Biopolymer-based Techniques for Encapsulation of Phytochemicals Bioacive in Food and Drug

OLIMPIA BUZIA DUMITRIU[1]*, STEFAN DIMA[2]

[1] Dunãrea de Jos University of Galati, Faculty of Medicine and Pharmacy, 3 Al. I. Cuza Str., 800017, Galati, Romania

[2] Dunãrea de Jos University of Galati, Faculty of Sciences and Environment, 111 Domneasca Str., 800008, Galati, Romania

Phytochemicals are biologically active chemical compounds found in plants. They were identified as displaying bioactivity in the prevention of cancer, heart disease and other diseases. The most important classes of phytochemicals are: polyphenols, carotenoids, essential oils, phytosterols etc. The encapsulation of phytochemical compounds in different matrices is required in order to prevent the degradation of phytochemical compounds under the influence of some factors such as oxygen, light radiations, pH and high temperature. Biopolymers with new functional properties, i.e. biodegradable, biocompatible and stimulus responsive, have been tested for encapsulation of phytochemicals bioactive. In this review, we are showing the most important biopolymer classes used as wall in the preparation of drug and food phytochemicals micro and nanoparticles.

Keywords: phytochemicals, polyphenols, carotenoids, essential oils, phytosterols

Lately, the requirements regarding the production and consumption of foods have considerably changed. Contemporary consumers are now aware that foods do not have solely the role of suppressing in the feeling of hunger bat they are also responsible for their health. International bodies such as World Health Organization (WHO) and Food and Agriculture Organization (FAO) have established recognition norms for the role of functional foods and alimentation in the prevention of some diseases such as: cardiovascular disease, diabetes, obesity, osteoporosis, cancer etc. [1]. In this context, the researches and food manufacturers have intensified their preoccupation towards the obtaining of innovative food products, using the special technologies which prevent the degradation of bioactive components, such as encapsulation, matrices and films immobilization etc.

Nowdays, the vegetal extracts are highly used in food production and in medicine as remedies for different diseases. All vegetables, fruits, and whole foods possess the thousands of potentially healthful constituents [2, 3]. Many researchers have associated the specific groups of phytochemicals with decreased risk of chronic degenerative disease .A number of in vitro and in vivo studies have been performed to confirm that consumption phytochemicals and phytochemical-rich products showed anti-cancer, anti-inflammation, and anti-diabetes, anti-obesity properties [4-6].

The phytochemical components are highly sensitive to the action of oxygen, light radiations, pH and high temperature. Also, some phytochemicals have a low water solubility, a poor stability, and a low absorbtion rate in the gastrointestinal tract (GIT). Therefore, the encapsulation of phytochemicals in different matrices is required in order to prevent these inconveniences.

In the present work, we have summarized the use of biopolymers to phytochemicals functionalization through various encapsulation techniques .

Phytochemicals in food and drug

Phytochemicals are plant chemicals that have protective or disease preventive properties, but are not essential nutrients. The potential effect of individual phytochemicals is based on the health effects of foods, because the most foods, such as whole grains, vegetables (cauliflower, cabbage, carrots, tomatoes, potato, broccoli), beans, fruits (blueberries, cranberries, cherries, apple) and herbs, contain many phytochemicals. It is recommended take daily at least 5 to 9 servings of fruits or vegetable. The main classes of phytochemicals are: polyphenols, carotenoids, phytosterols, organosulphurs etc. Each group of phytochemicals, exhibit a special bioactivity (table 1). Polyphenols are the most complex group of phytochemicals they belong: phenolic acids (chlorogenic acid and related compounds), flavonoids (flavonols, flavones), flavonols (quercetin and related compounds), flavones (luteolin and apigenin) and anthocyanins [7, 8]. Sources of polyphenols include tea, grapes/wine, beer, olive oil, coffee, chocolate, peanuts, and other fruits and vegetables. The health benefits of polyphenols is due of antioxidant, anti-inflammatory, antibacterial, antiviral properties. Most important carotenoids are. alpha-carotene, beta-carotene and lycopene; they are found in large quantities in carrot and tomatoes. Carotenoids have antioxidant activity and reduce the risk of developing certain types of cancer [9-12].

Some plants from *Allium* species, such as garlic, onion, shallot and leek contain organosulphurs (allicin, allin and other thiosulphinates) which decrease the low density lipoprotein (LDL).

Biopolymer-based methods for encapsulation of phytochemicals bioactive

Encapsulation is an innovative technology aimed at increasing the functionality of materials with applications in a wide range of areas: medicine, pharmacy, food industry, agriculture, etc. [3].

This technology achieves the entrapping of a solid, liquid or gaseous substance, called encapsulate, active, core, payload, fill or internal phase into another substance, called shell material, wall material, carrier material, coating material, external phase or support phase, under the form of particles of various sizes [14]. The particles with sizes

* email: buzia_olimpia@yahoo.com, Tel.: 0746222264

Source food	Phytochemicals	Health benefits
Carrots, tomatoes and tomato products	Carotenoids (lycopene, beta-carotenes)	Neutralization of free radicals that cause cell damage
Garlic, onions, leeks, olives, scallions	Sulfides, thiols	Decrease in LDL cholesterol
Broccoli and other cruciferous vegetables such as kale, horseradish	Isothiocyanates (sulforaphane)	Neutralization of free radicals that cause cell damage and protection against some cancers
Red Wine, Grape Juice, Grape Extracts, Cocoa	Proanthocyanidins and flavan-3-ols; Resveratrol,	Inhibition of LDL oxidation, inhibition of cellular oxygenases, and inhibition of proinflammatory responses in the arterial wall
Strawberries, Red Wine, Blueberries	Anthocyanins	Improvement of vision, inhibition of nitric oxide production, induction of apoptosis, decreased platelet aggregation, and neuroprotective effects Enhanced relaxation of calf aortas
Soy Beans, Soy Milk, and Tofu	Isoflavones (Genistein and Daidzein)	A reduction in blood pressure and increased vessel dilation
Aromatic plants (essential oils)	Terpene, perpenoide	Flavors, anti-inflammatory, antibacterial, antifungal, analgesic, sedative, spasmolytic etc properties; anticancer, antiviral and antidiabetic activities
Wheat bran	Sphingolipids, Phytosterols, Lignans, Phenolic acids	Protects against colon tumor

Table 1
POTENTIAL HEALTH BENEFITS FROM SOME PHYTOCHEMICAL COMPOUNDS

between 1nm and 100 nm are considered nanoparticles, and those with sizes between 100 nm and 1000 nm are considered microparticles [14, 15].

In terms of morphology, micro- and nanoparticles can be of two types: micro and nanoparticles type reservoir (or capsules) in which the active substance is included in a homogeneous the core/cores (microreservoirs), surrounded by a protective membrane, (fig.1a,b), and micro- and nanoparticles type matrix, called micro- and nanospheres, in which the active substance is dispersed in polymeric network spaces (fig 1.c).

Out of the many causes imposing the encapsulation of phytochemicals, the most important refer to: the protection of phytochemicals bioactive against environmental factors, such as: temperature, light, oxygen, enzymes, moisture, microorganisms, etc; the increase of the solubility of bioactive compounds; the isolation of bioactive molecules from the molecules in the drug and food matrix they may react with; the controlled release of bioactive components. (controlled-release drug delivery systems and targeted drug

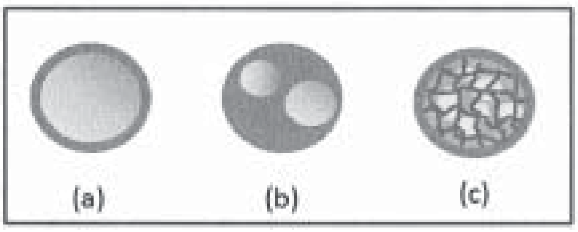

Fig.1. Schematic representation of microcapsules morphology: a, b) reservoir type microcapsules; c) polymer matrix (microspheres)

delivery systems); the increase of the bioavailability of bioactive compounds, due to the increase of bio-accessibility, as well as the absorption of biomolecules through biological barriers; masking the unpleasant taste and odour of some phytochemicals, such as vegetal peptides, saponins, flavones, etc; the conversion of liquids into free flowing powders whereby the biocomponent's mass and volume are reduced, and consequently the storage and transportation costs are cut down [14].

The carrier material influences the encapsulation efficiency, the microparticle characteristics, and the bioavailability of bioactive components.

The manufacture of micro and nanocapsules in food and drug industry employs carrier materials providing food safety (GRAS-Generally Regarded As Safe).

The carrier materials must be not toxic, tasteless and odourless, easily dispersible in solvents (water, ethyl alcohol). They must have emulsifying properties, ability to form thin or elastic films and a good resistance to physico-chemical agents: light, temperature, *p*H, ionic strength, enzymes, oxygen, moisture.

Biopolymer-based nanoparticles and microparticles are prepared by means of the main classes of natural biopolymers, generally recognised as safe, such as proteins and polysaccharides [14, 16]. Both polysaccharides and proteins provide a good protection for the bioactive compounds sensitive to physico-chemical factors and allow their controlled release into the gastrointestinal tract (GIT). For example, pectin may be used to manufacture polymer particles to release bioactive compounds in the colon, as it is not digested by the enzymes in the stomach, nor those in the small intestine, but it is easily degraded by the microflora in the colon [17].

Biopolymer nanoparticles or microparticles may be formed of individual polymers, or mixtures of biopolymers. The formation of polymer particles out of a single polymer is based on the assembly of polymer chains into a tridimensional network (gel) in whose pores various colloidal systems (conventional emulsions, nano-emulsions, microemulsions, solid lipid particles, liposomes) containing bioactive compounds may be entrapped [16, 22].

Globular proteins are jellified by heating at a temperature above the thermal denaturation temperature, when the self-assembly of polymeric chains occurs by hydrophobic interactions or disulphide bonds. Other proteins jellify by the variation of *p*H, ionic strength, or in the presence of chemical compounds (multivalent ions, glutaric aldehyde, genipin) or enzymes, such as transglutaminase. Casein jellifies by adjusting the *p*H in the proximity of the isoelectric point (*p*H 4.6), by adding Ca^{2+} ions or by adding rennet. The most commonly used proteins in manufacturing polymer particles are: gelatine, whey protein (β-lactoglobulin, α-lactalbumin), bovine serum albumin, casein, soy proteins, zein, gliadin [17, 18].

Polysaccharides jellify by three main mechanisms: ionotropic, cold-set and heat-set gelation [14, 19].

Ionotropic gelation is specific to polysaccharides whose structure consists of repeating different types of sugar units on which ionic groups ($-COO^-$, $-OSO_3^-$; $-NH_3^+$) are grafted.

Alginate, which is a block-copolymer formed of β-D-mannuronic acid and α–L-guluronic acid residues and pectin, which is a linear polysaccharide mainly formed of α(1-4)-D-galacturonic acid residues, form gels in the presence of multivalent cations (Ca^{2+}, Mg^{2+}). Alginate hydrogel particles have a *p*H dependent behaviour, allowing the release of bioactive compounds in certain GIT sectors. So, at a low *p*H (e.g. stomach) alginate beads shrink because of the protonation of the carboxyl groups and do not allow the release of biocomponents, while at a high *p*H (e.g. small intestine), the polyanionic chains electrostatically reject each other because of the large number of ionised carboxyl groups, while the alginate beads swell and favour the release of biocomponents [20, 23].

Carrageenan is another type of linear anionic polysaccharide in whose structure there are α-1,4 and β-1,3 linked anhydrogalactose residues with a different content of sulphate groups. According to the number of sulphate groups, there are many types of carrageenan, among which the most important are kappa (κ-), iota (ι-) and lambda (λ-) carrageenan. Due to the different number of negative charges, carrageenans have different structures, and different gelation mechanisms respectively. Thus, for ê-carrageenan gelation occurs in the presence of potassium ions, while for γ-carrageenan gelation occurs in the presence of the calcium ions. By heating, κ-carrageenan forms strong and rigid gels, and γ-carrageenan forms soft and elastic gels. λ-carrageenan does not form gels [21, 23].

Chitosan is a linear polysaccharide made up of units of glucosamine and N-acetylated glucosamine. It is a non-toxic, biocompatible and biodegradable polymer, with bioadhesive and antibacterial properties, obtained by the alkaline deacetylation of chitin. Its properties depend on the deacetylation degree. It is soluble at low *p*H, when the amino groups are protonated and may react with polyanions (alginate, carrageenan, tripolyphosphate) forming *p*H-sensitive hydrogels. The chitosan hydrogel swells at low *p*H due to the electrostatic repulsion among the protonated amino groups, and at high *p*H chitosan precipitates. This behaviour allows the use of chitosan in the manufacture of gastric delivery systems [21, 23].

In addition to the polysaccharides described above, the manufacture of polymer particles may also use other polysaccharides, such as: starch and derivatives, cellulose and derivatives, arabic gum, locust gum, dextran, inulin, agar etc.

To preparation the biopolymer-based micro-and nanoparticles, the mixtures of polyelectrolites with an opposite electrical charge, such as proteins and polysaccharides may be used.

Due to their zwitterionic structure, proteins are polyelectrolytes whose electrical charge is pH dependent. The pH value where the proteins' net electrical charge is zero is called isolelectric point (pI). At *p*H < < pI, proteins have positive net charge and form precipitates with anionic polysaccharides. Such phenomena also occur when anionic polysaccharides are mixed with cationic polysaccharides: e.g. the chitosan mixed with carboxy-methylcellulose (CMC), xanthan, carrageenan, alginate (extracted from brown algae), pectin, heparin, hyaluronan, sulfated cellulose, dextran sulfate, chondroitin sulfate etc.

The formation of non-covalent protein-polysaccharides complexes is a method to diversify the types of particles applied to functional food production [16].

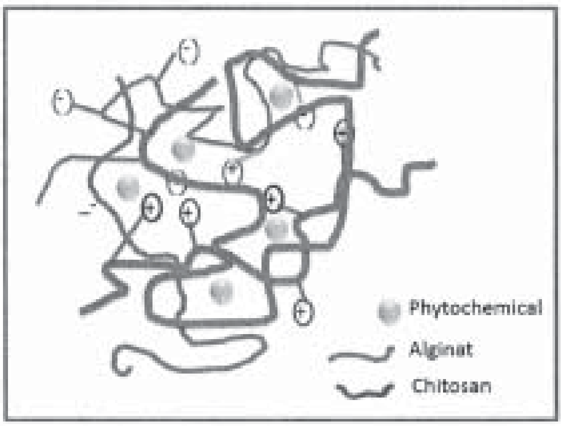

Fig.2. Chitosan/alginate polyelectrolyte complex (complex coacervates)

The preparation of biopolymeric-based micro and nanoparticles employs a large variety of techniques. The following will briefly describe the main methods of entrapping phytochemicals bioactive into polymeric particles, applied to functional food production.

External gelation is a simple method of preparation micro and nanospheres loaded with hydrophilic or lipophilic phytochemical bioactive. The encapsulation of bioactive components by external gelation method takes place by covering the following stages: a) preparing biopolymer solutions (alginate, pectin, κ-carrageenan, chitosan) in which phytochemicals bioactive (polyphenols, essential oils, carotenoids, etc.) are included; b) dropping droplets of biopolymer solution and bioactive into a gelation bath, by means of various devices: pipette, syringe, vibrating nozzle, spraying nozzle, jet cutter, atomizing disc, electric field, coaxial air flow and concentric nozzle; c) gelation of polymer droplets which may occur in the presence of multivalent ions (Ca^{2+}, Mg^{2+}, K^+, sodium tripolyphosphate); d) separation, drying and storage of polymer microspheres. This method yields microspheres with sizes ranging within 10-800μm. The characteristics of the microspheres obtained by extrusion are influenced by the viscosity of the biopolymer solution, the concentration of the gelation agents, the dropping method [22].

Emulsion-internal gelation method, is applied to the encapsulation hydrophilic biocomponents. The method is based on preparing a W/O emulsion, whose discontinuous phase is made up of droplets containing the biopolymer aqueous solution, the bioactive component and a source of multivalent ions producing the gelation of biopolymers (e.g. $CaCO_3$). They are released from their salts by adding an acid (acetic acid) in the W/O emulsion. The external phase of the W/O emulsion may be a vegetable oil where a lipophilic surfactant (Span 80) was solubilised. The most difficult stage of this method is the separation of the microspheres in the system. The microspheres obtained have sizes between 0.5 and 100μm [20].

Complex coacervation consists in entrapping bioactive compounds into a protein-polysaccharide complex or anionic – cationic polysaccharides complex. Biopolymer complex coacervates are formed by the electrostatic attractions between the electrical charges of the two biopolymers in specific *p*H and ionic strength circumstances. Gelation of the protein-polysaccharide complex occurs either by cooling or by the thermal denaturation of protein. To solidify the particles one may use enzymatic reticulation agents (transglutaminase) or chemical reticulation agents (glutaraldehyde, adipic acid derivatives, malonic acid derivatives, epichlorohydrin).

Our laboratory has recently encapsulated pimenta dioica essential oil in a chitosan-κ- carrageenan complex, studying the influence of *p*H and temperature on the swelling and release processes [21].

Spray drying is one of the earliest and most common methods to encapsulate bioactive compounds. A suspension or solution of biopolymers in water containing bioactive component is atomized and sprayed in a drying chamber, where the water in the fine droplets is suddenly vaporized, and thus produces microparticles whose size is in the range 10-100μm [22]. The characteristics of microparticles are influenced by the physico-chemical properties of the materials (biopolymer concentration, the water content, the wall material-core material ratio, viscosity, etc), and the technological parameters (atomizing gas flow rate, drying gas flow rate, inlet temperature, outlet temperature, etc). The disadvantages of this method consist in the restrictive use of only the hydrophilic wall material and the application of high temperatures which may degrade thermosensitive components.

Conclusions

Today's consumers also consider food as a source of health. They require that food bring an important contribution to preventing disease and improving health.

People who eat the recommended amounts of a variety of fruits and vegetables may cut their risk of many diseases by as much as half. The encapsulation of phytochemical components has led to the diversification of functional food and the improvement of their quality. The interest in developing new encapsulation technologies and new and functional carrier materials ensuring the ever need for food-grade micro and nanoparticles has been on the rise lately.

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