial Control, Food Industry, Fungi, Legislation, Lysozyme, Membrane, Microbial Control, Nisin, Organic acids, Pediocin, Preservation, Resistance, Spoilage, Stability, Technology, Yeast.

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INTRODUCTION

Food is susceptible to spoiling, and the need to preserve food quality is indissociable of the commercialization of processed foods. Microorganisms are ubiquitous, and processes guaranteed to reduce the microbial count in foods involve thermal processing, or other sterilization methods to a lesser extent. This means that products such as those minimally processed (*e.g.* vegetables, fruits, meat, dairy products, juices *etc.*) are *expected* to have low levels of contaminants, which may be controlled but not completely avoided during processing. Several traditional foods are stabilized, resulting in controlled development of microorganisms (*e.g.* wine, beer, cheese, yogurt), or results of physicochemical conservation methods (*e.g.* salting, concentrating, drying). With the development of food technology, thermal processing (pasteurizing, sterilizing, or freezing) became popular. However, most of these methods may dramatically alter food flavor or structure. Thus, in the perspective of the hurdle technology [1], food safety can be improved by combination of different food preservation methods, including the use of natural antimicrobial compounds. The combined use of those compounds with adequate methods of food processing can also contribute to reduce the risks of contamination by foodborne pathogens.

Antimicrobial compounds are produced by animals, plants and microorganisms. They occur naturally in foods or may be used as food additives. The first antimicrobial compounds used were spices and extracts from plants such as cinnamon, clove, garlic, mustard and onion [2], followed by fermentation products such as vinegar. With the development of chemistry, even inorganic substances such as hydrofluoric acid, fluorides, chlorates and other chemical compounds were used as preservatives [3]. But the development of efficient food additives was possible only with the advent of antibiotics, – which are highly effective and specific antimicrobial agents.

The use of antimicrobial compounds in foods is tightly regulated, for good reason: besides the usual problems associated with any additive – toxicity in high doses, allergies, other bioactivities – antimicrobial compounds may dramatically alter microbiota, and also may bioaccumulate in the environment. In addition, in the food chain, the selective pressure exerted mainly by inadequate use of antibiotics in primary production is responsible by appearance and spread of bacterial resistance [4]. Therefore, direct and indirect factors, such as cross-contamination during food processing, intentional addition of probiotic or others technological process (*e.g.* fermented foods), are issues for concern.

Actually, it was already known in 1948 that milk from cows treated for mastitis contained enough antibiotics to inhibit cheese-starter cultures [5], and regulation was issued to prevent or alert for the possibility of antibiotics in milk reaching the public at large. There are more than 30 permitted antimicrobial additives for food uses in EU, most of them organic acids or their salts (17), sulfites (8), nitrites (4) or phenols (3). Part of these preservatives is not permitted in processed food itself, but rather as a post-harvest treatment, as is the case of biphenyl or thiabendazole. Table **1**, presents the additives listed by the Codex Alimentarius and their typical dosage.

Table 1. Food additives with antimicrobial (preservative) action. The list covers most additives permitted by regulating agencies worldwide[1,2].

INS	Additive name	Typical maximal dosage (mg/kg)[3]	Applications other than as preservative
200	Sorbic acid	15-3000	Antioxidant, Stabilizer
201	Sodium sorbate		
202	Potassium sorbate		
203	Calcium sorbate		
209	Heptyl para-hydroxybenzoate	12 (EUA, beverages)	
210	Benzoic acid	15-5000	
211	Sodium benzoate		
212	Potassium benzoate		
213	Calcium benzoate		

CHAPTER 11

Use of (Bio) Surfactants in Foods

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Abstract: Surfactants are surface-active microbial products with an amphiphilic structure that allows these to act as emulsifiers, surfactants, foaming and dispersing agents. Biosurfactants have advantages over their chemically synthesized counterparts, such as biodegradability, lower toxicity, activity at extreme temperatures and pH values, besides the ability to be produced using sustainable by-products. Thus, biosurfactants have potential applications in the chemical, petroleum, environmental and pharmaceutical industries. Biosurfactants may also be used as food additives (which are compounds added to enhance the characteristics of food), acting as thickeners, emulsifiers and stabilizers. This chapter discusses the importance of the microbial biosurfactants, their characteristics and potential industrial applications, particularly as food additives.

Keywords: Amphiphilic, Bioemulsifiers, Biosurfactants, Dispersing, Emulsion, Foaming, Food additives, Interface, Solubilizes, Surface-active, Surfactants, Thickeners.

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INTRODUCTION

Surfactants are surface active agents with amphiphilic molecules that have a distinct hydrophilic head moiety, often a sugar or amino acid, and a hydrophobic hydrocarbon/lipid tail moiety. The general ability of surfactants to be located at the interfaces between liquid phases with different degrees of polarity and hydrogen bridges, such as air-water, oil-water or water-solid interfaces, leads to a reduction in surface and interfacial tensions. The presence of both hydrophilic and lipophilic moiety provide the peculiar properties of the surfactants. The hydrocarbon chains are often the non-polar portions, while the polar portions can be ionic, non-ionic or amphoteric [1]. Biological origin surfactants have an advantage over synthetic chemical ones in terms of lower toxicity and biodegradability.

Therefore, surfactants change the surface and interfacial properties of a liquid [2]. These characteristics allow the formation of a microemulsion (oil-in-water or water-in-oil microbubbles) [3, 4]. This triggers industrial applications such as the formation of emulsions, wetting, foaming, solubilisation moisture retention, lubrication, detergency, phase dispersion and viscosity reduction [5 - 8]. Surface tension in an aqueous environment is reduced when the concentration of the surfactant is increased, forming micelles, which are aggregated to the amphipathic molecules. Within aqueous environments, the hydrophilic portion of a micelle is turned toward the outer portion of the molecule and the hydrophobic portion is positioned towards the inner part. The critical micelle concentration (CMC) is defined as the minimum surfactant concentration needed in order to initiate the formation of micelles [9 - 12]. At concentrations higher than the CMC, no further reduction in the surface or interfacial tension is detected. At the CMC, surfactant monomers are spontaneously incorporated into the micelle vesicles or bilayer-structured aggregates, which are produced due to the several weak physicochemical (hydrophobic, van der Waals and hydrogen bonding) interactions [13, 14]. The CMC is the most common measurement of the activity of a surfactant, especially regarding solubility in the aqueous phase [12]. The efficiency of a surfactant is determined by the CMC and this effectiveness reflects the ability of a given surfactant to reduce surface and interfacial tensions [2].

Surfactants have applications in the petrochemical, food, beverage, cosmetic, pharmaceutical, mining, paper, metallurgical, textile and agrochemical industries as well as in the bioremediation of contaminated soil and water [5, 15 - 19]. Moreover, they are used in soaps and detergents [20]. In 2012, the worldwide surfactant production exceeded three million tons a year, with the majority of these products being employed in domestic detergents.

This chapter describes the importance of biosurfactants (mainly those ones with microbial origin), their properties, characteristics and industrial applications particularly as potential food additives.

SURFACTANTS IN FOOD PRODUCTS

Food additives (vitamins, flavours, enzymes, antioxidants, *etc.*) play an important role in the functional properties, stability and quality of foods. For instance, amphiphilic molecules affect the physical and textural stability of the microstructure of a product. Moreover, many types of multifunctional amphiphilic macromolecules are very important as surface-active agents. A large number of researchers have recognised proteins, glycolipids, polysaccharides, lipoproteins and polar lipids as suitable stabilisers, emulsifiers or foaming agents in manufactured and natural foods [21].

Surfactants have been used in foods for centuries [22]. It can be noticed a growing awareness regarding the importance of colloid and surface science in solving technological problems and properties related to food formulas. Foods are mainly comprised of lipids, proteins and carbohydrates in a variety of complex mixtures. Colloidal systems in foods have an infinite number of combinations and are organized in complex microstructures in the form of dispersal agents, foams, emulsions, gels, *etc.* [23]. Such aggregated structures are composed by particles or by the combination of polymers and surfactants and are determined by attraction (van der Waals forces) and repulsion forces. The repulsion force can be electrostatic or steric depending on the food formulation composition. An electrostatic force is caused by charged interfaces. An example of this is seen in ionic surfactants. Steric repulsion is due to adsorbed polymers or vesicles in the continuous phase. An example of such event is a surfactant with a polymeric polar

CHAPTER 12

Production and Applications of Food Enzymes

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Abstract: Enzymes important biocataçysts widely used in food processing and are also useful for generating food ingredients. They may be recovered from animals, plants and mainly microorganisms, which represent the main source of industrial enzymes. This chapter seeks to link the development of new enzymes for food processes involving the requirements of the food industry, including: main companies producing enzymes; main enzymes used for food processing; food enzyme applications, emerging enzymes and production for food processing.

Keywords: L-asparaginase, Baking, Biotechnology, Dairy products, Enzymes, Food processing, Market, Phytase, Starch, Tannase.

INTRODUCTION

Enzyme technology has passed through a maturation phase in which research and development discovered new applications for biocatalysts in chemical, pharmaceutical and food industries. The advances in biotechnology, mainly in genetics, protein engineering and direct evolution have inaugurated a new era for enzymes and its use increased in research and industrial scale due to variety of reactions in environmental conditions. Moreover, the capacity to replace harmful chemical reactions and the trend for developing cleaner technologies enhances the importance of enzymes for the green chemistry and the biobased economy society towards the creation of new processes and product innovation in the market. There are many advantages in using enzymes, including higher product quality,

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lower manufacturing cost, less waste and lower energy consumption [1].

Enzymes have been applied in food fermentation since earliest times for the manufacturing of cheese, sourdough, beer, wine and vinegar, besides non-food products, such as leather, indigo and linen. For their empirical usages, enzymes were generated by spontaneously growing microorganisms or by animal and vegetable preparations, such as calves' rumen or papaya fruit [2].

The commercial production of enzymes for food use have been reported since 1874, when the scientist Christian Hansen manufactured cheese using chymosin obtained from calves' stomachs [3]. This enzyme is currently produced using the recombinant DNA technique with the bovine chymosin gene expressed in *Escherichia coli* K-12. This was the first recombinant enzyme approved by the US Food and Drug Administration (FDA) for use in food [4]. In the 1930s, pectinases were used for clarification of juices, and some years later, invertase was also used for sacarose hydrolysis to produce inverted sugar syrup applying a pioneer process with immobilized enzymes. The application of enzymes in large scale in the food industry began in 1960s, when amyloglucosidase was used for starch hydrolysis to produce glucose syrups [5, 6]. This process was used to substitute the acid hydrolysis due to many advantages, *e.g.* higher product yields, increased degree of purity and easier crystallization. In 1973, the production of fructose syrup became feasible using immobilized glucose isomerase. After that, the industrial application of immobilized enzymes became true.

In the meanwhile, developments of recombinant DNA techniques permitted an improvement in enzyme production by microbial fermentation, increasing microbial stability and altered specificity and selectivity of enzymes. Those techniques showed excellent results to modelling fungi and bacteria for specific enzyme production and are continuing to broaden the applications of enzymes in food technology and different sectors of industry [7 - 10]. The objective of this chapter is to provide an overview on the current enzyme global market and the food applications of enzymes, emphasizing the traditional commercial applications, enzyme production processes and emerging enzymes.

MAIN COMPANIES, COMMERCIAL AND MARKET ASPECTS OF ENZYMES

The necessity of product innovation as a trend for a sustainable market, inspired the technological development stimulating the creation of new applications for enzymes in different industrial sectors in recent years. There are four major sectors of industrial enzymes, *i.e.* detergent (household care), technical (textile, leather, pulp and paper, ethanol and others), food and feed enzymes. The most important enzymes in industrial market are protease, amylase, lipase, ligase, phytase, cellulase, xylanase *etc.* [11]. To illustrate the enzyme market, 75% of industrial enzymes are consumed by food and brewing, feed and detergent industries [12]. Food enzymes constitute the major market share of industrial enzymes. Nevertheless, the segment of technical enzymes has increased fast in last 4-5 years due to the fuel ethanol enzymes (c.a. 11% of the global market in 2009), whose market has increased 15%-20% each year in the last years [11]. In fact, enzyme market has been expanding in recent years. In 2011, this market reached US$ 3.5 billion, with an annual increase of 6.1% [12].

The global industrial enzyme market operates as an oligopoly market, with the presence of three major suppliers: Novozymes, Genencor International (DuPont) and DSM [11]. In 2011, Novozymes and Genencor, presented market share of 47% and 21%, respectively. After several years of development, industrial enzyme market from China has become an important production base with about 10% of the global market. The expectations from the industrial market from China, considering its economic situation and domestic demand, indicates a faster growth rate of 10% or the global 6-8% for the next three years. The Chinese R&D delay and the foreign companies patent monopoly in China market have let the Chinese enterprises with a low competitiveness. Therefore, Novozymes, DuPont (Genencor) and other foreign brands still dominates the Chinese market. However, the Chinese VTR occupy a significant position, with 16.5% (in 2011) in market segments, *e.g.* feedstuff share in Chinese feed enzyme market [12].

Novozymes reported in 2012 that this company still retained its global market share of 47% and that total sales increased by 7% compared to 2011, with an estimated enhancement of 7% for global market for industrial enzymes. The major

SUBJECT INDEX

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