

## NANOCAPSULES BASED ON PLANT PROTEINS

Danijela Rajić<sup>1\*</sup>, Srđan Vuković<sup>1</sup>, Aleksandar Vuković<sup>2</sup>,  
Vesna Gojković Cvjetković<sup>1</sup>, Svetlana Pelemiš<sup>1</sup>

<sup>1</sup>University of East Sarajevo, Faculty of Technology Zvornik, Republic of Srpska, Bosnia and Herzegovina

<sup>2</sup>University of Novi Sad, Faculty of Technical Sciences, Novi Sad, Serbia

\*Corresponding author: danijelarajic@tfzv.ues.rs.ba

**Abstract:** Recently, nanobiotechnology has a very wide range of applications, and one of them is the application of nanoparticles for delivery systems of active substances. Nanoparticles are one of the most unique entities that enhance performance, expand applications, and provide an opportunity to develop materials that can address many scientific challenges. Hollow nanoparticles-nanocapsules built from plant proteins have suitable physico-chemical and functional properties, which enable them to encapsulate hydrophilic and hydrophobic bioactive compounds. Apart from their essential function of providing amino acids for human consumption, proteins also play a prominent role in the preparation, processing, storage and consumption of food and contribute to the quality and sensory properties of food products. The development of nanoparticles with specific properties is encouraged by their application in nanotechnology, food and agricultural systems, especially the development of bioactive components of functional food, with improved solubility, physico-chemical stability, oral bioavailability and sensory characteristics. This paper provides an overview of plant proteins that can be used for the production of nanocapsules, their functional properties, as well as the methods that can be used to obtain nanocapsules based on plant proteins.

**Keywords:** nanocapsules, nanobiotechnology, plant proteins, functional food.

### 1. INTRODUCTION

Nanoparticles made of metals, carbon, organic and inorganic polymers, and their mixtures have been extensively studied and used for medical, environmental, food, energy, and other applications. Nanoparticles of various shapes and sizes have been developed, including solid, hollow (nanocapsules), and core-shell structures. Nanoparticles are also classified based on their source, such as polymeric nanoparticles, magnetic nanoparticles, liposomes, carbon nanotubes, quantum dots, dendrimers, metal nanoparticles, etc. Nanocapsules have an advantage over most other colloidal carriers due to their small size, higher encapsulation power, better penetration ability, and targeted delivery of active substances [1-3].

When used for medical applications, nanoparticles have been able to avoid detection by the immune system and accumulate in tumors and organs, so they are therefore considered ideal for targeted drug delivery. The larger surface area and the ability to load entities inside the hollow particles give them an extraordinary load capacity. Such nanoparticles have also been used to remove dyes and chemicals from polluted water. Compared to carbohydrates or metals, protein-based nanoparticles are considered ideal for medical, food, cosmetic, and other applications.

Proteins offer the possibility for surface modifications and binding of drugs and other biomolecules through covalent, ionic, hydrogen bonding and other associations. Plant proteins have been extensively studied and made into nanoparticles using var-

ious techniques. Compared to animal proteins, plant proteins do not cause immunogenicity and are therefore preferred for the development of nanomaterials. Although protein-based nanoparticles offer unique properties, there are significant limitations in their preparation and application

First of all, most proteins do not dissolve in aqueous solvents or common chemicals, and are therefore difficult to convert into nanoparticles. The use of toxic solvents or protein hydrolysis leads to the loss of many characteristics. In addition to being biomolecules, the stability and lifetime of the particles are also a problem, and obtaining a uniform particle size or a narrow size distribution is also a challenge. However, the benefits and scope of use of protein nanoparticles, especially those made from plant proteins, outweigh their limitations.

The biggest advantage of colloidal food or drug carrier systems is the ability to target food (drugs) with modified body distribution, as well as improving the cellular uptake of a number of substances and their ability to deliver a wide range of drugs to different parts of the body over a long period of time [2, 4, 5].

## 2. PLANT PROTEINS

Plant protein refers to proteins of terrestrial plant origin. In most cases, plant proteins are found in seeds and grains that store most of the nitrogen sources. In the context of human protein nutrition, the most important plant species are cereals and legumes, including oilseed legumes, which are consumed as part of grain components (e.g. milled from grains) or as enriched protein ingredients as co-products of oil extraction or in starch production (e.g. soy proteins and gluten). The protein content in corn is between 9 and 12% (Table 1). About half of the produce is used directly as animal feed, and a quarter is used for ethanol production in the US. Only a small percentage of the total corn production is used for human consumption, most commonly for products such as corn chips and tortillas and the production of corn syrups. Cornmeal is one of the main products resulting from the dry milling process of corn and is used in a variety of food products such as pancake mixes, muffins, donuts, and dough, as well as baby food, meat products, cereals and some fermented products

*Table 1. Typical protein content of major cereals, legumes and plant sources [6]*

	<b>Protein content</b>	<b>Other constituents</b>
<b>Wheat</b>	8-15%	~75% starch; 1-2 % lipids
<b>Flour</b>		~5% non-starch polysaccharides
<b>Rice</b>	7-9%	90% starch
<b>Corn</b>	9-12%	70-75% starch; 3-18% oil from the germ
<b>Barley</b>	8-15%	60-64% starch; 2-3% lipids
<b>Dehulled barley</b>		3-10% soluble dietary fibre and 11-14% insoluble dietary fibre
<b>Sorghum</b>	9-17%	~ 2% lipids; 70-75% starch
<b>Soybean</b>	35-40%	~ 20% oil; ~ 30% non-starch polysaccharides
<b>Pea</b>	20-30%	60-65% starch; ~ 5% non-starch polysaccharides
<b>Chickpea</b>	20-25%	~60% starch; ~10% non-starch polysaccharides
<b>Lupin</b>	35-40%	~ 10% oil; 35-40% non-starch polysaccharides
<b>Canola</b>	17-26%	40% oil; 12-30% non-starch polysaccharides

## 2.1. Types of plant proteins

Storage plant proteins were first classified by Osborne (1924) based on their solubility and extractability in various solvents. The four main classes of proteins that have since become known as “Osborne fractions” are: albumins, globulins, prolamins, and glutelins. Albumins are soluble in water and coagulable by heat, while globulins are insoluble in water but soluble in salt solutions. Prolamins are not soluble in either water or saline solutions, but can be extracted with concentrated aqueous alcohol solutions (60-70% v/v) [7]. Osborne’s classification is still in use, but over the years, fractionation methods have improved, and it has become known that each of these classes contains a complex mixture of proteins and that there is a lot of overlap between the classes [8].

Current practice uses the combined albumin-globulin fraction as the salt-soluble protein, while the prolamins are extracted with an aqueous solution of 1-propanol or 2-propanol with a reducing agent. This method is suitable for the study of basic genetic products. It is quite inappropriate from a technological point of view, because reducing agents lead to the re-establishment of new disulfide bonds that change the solubility of the fractions. To prevent denaturation of the glutelin fraction by alkaline extraction, alternative extracts such as buffers containing the detergent sodium dodecyl sulfate (SDS) at pH 10 are used. The relative proportion of Osborne protein fractions in wheat, barley, maize, and rye seeds is different in different seed cereals (Table 2) [7].

In addition to knowing which types of proteins are found in cereals, the amino acids that are an indispensable part of cereals as well as those that are occasionally found in their composition are also shown in one place (Table 3).

### Albumins

Albumins are generally present in relatively small amounts as part of the storage proteins of cereal seeds, but more so in oilseeds and legumes. Therefore, they have been studied more in oilseeds (canola and sunflower) and legumes (lupine and peas) than in cereals. They are compact globular proteins consisting of two polypeptide chains with molecular weight (Mw) values of 4000 and 9000 Da that form a disulfide cross-linked protein. Importantly, albumins account for more than half of the total sulfur in the seeds of legumes such as peas and lupins, despite accounting for only 10-30% of total proteins [10].

### Globulins

Globulins are the most abundant protein fraction in the seeds of legumes such as soybeans, peas, and lupins, but they are scarce in cereal grains. However, the concentration of globulin can vary considerably depending on the different cultivars and ranges from 40-80% of total soybean protein, 65-80% of total pea protein, and about 75% of total lupine protein. Unlike albumin, globulin proteins from plants contain relatively low levels of the sulfur-containing amino acids cysteine and methionine. There are two types of globulins in soy: glycinin and conglycinin, with sedimenta-

**Table 2.** Relative percentage of Osborne protein fractions in cereal seeds [9]

Cereals	Nonprotein N	Albumins	Globulins	Prolamins	Glutelins	Remains
Barley <sup>a</sup>	11.6		15.6	45.2	18.0	9.6
Wheat <sup>b</sup>		33.1		60.7		6.2
Corn <sup>a</sup>	4.4	0.9	1.5	55.4	22.9	14.9
Rice <sup>c</sup>		15.7		6.7	61.5	16.1
Oat <sup>d</sup>	11		56	9	23	1
a) Total seed N (%) b) Recovered seed N (%) c) Total protein (%) d) Recovered protein (%)						

**Table 3.** Amino acid content (mg/g proteins) of plant proteins of different cereals, legumes, and oil seeds [10]

Amino acid content (mg/g protein)										
	Wheat	Barley	Corn	Rice	Sorghum	Soybean	Lupin	Pea	Chickpea	Canola
<b>Indispensable amino acids</b>										
<b>Arginine</b>	48	50	43	79	34	73	98	102	98	58
<b>Histidine</b>	24	22	28	24	22	26	27	25	28	31
<b>Isoleucine</b>	34	38	38	44	41	46	45	46	46	23
<b>Leucine</b>	69	71	126	86	138	79	74	73	78	71
<b>Lysine</b>	30	37	27	38	21	65	55	81	71	56
<b>Methionine</b>	16	18	20	22	14	13	8	10	11	21
<b>Cystine</b>	26	24	16	16	16	13	14	12	12	24
<b>Phenylalanine</b>	47	54	50	50	51	50	38	49	60	38
<b>Tyrosine</b>	31	33	39	33	28	32	37	29	31	32
<b>Threonine</b>	30	35	37	34	31	39	38	44	39	44
<b>Tryptophan</b>	11	16	7	27	13	13	10	10	9	13
<b>Valine</b>	46	53	50	60	52	49	42	51	47	55
<b>Non-indispensable amino acids</b>										
<b>Alanine</b>	37	42	77	59	87	43	37	44	45	44
<b>Aspartic acid</b>	51	60	64	99	65	119	113	118	121	7342
<b>Glutamic acid</b>	309	249	194	199	219	190	227	174	165	181
<b>Glycine</b>	41	41	38	45	31	42	43	44	42	49
<b>Proline</b>	103	115	92	49	84	56	42	42	44	60
<b>Serine</b>	48	43	51	48	43	52	52	47	53	40

tion coefficients of about 11S and 7S, respectively. 11S glycine is the major protein in soybeans [10].

#### Prolamins

Prolamins are the primary storage proteins in cereals, accounting for approximately 50% of total grain proteins, with the exception of rice, where prolamins account for only about 4%. The name prolamins was used by Osborne (1924) to reflect the high content of proline and glutamine in cereal proteins. Four main prolamins have been found, namely gliadins in wheat, hordeins in barley, zeins in maize, and kafirins in sorghum. In the original Osborne classification, three fractions were classified as glutelins: i.e. wheat glutenins, corn glutenins, and rice glutenins. Osborn's classifications of prolamins and glutelins have since been modified and revised in light of scientific advances in understanding the structures and relationships of individual proteins. Thus, in wheat, prolamins are composed of gliadin and glutenin, the two main components of wheat gluten. Prolamin proteins in barley are hordeins, and in corn they are zein proteins. Prolamins vary widely in mo-

lecular weight, both within and between plant species. The most studied prolamins are gluten proteins from wheat. They represent ~85% of wheat proteins. About half of the gluten proteins are monomeric gliadins, and the rest are disulfide-linked polypeptides that form the polymer fraction of gluten, the size of which can range up to tens of millions of Da. The gliadin fraction contains mainly single polypeptide chains of molecular weight in the range of 30-75,000 Da [8, 10]. Although zein is one of the few cereal proteins that is industrially produced in a relatively pure form, mainly in China, it is rarely used directly for human consumption due to its poor water solubility. The main applications of zein are as a polymer material for films, a carrier for nanocapsules, coatings, and in the composition of plastics [10].

#### Gliadins

Gliadins associate with each other and with glutenin proteins through noncovalent hydrogen bonds and hydrophobic interactions.  $\omega$ -gliadin contains a significant amount of glutamine, proline, and phenylalanine, but not cysteine. In contrast,  $\alpha$ -

$\beta$ - and  $\gamma$ -gliadins have less proline, glutamine, and phenylalanine, but 2–3 mol.% cysteine plus methionine. Glutenins are divided into two groups, high molecular weight (HMW) and low molecular weight (LMW) subunits. Their size ( $M_v > 100,000$  Da) and their ability to form an intermolecular network give gluten the framework of its structure. Thus, HMW subunits are largely responsible for determining the viscoelastic properties of gluten. Glutenins are structurally similar to  $\alpha/\beta$ - and  $\gamma$ -gliadins. However, their ability to form intermolecular disulfide bonds with each other and/or with HMW glutenins is important for glutenin macropolymer formation.

#### Glutelins

Unlike other cereals, which accumulate prolamins as their primary nitrogen reserve, the main storage proteins in rice are glutelins, which account for up to 80% of total rice proteins. They have a high molecular weight, ranging from 45,000 to 150,000 Da. Rice glutelins have been difficult to study due to their extensive aggregation, cross-linking of disulfide bonds, and glycosylation that make them generally insoluble except in dilute alkalis and therefore difficult to extract [10].

### 2.2. Functional properties of plant proteins

Proteins have a number of important functions in human nutrition. Apart from their essential function of providing amino acids for human consumption, proteins also play a prominent role in the preparation, processing, storage, and consumption of food and contribute to the quality and sensory properties of food products. The most important functional properties of proteins in food include their solubility, water and fat binding ability, gel formation, rheological behavior, emulsifying abilities, foaming, and fogging abilities. These properties relate to the way proteins react with large (carbohydrates, lipids, and proteins) and small (gases, salts, volatile substances, and water) molecules, as well as the size of the molecules, the structure (primary amino acid sequences, secondary, and tertiary conformations), and the charge distribution of protein molecules. Changes in protein structure as a result of the environment to which the protein is exposed during food processing or under the influence of the food matrix will also affect the functional properties of plant proteins [10].

#### Solubility

The solubility of proteins in aqueous solutions is often a precondition for their other functional properties, such as emulsification and foaming. Factors that affect protein solubility are pH, ionic strength, type of solvent, and temperature. Proteins are least soluble at their isoelectric point. The usual method used to isolate most soluble plant proteins (mainly albumin and/or globulin) is based on the principle of the isoelectric point, i.e. proteins are dissolved using acid, alkali or solvent beyond their isoelectric point and then precipitated by adjusting the pH. Isolated proteins (e.g. prepared protein isolates from soy, pea, and canola) have good solubility at neutral pH. However, for most plant proteins, especially those cereal proteins containing high levels of prolamins and glutelins, solubility at neutral pH is extremely low due to the low content of charged amino acid residues. This low solubility has limited the use of these proteins in a much wider range of food applications, other than dough-based products [10].

#### Emulsification

Food proteins, especially those from milk and eggs, are commonly used to stabilize food emulsions and foams. Their amphiphilic nature allows them to adsorb at oil/water and air/water interfaces and form an interfacial layer that reduces surface tension and inhibits coalescence of oil droplets or air cells during food processing and storage. The ability of a protein to function as a food emulsifier depends on its structure and properties. The structure of plant proteins, as well as their absence or changes during adsorption on the surface, can represent a potential opportunity to form protein particles and improve emulsion stability [10].

#### Gelation

Food proteins, especially globular proteins, can form gels when heat-denatured in aqueous solutions when protein-protein and protein-solvent interactions are balanced. The temperature of gel formation and its resulting properties are determined by the molecular structure of the protein, protein-protein and protein-solvent interactions [10].

#### Foaming

The ability of food proteins to stabilize foams is related to the propensity of proteins to adsorb at



air-water interfaces and their ability to reduce surface tension and form strong interfacial membranes through protein-protein interactions at the air-water interface. Natural plant proteins, due to their compact structure, have limited foaming properties. Plant protein fractions that are rich in albumins (e.g. those from peas and lupins) have shown good foaming properties equivalent to those of egg whites). The foam properties of soy proteins are by far the most studied compared to proteins from other plant sources. However, structural modification, either by pH change or limited enzyme hydrolysis, is often required for plant proteins to achieve their potential as active foaming agents. It should be noted that a change in pH affects the conformation of glycinin at the air-water interface. and this can have a major impact on the rheological and foaming properties of these proteins [10].

### 3. METHODS OF PREPARATION OF NANOCAPSULES

There are several methods for the preparation of composite nanocapsules based on natural proteins as carriers. The choice of the method itself depends on several factors, but mostly on the physical and chemical properties of the biopolymer. The choice of method is also influenced by the components that are added to the product itself and their interaction with the biopolymer as a carrier. Most of the modern methods described in the literature are based on the precipitation of macromolecules, which occurs by reducing the solubility in a given solvent. Apart from the conventional methods of preparing nanoparticles by co-precipitation or phase separation, newer methods such as atomization and high-temperature shearing provide better options for obtaining nanoparticles with the desired characteristics [11].

Various techniques including desolvation, coacervation, emulsification, nanoprecipitation, nanospray drying, self-assembly, electrospray, and cross-linking have been discussed in the literature for the production of composite nanocapsules. The choice of method for the preparation of nanoparticles depends on various aspects such as the composition of amino acids and the physicochemical properties of the protein used as a nanocarrier, as well as the characteristics of the bioactive molecules that will be nanoencapsulated [12].

An important parameter when studying the behavior of macromolecules in solutions from a thermodynamic point of view is the Flory X-parameter, which, as a key factor, regulates the thermodynamic behavior of macromolecules in solutions. This parameter represents the change in Gibbs free energy ( $\Delta G$ ) per solvent when moving from a solvent-solvent contact to a solvent-macromolecule contact.

Flory X-parameter is given by equation:

$$X = \frac{\Delta G}{k_B T} = \frac{\Delta H - T\Delta S}{k_B T} = \frac{1}{2} - A\left(1 - \frac{\theta}{T}\right) \quad (1)$$

Where  $k_B$  is Boltzmann constant,  $T$ , temperature, and  $A$  and  $\theta$  parameters given as:

$$A = \frac{2\Delta S + k_B}{2k_B} \quad (2)$$

$$\theta = \frac{2\Delta H}{2\Delta S + k_B} \quad (3)$$

From these equations, the parameter  $A$  is directly related to the change in entropy ( $\Delta S$ ), while the temperature  $\theta$  is related to both entropy ( $\Delta S$ ) and enthalpy ( $\Delta H$ ). When the temperature  $\theta$  is equal to  $T$ , then the X parameter, according to Eq. 1, equals 1/2. It can be determined by measuring the light scattering of a dilute solution of macromolecules. In that case, the solvent is suitable for dissolving that particular macromolecule if X is less than 1/2 and is bad if X is greater than 1/2 [13].

#### 3.1. Coacervation

The phenomenon of coacervation is the layering of the original solution and the separation of two liquid phases of different concentrations of the same lyophilic colloid. One phase (the original solution) is richer in solvent, and the other (coacervate) contains more colloids, so it is thick and viscous. This phenomenon occurs when a non-solvent is added to the system, that is, a substance that disrupts the equilibrium of the solution and reduces solvation and solubility.

Coacervation can occur by changing temperature, pH, adding macromolecular substances, salts, non-solvents, resorcinol [12]. The occurrence of coacervation and coagulation are interconnected, so when the conditions change, coagulation

and separation of solid sediments can occur instead of coacervation. It is considered that coacervation occurs with the addition of smaller amounts of flocculants, which results in discharge and a certain decrease in solvation, but not enough to cause precipitation. If the phase separation in the solution of a macromolecule is caused by the addition of another macromolecule, it is a case of complex coacervation or thermodynamic incompatibility [14]. In complex coacervation, coacervate formation occurs, when two macromolecules present do not tend to associate, but one macromolecule tends to push the other out of the solution. In this case, each of the two resulting phases is rich in only one macromolecule. Nanoparticles are formed in one of these three ways in the coacervate phase, with or without a crosslinker.

This method can be used to obtain nanoparticles of zein or protein from legumes. Cross-linking can be used as an auxiliary technique in the preparation of nanoparticles using the coacervation technique in order to increase the stability of biopolymer particles, and for this purpose different cross-linkers can be used: chemical, ionic, thermal, and enzymatic. They are usually efficient and have a positive impact on particle stability, but in some cases they are difficult to remove and can be toxic. Sometimes it is necessary to use a dialysis process to remove them, which can make the process of preparing nanoparticles more expensive [15].

### 3.2 Precipitation in a non-solvent

Non-solvent precipitation, also known as nanoprecipitation, has been used since 1969 [16]. Since then, it has been improved and widely used for making a wide range of nanoparticles, including drug nanoparticles, polymer nanoparticles, protein nanoparticles as carriers of active substances, as well as inorganic nanoparticles. Nanoprecipitation has been explored to formulate a wide range of hydrophobic components including hydrophobic drugs, polymers, hydrophobic proteins, etc. About 40% of clinically approved drugs and 90% of drugs in development are reported to be hydrophobic with low water solubility. Therefore, nanoprecipitation serves as a powerful tool for formulating these hydrophobic drugs. Formulating hydrophobic drugs into nanoparticles provides several advantages, including that drug nanoparticles can improve the solubility and dis-

solution rate of insoluble drugs, thereby increasing their bioavailability. Formulation into nanoparticles can improve the chemical stability of some drugs and control the kinetics of drug release; drug nanoparticles can be further modified to have additional functions and desirable properties [17]. The method involves the use of two mutually miscible solvents, where it is necessary that the biopolymer is dissolved in one and undissolved in the other. During the precipitation process, sudden desolvation of the biopolymer occurs when the biopolymer solution is added to the non-solvent. For example, a 70-80% ethanol solution of proteins can, with stirring, be added to water, which is a non-solvent and possibly contains dissolved surfactants. When the alcohol concentration drops below the critical point of protein solubility, the proteins become insoluble and precipitate, forming nanoparticles. Nanoparticles of hydrophobic proteins such as zein, gliadin from gluten, etc. can be formed in this way [15]. Precipitation in a non-solvent is a fast and simple technique that enables the preparation of very small particles with a monomodal size distribution and requires little energy consumption.

In the case of precipitation, the physico-chemical properties of nanoparticles largely depend on the conditions under which the biopolymer solution is added to the aqueous phase, including the rate and method of addition, the speed of mixing during the procedure, and the solvent/non-solvent ratio. Also, the characteristics of nanoparticles are influenced by the properties and concentration of the components they are made of. Three widely used nanoprecipitation methods include traditional nanoprecipitation, flash nanoprecipitation, and microfluidic-based nanoprecipitation [17].

### 3.3. Electrospraying

The electrospray technique is one of the most effective techniques for the preparation of nanoparticles or nanospheres. The experimental setup consists of a syringe pump with a polymer solution connected to a high-voltage power supply that forms a functional electrode. A metal foil collector placed opposite serves as a grounding electrode. The flow rate and applied voltage are optimized depending on the type of solution used for electrospraying. The liquid exiting the nozzle into the electric field forms a custom cone due to surface tension [18].

By increasing the electric field, the Taylor cone is broken into highly charged droplets. By selecting the appropriate conditions, these droplets approach the micro- or nano-sized level. Solid particles can be produced by evaporation of the solvent. Needle gauge diameter, applied voltage, flow rate, and working distance vary for respective drug delivery systems. The principle of electrospraying is to apply a high voltage to a polymer solution to force the polymer to exit the syringe in the form of nanoparticles [19].

Electrospraying has emerged as a similar technique to electrospinning that uses an analogous technology to produce nanostructures. Nanoparticles may be useful for numerous biological, medical, or pharmaceutical applications due to their zero-dimensional nature, while nanofibers may only be useful for their two-dimensional applications. Even then, the research work quantified in terms of journal publications on electrosprayed nanoparticles is rather less compared to that of electrospun nanofibers. This method is not only suitable for the synthesis of nanoparticles of synthetic polymers, but also for nanoparticles of natural polymers, either proteins or carbohydrates and was found to produce stable nanoparticles without losing their bioactivity of either the drug or the encapsulated biomolecules. The electrical sputtering technique is also called electrodynamic sputtering or electrohydrodynamic atomization [19].

#### 3.4. Emulsification

In recent years, the potential of nanostructured systems obtained by emulsification for encapsulation, protection, and release of active ingredients has been intensively investigated. Incorporation of natural antimicrobial agents for food preservation or bioactive compounds for food fortification can be effectively enhanced using emulsion-based delivery systems. Recently, it has been described that reducing the droplet size to the nanometer range can improve the activity of functional ingredients due to their larger active surface. There are several types of emulsion-based nanostructures described as functional ingredient delivery systems.

In general, they consist of lipid droplets dispersed in an aqueous medium. In the case of lipophilic active ingredients, they are dissolved within the oil core of the dispersed droplets. After removing the solvent, the solid nanoparticles are separated us-

ing an ultracentrifuge. Depending on the state of the lipid core, whether it is liquid oil or solid fat, nanoemulsions or solid lipid nanoparticles can be formed. Overall, the design of such nanostructures obtained by emulsification opens up new possibilities for the protection of active ingredients that are incorporated into food products, ensuring optimal functionality during the product's shelf life and during their digestion [15, 20].

#### 3.5. Spray drying

Spray drying is a technique based on the transformation of a liquid into a dry powder by atomization in a hot stream of drying gas, which is mostly air. The spray drying process consists of four basic steps, namely atomization of the liquid raw material, drying in a stream of hot air, formation of dry particles, and separation and collection of the dry product from the drying gas. First, the liquid is fed into the drying chamber by a peristaltic pump through a sprayer or nozzle that can be a rotary sprayer, a pressure nozzle, or a two-piece nozzle, and the atomization takes place by centrifugal, pressure, or kinetic energy. The resulting small droplets (micrometer scale) are subjected to rapid evaporation of the solvent, which leads to the formation of dry particles that are separated from the drying gas by means of a cyclone or bag filter that deposits them in a glass collector located in the lower part of the device [21].

A nanospray drying approach was effectively used to prepare bovine serum albumin (BSA) nanoparticles with a size of 460 nm. Furthermore, the successful use of this approach for the preparation of proteins for pulmonary, nasal, and controlled oral delivery has been demonstrated. New nano spray dryers use vibrating mesh technology to create fine droplets. In short, the spray head contains piezoelectric crystals responsible for vibration and movement. This head is fitted with a spray cap that contains a thin perforated membrane with precise hole sizes. When an ultrasonic frequency is introduced, the crystals will vibrate and generate a piezoelectric effect, which in turn causes the grid to vibrate up and down in sync with the generated current. This consequently leads to the injection of fine and precise droplets from the holes and the generation of aerosols [15, 21].



#### 4. CONCLUSION - APPLICATION OF NANOCAPSULES

Nanoparticles are one of the most unique entities that improve performance and provide an opportunity for the development of new materials in various fields. Nanoparticles made of metals, carbons, organic and inorganic polymers, and their mixtures have been extensively studied and used for medical, environmental, nutritional, energy, and other applications. In medicine, they represent a significant invention for targeted drug delivery. The larger specific surface area and the ability to encapsulate active substances provide them with the capacity for application in many areas, from medicine to agriculture, as well as the removal of dyes and chemicals from polluted water.

Compared to carbohydrates or metals, plant protein-based nanoparticles are ideal in the medical, food, and cosmetic industries. Nanoparticles made from plant proteins have proven effective for encapsulation and delivery of drugs and other biomolecules, bioimaging, increasing food stability and shelf life, and removing toxic components in water [2]. Nanocapsules have advantages over hydrogels, organogels, liposomes, and microparticles due to smaller particle size, higher encapsulation efficiency, higher penetration power, and targeted delivery [1]. The development of nanoparticles with specific properties is encouraged by their application in nanotechnology, food, and agricultural systems, especially the development of bioactive components of functional food, with improved solubility, physico-chemical stability, oral bioavailability, and sensory characteristics.

#### 5. REFERENCES

- [1] C. Yang, et al., *Oat protein-shellac beads: Superior protection and delivery carriers for sensitive bioactive compounds*. Food Hydrocolloids 2018. 77: p. 754-763.
- [2] N. R. Rapisarda, *Properties and Applications of Nanoparticles from Plant Proteins*. Materials 2021. 14.
- [3] S. Chen, et al., *Fabrication and characterization of binary composite nanoparticles between zein and shellac by anti-solvent co-precipitation*. Food and Bioproducts Processing, 2018. 107: p. 88-96.
- [4] C. Weber, et al., *Desolvation process and surface characterisation of protein nanoparticles*. International Journal of Pharmaceutics 2000. 194: p. 91-102.
- [5] M. Rahimnejad, et al. *Production of protein nanoparticles for food and drug delivery system*. African Journal of Biotechnology 2009. 8(19): p. 4738-4743.
- [6] N. Reddy and M. Rapisarda, *Properties and Application of Nanoparticle from Plant Proteins*, Materials, 2021
- [7] A. Evans, et al., *The Carbohydrate Composition of Cotyledons and Hulls of Cultivars of Lupinus angustifolius from Western Australia*. Journal of the Science of Food and Agriculture, 1993. 61: p. 189-194.
- [8] K. Zhou, M.S., Herman Lutterodt, Monica Whent, N. A. Michael Eskin, Liangli Yu, *Cereals and Legumes*, in *Biochemistry of Foods*. 2013, Elsevier Inc.
- [9] P. R. S. Tatham, *The prolamin storage proteins of cereal seeds: structure and evolution*. Biochemical Journal, 1990. 267: p. 1-12.
- [10] L. Day, *Proteins from land plants – Potential resources for human nutrition and food security*. Trends in Food Science & Technology, 2013.
- [11] M. Mohammadian, et al., *Nanostructured food proteins as efficient systems for the encapsulation of bioactive compounds*. Food Science and Human Wellness, 2020.
- [12] K. Miladi, et al., *Particles from preformed polymers as carriers for drug delivery*. EXCLI Journal 2014. 13: p. 28-57.
- [13] Lj. Đaković, *Kolidna hemija*. 2006, Beograd: Zavod za udžbenike i nastavna sredstva Beograd.
- [14] M. Tarhini, et al., *Protein-based nanoparticles: From preparation to encapsulation of active molecules*. International Journal of Pharmaceutics 2017.
- [15] H. Fessi, et al., *Nanocapsule formation by interfacial polymer deposition following solvent displacement*. International Journal of Pharmaceutics, 1989. 55.
- [16] Y. Liu, et al, *Formulation of nanoparticles using mixing induced nanoprecipitation for drug delivery*. Industrial & Engineering Chemistry Research, 2019.
- [17] N. Bock, et al., *Electrospraying, a Reproducible Method for Production of Polymeric Mi-*

*crosspheres for Biomedical Applications*. *Polymers* 2011. 3: p. 131-149.

[18] R. Ramakrishna, *Electrosprayed nanoparticles for drug delivery and pharmaceutical applications*. *Biomatter*, 2013. 3.

[19] L. Salvia-Trujillo, et al., *Emulsion-Based Nanostructures for the Delivery of Active Ingredients in Foods*. *Frontiers in Sustainable Food Systems* 2018. 2.

[20] A. Sosnik, et al., *Advantages and challenges of the spray-drying technology for the production of pure drug particles and drug-loaded polymeric carriers*. *Advances in Colloid and Interface Science*, 2015.

[21] S. H. Lee, et al., *Nano spray drying: A novel method for preparing protein nanoparticles for protein therapy*. *International Journal of Pharmaceutics*, 2011. 403.

## НАНОКАПСУЛЕ НА БАЗИ БИЉНИХ ПРОТЕИНА

**Сажетак:** Нанобиотехнологија у посљедње вријеме има веома широк спектар примјене, посебно у производњи наночестица за системе испоруке активних материја. Наночестице су један од најјединственијих ентитета који побољшавају перформансе, проширују апликације и пружају прилику за развој материјала који могу да одговоре на многе велике научне изазове. Празне наночестице-нанокапсуле изграђене од биљних протеина имају погодне физичко-хемијске и функционалне особине, које им омогућавају инкапсулирање хидрофилних и хидрофобних биоактивних једињења. Осим своје основне функције обезбијеђивања аминокиселина за људску исхрану, протеини такође имају истакнуту улогу у припреми, преради, складиштењу и потрошњи хране и доприносе квалитету и сензорним особинама прехранбених производа. Развој наночестица специфичних својстава подстакнут је њиховом примјеном у нанотехнологији, прехранбеним и пољопривредним системима, поготово развојем биоактивних компонената функционалне хране, са побољшаном растворљивошћу, физичко-хемијском стабилношћу, оралном биорасположивошћу и сензорним карактеристикама. У овом раду дат је преглед биљних протеина које је могуће користити за производњу нанокапсула, њихове функционалне особине као и методе којима је могуће добити нанокапсуле на бази биљних протеина.

**Кључне ријечи:** нанокапсуле, нанобиотехнологија, биљни протеини, функционална храна.

---

Paper received: 31 August 2022

Paper accepted: 21 November 2022

---