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Review Article

Bioactive Compounds of Vegetable Origin

Sebastian-Cristian Strugari, Monica Butnariu*

Chemistry & Biochemistry Discipline, Life Sciences University "King Michael I" from Timisoara, 300645, Calea Aradului 119, Timis,

***Corresponding Author:** Monica Butnariu, Chemistry & Biochemistry Discipline,Life Sciences University "King Michael I" from Timisoara, 300645, Calea Aradului 119, Timis.

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Abstract

Plants are consecrated sources of pharmaceutical, aromatic and industrial compounds, civilization being inextricably linked to the world of plants, for millennia they have been the major source of bio-products essential for the survival of the entire animal kingdom. True biochemical organisms powered by solar energy, plants synthesize not only the basic compounds necessary for their survival, from the category of glucides, proteins and lipids, but also a wide range of organic substances that can be extracted in sufficient quantities to be of significant importance as raw materials with various scientific, technological and commercial applications. Even today, the botanical endowment of the planet stores numerous resources that are still insufficiently known, which can represent cost-effective alternatives for obtaining scarce raw materials in various economic fields. The vegetable kingdom continues to represent the main supplier of phytochemical compounds used in various industrial branches such as pharmaceuticals, food, cosmetics, agrochemicals, with commercial values expressed in billions of dollars. Plants are irreplaceable sources of industrial oils (volatile and fixed), flavors, perfumes, resins, hydrocolloidal gums, saponins and other surfactants, dyes, pesticides, natural rubber, medicinal substances and many other special compounds.

Key Words: bioactive compounds; phytochemical compounds; essential oils; polyphenols

Introduction

Bioactive compounds (BCs) of vegetable origin are a diverse class of compounds produced by plants that perform many important functions. Unlike primary metabolites, these BCs are not essential for the plant's basic metabolism, but they still perform significant functions that allow the plant to adapt and thrive in its living environment. Because plants lack certain fundamental characteristics that we find in humans and animals, they must have means to deal with additional challenges. For example, plants do not have an immune system to eliminate disease-causing microbes, cannot actively seek a mate for reproduction, cannot run or hide to escape predators, and do not have a skeleton to protect them of injuries. BCs provide a solution to all these problems and more. All plants taken together produce over 100,000 different varieties of BCs that serve a multitude of purposes. The main categories of BCs are terpenes and terpenoids, phenols and alkaloids. Compounds are grouped into one of these categories based on their chemical structure. Among the BCs are also polyphenolic compounds and essential oils (EOs). Polyphenols are compounds containing one or more aromatic rings with one or more hydroxyl groups. They are found in a wide variety of plants and are the most abundant secondary metabolites (SMs). Polyphenols are very rarely found in free form, most of them being isolated in conjugated forms, most often having linked glucosidic groups. BCs in plants include phenolic acids, phenolic alcohols, flavonoids, tannins, stilbenes, and lignans. EOs are complex mixtures of volatile BCs that are found in a wide variety of aromatic plants and are important in plant physiology and ecology. They can be classified into two main groups: hydrocarbons and oxygenated compounds. Hydrocarbons include terpenes, sesquiterpenes and diterpenes, and oxygenated compounds esters, aldehydes, ketones, alcohols, phenols, oxides, acids and lactones. Some EOs may also contain nitrogen or sulfur compounds [1].

As a result of the photosynthesis process, glucides are biosynthesized in green plants: trioses and hexoses. Amino acids, hormones, vitamins etc. are biosynthesized from these substances at the level of the leaves. which are transported through the phloem to all plant cells. At the same time, sucrose is synthesized and transported to all the organs and cells of the plant where it is used as an energy substrate or as a primary product for the biosynthesis of other organic substances characteristic of autotrophic photosynthesizing plants. the substances resulting from these transformations have different roles in plant life: as plastic substances, reserves, active substances or SMs. The biosynthesis, interconversion and biodegradation of glucides, lipids and proteins were described in detail in the previous chapter, so in this chapter references will be made mainly to the biochemical cycles of biosynthesis and intertrans formation of pigments, hormones, terpenes [2].

Bioactive compounds - biochemical cycles of biosynthesis

In the γ-Aminolevulinic acid cycle, which takes place in chloroplasts, $C_{55}H_{72}O_5N_4Mg$ pigments are biosynthesized. The substances that are used in this biosynthetic cycle are: the AA glycine and succinyl CoA, produced in the Krebs cycle. From these two compounds glutamic acid → 5-aminolevulinate → porphobilinogen → uroporfobilinogen → coproporphyrinogen → protopofyrinogen is formed from which $C_{34}H_{34}N_4O_4$ is formed. By incorporating a Mg^{2+} ion, in the presence of chelates, Mg-$C_{34}H_{34}N_4O_4$ results. This turns into Monovinyl protochlorophyllide a [$C_{35}H_{30}MgN_4O_5^{-2}$], which in the presence of light, NADPH, H^+ and protochlorophyllide oxidoreductase forms Chlorophyllide a [$C_{35}H_{34}MgN_4O_5$]. After esterification with phytol ($C_{20}H_{39}OH$) it forms $C_{55}H_{72}O_5N_4Mg$. The metabolism of terpenes takes place in the mevalonate cycle and has as initial product acetyl CoA. In this cycle, monoterpenes, sesquiterpenes, diterpenes, triterpenes and tetraterpenes are synthesized, among which we can mention: linalool, limonene, gibberellins, abscisic acid, ubiquinone, carotenoid pigments, etc. The biosynthesis of these substances is genetically coordinated, by coding the synthesis of the enzymes that catalyse the reactions in this cycle: phytoene-desaturase, phytoene-synthase, hydroxymethylglutaryl-CoA reductase, geranylgeranyl-pyrophosphate synthase, lycopene cyclase, capsanthin-capsorubin synthase. In the first stage of terpene biosynthesis, the formation of 3-hydroxy-3-methylglutaryl-CoA takes place, from three molecules of acetyl CoA, a reaction catalyzed by an enzyme that cofactors with iron and quinone. In the second step, catalysed by hydroxymethyl-glutaryl CoA reductase, mevalonate is formed. Through decarboxylation and phosphorylation, mevalonate yields Isopentenyl pyrophosphate (IPP) (C5), from which Geranyl pyrophosphate (GPP) (C10), Farnesyl pyrophosphate (FPP) (C15) and geranylgeranyl pyrophosphate (GGPP) (C20) are formed. These substances are precursors for the various terpenes. Thus, monoterpenes are formed from GPP: linalool and limonene. Sesquiterpenes and squalene are formed from FPP. Diterpenes are synthesized from GGPP, which is the precursor of gibberellins and abscisic acid, and phytoene, which is the precursor of carotenoid pigments [3].

Localization at the cellular level of the various steps of the terpene synthesis process is difficult. The IPP can be biosynthesized in all cellular structures where terpenes are synthesized. The specific site for the synthesis of monoterpenes is the plastids. The synthesis of farnesyl-pyrophosphate and derived sesquiterpenes, as well as triterpenes including phytosterols, takes place in the cytoplasm and at the level of the endoplasmic reticulum. Diterpenes are biosynthesized in plastids, where the activity of GGPP synthase was identified. In chloroplasts, the activity of Ent-kaurene ($C_{20}H_{32}$) synthase was identified, which catalyzes the synthesis of $C_{20}H_{32}$, the precursor of cytokinins. Carotenoid pigments and tocopherols are also biosynthesized in chloroplasts, and ubiquinone is biosynthesized in mitochondria and microsomes. Diterpenes are biosynthesized in plastids, where the activity of GGPPS was identified. In chloroplasts, the activity of $C_{20}H_{32}$ synthase was identified, which catalyzes the synthesis of $C_{20}H_{32}$, the precursor of cytokinins. Carotenoid pigments and tocopherols are also biosynthesized in chloroplasts, and ubiquinone is biosynthesized in mitochondria and microsomes. Terpene biosynthesis occurs in all plant cells. In the case of species that synthesize large amounts of terpenes, their synthesis takes place in specialized cells such as the resiniferous canals in pine leaves or the resiniferous cells isolated from Thuja. In the case of angiosperm plants, monoterpenes are synthesized in glandular hairs on Mentha leaves, in petals or pistils of flowers (e.g., linalool), and latex in laticifers. Numerous terpenes with 10 or 15 carbon atoms, with a high degree of volatility, are known as volatile oils and give the characteristic aroma to some plant organs: flowers, fruits, seeds, etc. Sterols are made up of 5 isoprene units, such as: cholesterol, sitosterol, stigmasterol and campesterol and are found in the composition of plasma membranes, having a role in regulating their permeability. Some isoprenoid compounds secreted by the roots of plants are toxic to the roots of other plants, being considered allelopathic substances. The shikimate pathway (shikimic acid pathway) (SA pathway) has as its substrate erythroose-4-phosphate, produced in the pentose phosphate pathway (PPP). It results in phenols, lignins, anthocyanins and phenolic AAs such as tryptophan from which auxin is biosynthesized. It is considered that under normal conditions approximately 20% of the carbon fixed by plants is used in the SA pathway [4].

In this cycle, phosphoenolpyruvate and erythrose-4-phosphate condense into a compound with 7 carbon atoms: 3-deoxy-D-arabino-heptulosonate-7-phosphate (DHAP), resulting in chorismate, in the following way: DAHP → 3 dihydroquinate → 3 dihydroshikimate → shikimate → chorismate. $C_{10}H_{10}O_6$ represents the initial compound for 3 cycles: - from the first, phenylalanine, lignins and flavonoids result, - from the second, tryptophan, auxins, glucosinolates, phytoalexins and alkaloids - from the third, tyrosine and melanins. The key enzyme of the SA pathway is phenylalanine ammonium lyase (PAL), which is linked to the membrane of the endoplasmic reticulum, chloroplasts, mitochondria and plasma membranes. Mechanical injuries and pathogenic attack induce the formation of mRNA that encodes the synthesis of DAHP synthase, stimulating the SA pathway. The enzymes that catalyze the reactions in this cycle are synthesized in the ribosomes in the cytoplasm. The activity of these enzymes has been highlighted in chloroplasts and its identification in the cytoplasm is uncertain. Part of the AAs that are formed in this cycle are, in turn, precursors of other substances. Tryptophan is the precursor of the growth hormone auxin, phenylalanine is the precursor of flavonoid pigments and lignins, tyrosine is the precursor of ubiquinone, a substance transporting electrons in the respiration process. In the SA pathway, which takes place in the endoplasmic reticulum and in the cytoplasm, phenolic acids are also biosynthesized: p-coumaric, cinnamic, caffeic, ferulic, chlorogenic, as well as gallotannins. Phenolic substances can stimulate or inhibit the action of hormones, inhibit the synthesis of ATP in the mitochondria, as well as the activity of some enzymes, or of the cytoplasmic currents in the cells of the absorbing hairs [5].

Some phenolic substances (ferulic, lunularic, chlorogenic acid and catechins) have an inhibitory effect on seed germination. Through the oxidation of substances such as $C_9H_{11}NO_3$ from Solanum tuberosum tubers, $C_8H_{11}NO_2$ from Musa acuminata, phenolic acids (PAs) from Malus domestica, black melanins [$C_{18}H_{10}N_2O_4$] are formed in the presence of phenolases, which give the characteristic color to mechanically damaged or senescent fruits. Some phenols are allelopathic substances: $C_{10}H_6O_3$ produced by Juglans regia, $C_7H_6O_3$ produced by Quercus falcata, $C_{10}H_{10}O_4$ produced by Adenostoma, etc. $C_9H_8O_2$ derivatives were identified in vacuoles and chloroplasts. The enzymes involved in the biosynthesis of flavonoid substances: chalcone-flavone isomerase and flavonoid hydrolase, were identified in the endoplasmic reticulum and in chloroplasts, and the flavonoid substances were identified in vacuoles, the extraplasmic space and chloroplasts.

The biosynthesis of flavonoid substances is genetically coordinated. Anthocyanins can be synthesized in any plant cell, being located in the cytoplasm for monomers, dimers and trimers and in vesicles, improperly called anthocyanoplasts, for the final products. The formed anthocyanins are transported from these vesicles, into the vacuole, through a process of pinocytosis, and the membranes of the vesicles can be incorporated into the tonoplast. Organic acids result from the Krebs cycle, and phenolic substances from the SA pathway [6].

Alkaloids are heterocyclic nitrogenous substances, which are found in over 13,000 species. Most plant alkaloids come from amines or AAs and only a part come from isoprenoid precursors in which nitrogen is incorporated in a late stage of the biosynthetic cycle. This is the case of $C_{45}H_{73}NO_{15}$ from Solanum tuberosum and $C_{27}H_{45}NO_2$ from Lycopersicon esculentum. Alkaloids were identified in vacuoles, chloroplasts and the extraplasmic space. The enzymes involved in the synthesis of these substances were identified in the membranes of the endoplasmic reticulum, in the plasmalemma and in the tonoplast. Among the alkaloids, nicotine has the best studied biosynthetic cycle. The primary compounds are $C_6H_{14}N_4O_2$ and $C_5H_{12}N_2O_2$ which are decarboxylated and metabolized to the conjugated form of $C_4H_{12}N_2$. The biosynthesis of $C_{10}H_{14}N_2$ takes place in small vesicles, which come from the endoplasmic reticulum or the Golgi complex and which contain the enzymes involved in this process. The membrane of these vesicles is permeable for tertiary compounds that can be synthesized in other sites. The formed quaternary compounds are released in the vacuole, following its fusion with vesicles. Cyanogenic glycosides (CNglcs) such as $C_{20}H_{27}NO_{11}$ and $C_{14}H_{17}NO_6$ were identified in the cell vacuoles, and the enzymes involved in the synthesis of some CNglcs were identified in the endoplasmic reticulum membrane. CNglcs have been identified in over 1,000 species, 500 genera and 100 families of plants. Among these substances, the best known are: $C_{20}H_{27}NO_{11}$ and $C_{14}H_{17}NO_6$ from Rosaceae and $C_{14}H_{17}NO_6$ from Caprifoliaceae. Biogenic amines are widespread in plants both as simple amines (primary, secondary, tertiary) and as amines with different functional groups (alcoholic, phenolic, carboxylic, etc.). Among the amines, $C_4H_{12}N_2$ is important, which is formed from $C_5H_{12}N_2O_2$ (in Pisum, Nicotiana), $C_5H_{14}N_2$, which is formed from $C_6H_{14}N_2O_2$ (in Lupinus, Pisum), $C_{10}H_{12}N_2$, which is formed from $C_9H_{11}NO_3$ (in Hordeum, Lolium), $C_8H_{11}NO_2$, which is formed from L-DOPA, $C_9H_{11}NO_4$. $C_4H_{12}N_2$, $C_{10}H_{26}N_4$ and $C_7H_{19}N_3$ interact with nucleic acids and can thus be involved in protein biosynthesis. $C_4H_{12}N_2$, cadaverine, $C_{10}H_{26}N_4$ and $C_7H_{19}N_3$ in a concentration of 10-4 - 10-6 M stimulate the growth process, and $C_8H_{11}NO_2$ is the precursor for the formation of melanoid compounds in bananas. The volatile substances that give fruit aroma are intermediate compounds of metabolism: alcohols, aldehydes, ketones, esters, ethers, etc., substances with a high degree of volatility [7].

Plants are irreplaceable sources of EOs (volatile and fixed), flavors, perfumes, resins, hydrocolloidal gums, saponins and other surfactants, dyes, pesticides, natural rubber, medicinal substances and many other special compounds. The best-known phytochemicals include drugs such as morphine and codeine (analgesic alkaloids derived from the latex of Papaver somniferum), cocaine (local anesthetic alkaloid derived from coca leaves), quinine (antimalarial alkaloid derived from Cinchona bark), curare and digitalin; perfumes and essences such as rose oil and jasmine; industrial raw materials such as fatty acids, pine oil and natural rubber; pesticides such as pyrethrins from Chrysanthemum cinerariifolium and nicotine. Although there are often relevant differences in the synthesis and accumulation of BCs in different tissues or in different phases of plant development, the genome of each cell contains the necessary information to trigger the full potential of BCs characteristic of the species [8].

Metabolism consists of closely coordinated series of chemical reactions mediated by enzymes that take place within the plant organism, resulting in the synthesis and use of a wide variety of molecules from the category of glucides, AAs, fatty acids, nucleotides and polymers derived from them (polysaccharides, proteins, lipids, DNA, RNA, etc.). The totality of these processes is defined as primary metabolism and the respective compounds, which are essential for the survival of the plant, are described as primary metabolites. In addition to the "primary" metabolites, with a major role in maintaining the viability of the plant (proteins, glucides and fats), a series of compounds including terpenes, steroids, anthocyanins, anthraquinones, phenols and polyphenols are synthesized, which belong to the so-called metabolism "secondary". BCs are present only in certain species, often exhibiting organ or tissue specificity, can be identified only at a certain stage of growth and development within a species, or can be activated only during periods of stress, caused by e.g., by the attack of some microorganisms or the depletion of nutrients. Their synthesis seems without direct significance for the synthesizing cell, but it can be decisive for the development and functioning of the organism as a whole. Although their synthesis is not an indispensable part of the program of gene expression and development, these metabolites are not simple catabolic products, because they have a very diversified structure and can often be re-included in metabolic processes. In fact, the demarcation between primary and BCs is uncertain, as many of the intermediates of primary metabolism fulfill similar roles in BCs as well. Thus, some obscure AAs are clearly SMs, while sterols are essential structural compounds of many organisms and must therefore be considered primary metabolites. The overlap of the roles of many compounds ensures a close interrelationship between primary and BCs, and the interpretation of the demarcation between these processes must be done with caution. BCs are compounds that are derived through pathways from primary metabolic pathways and are not essential to sustain cell life. These compounds do not have continuous production. Very often, BCs are produced during the non-growing phase of cells. BCs are the end products of primary metabolites such as alkaloids, phenols, steroids, essential oils, lignins, resins and tannins, etc. [9] What are the differences between primary metabolites and BCs?

1. Unlike BCs, primary metabolites are essential for cell growth and are directly involved in metabolic reactions such as respiration and photosynthesis.

Most primary metabolites are identical in most organisms, while BCs are numerous and widespread, unlike primary metabolites.

2.BCs are derived by pathways involving primary metabolites. Therefore, BCs are considered to be the end products of primary metabolites.

3.Primary metabolites are produced during the growth phase of the cell, while BCs are produced during the non-growth phase of the cell.

4.BCs are accumulated by plant cells in very small amounts than primary metabolites.

5.The phase of growth in which primary metabolites are produced is sometimes called "trophophase", while the phase in which BCs are made is called "idiophase".

6.Most BCs are involved in defense reactions, unlike primary metabolites.

7.Proteins, carbohydrates and lipids are the main primary metabolites, while BCs are alkaloids, phenols, sterols, steroids, essential oils and lignins, etc. [10].

Plant colors are BCs of food products that are not consumed as food and are not used as ingredients to produce a food. The category of natural dyes includes preparations obtained from food raw materials and other edible natural sources obtained by physical and/or chemical extraction methods, leading to a selective extraction of pigments in relation to nutritional or aromatic constituents. Chlorophylls, carotenoids, anthocyanins, and betanin are four main classes of plant-derived pigments that account for most natural dyes. Chlorophylls are green pigments present in all photosynthetic plants and algae. The main sources used for the production of these natural dyes are alfalfa, spinach, nettles, etc. This pigment formed is sensitive to light, pH and heat. Carotenes can be extracted from a large number of sources, including algae, carrots and palm oil, or produced by fermentation of microorganisms. Small carotenes are a combination of α-carotene and ß-carotene. ß-Carotene is one of the most common carotenoids, being widely used in the food industry as a food coloring. [11].

Lutein is a carotenoid pigment found in high concentrations in green leafy vegetables, alfalfa and the petals of marigold (Tagetes erecta L.). The chemical structure of lutein is similar to that of ß-carotene, but it has better stability to heat treatments and oxidation. Discoloration can be prevented or reduced by adding antioxidants in food manufacturing technologies. It is stable to pH variations, with a constant color shade. Lycopene is a carotenoid pigment with a structure similar to carotene that is found in small amounts in many fruits and vegetables. Tomatoes are the main source of this pigment. In the presence of heat and fat, the lycopene crystals dissolve, which leads to a change in color from red to an orange hue. The color change depends on the fat content, the saturation level of the fat and the treatment temperature. Anthocyanins are the largest group of water-soluble pigments in the plant kingdom, belonging to the flavonoid family. The color of anthocyanins depends on their pH. As the pH value increases, the color of anthocyanins changes from red to blue through purple. Anthocyanin pigments are water soluble and able to withstand short periods of moderate heating. Anthocyanins will discolor on exposure to light over time. [12].

Almost 300 anthocyanins with different chemical structures are described in the specialized literature. Betanin is a water-soluble, heat-sensitive pigment and may be prone to oxidation in foods with high water activity. Optimum stability of betanin is at pH 4.5. The main edible source of betanin is beetroot. This pigment is recommended for food products with a short shelf life. At high sugar content and low water activity, betanin pigment is more stable to heat treatments. To prevent oxidation of betanin and loss of color, antioxidants can be included in foods. BCs are used in alternative medicine, having beneficial effects on health. The process of replacing synthetic dyes by natural food dyes in food is quite complex and depends on the food matrix, ingredients, pH, exposure to heat treatments, light and oxygen. BCs significantly lose their effectiveness during processing and storage. Heat treatments are the main cause of the destruction of natural antioxidants. [13].

The oxidative degradation of BCs depends on the value of the redox potential, the composition of food matrices, the concentration of oxygen, the activity of water and the degree of thermal processing. Epoxy carotenoids are less sensitive to thermal treatments such as lutein, - and β-carotene, lycopene, phytofluene. The decrease in the content of L-hydroascorbic acid and total polyphenols depends on the quality of the raw material, the processing methods, the duration and conditions of storage and the quality of the packaging. Important factors influencing the stability of anthocyanins are enzymes, copigmentation, oxygen and L-ascorbic acid, sulfur dioxide, pH, metal ions, water activity, sugar content, temperature and light. [14].

EOs are generally liquid substances with an oily appearance, insoluble in water, soluble in alcohol and organic solvents. They have the smell of the volatile substances they contain, which give the characteristic fragrance to plants, flowers, fruits, seeds, bark of trees. From a chemical point of view, EOs are complex mixtures of aliphatic and aromatic hydrocarbons, aldehydes, alcohols, esters and other constituents, but compounds from the terpenoid class predominate. Although they are called oils, these substances do not contain fatty substances: a drop of essential oil placed on a sheet of paper will not leave a mark, unlike a drop of vegetable oil. Essential oils contain several hundred different types of molecules, each with specific properties (antiseptic, antibacterial, immunostimulatory, decongestant, etc.). [15].

For example, sage contains 250 different molecules, of which 75% are from the ester family and 15% are monoterpenes. Their molecules act in synergy, which explains the polyvalence of essential oils and their broad spectrum of action. Once the properties of the family of compounds and their concentration in essential oils are known, their effects (beneficial or harmful) can be determined. The biological properties of EU are determined by their main components [16]. The widespread application of EU in aromatherapy reveals their effect on the immune system of the human body. Given the fact that essential oils are natural antioxidants and possess properties to eliminate radicals that form in the body under the action of stress, including ionizing radiation (IR) [17], it allows their use in reducing the effects of IR action by stimulating the system immune. Unlike many other preparations, used to activate the immune system, EU in low concentrations exhibits a long-lasting immunostimulating action, side effects being absent [18]. EOs of basil (Ocimum basilicum L.), jasmine (genus Jas minum L.), clove (Syzygium aromaticum L., Merrill & Perry L. M.) and sage (Salvia officinalis L.) are known to possess a wide range of properties immunomodulators. Laurel EOs (Laurus nobilis L.) acts selectively in the formation of T-cell immunity, while the oils of eucalyptus (genus Eucalyptus L.), fir (genus Abies Mill), anise (Pimpinella anisum L.) and St. John's wort (Artemisia abrotanum) L.) acts selectively on cells of the B immune system [19].

Conclusions

Research conducted in the last decade reveals the effect of BCs of plants – EOs and plant extracts – on the immune system and some indicators of the health of animal and human organisms. Since BCs are natural antioxidants and have properties to eliminate radicals that form in the body under the action of stress, including ionizing radiation, they can be used to reduce the effects of ionizing radiation by stimulating the immune system. Unlike many other preparations used to activate the immune system, BCs in low concentrations have a long-lasting immune stimulating action, and no side effects have been established during their administration.

Conflicts of interest

The author declares: no conflicts of interest.

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