Importance of lecithin for encapsulation processes

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The crude soy and rice lecithin are used in studies of liposome formulation. They have been used as flavor encapsulators, flavor masking, antioxidants, and nutrient protective against degradations in the gastrointestinal tract. This study aimed to show the importance of rice and soy lecithin in its varied uses as liposomes in different areas. The paper indicates the importance of soy and rice lecithin, both crude and purified (phosphatidylcholine) ones, as feasible for the encapsulation of various materials, from medical and pharmaceutical to food ones.

Key words: Lecithin, phospholipids, vesicles.

INTRODUCTION

Lecithin is the name given to a mixture of glycolipids, triglycerides, and phospholipids (for example phosphatidylcholine, phosphatidylethanolamine, and phosphatidylinositol). However, in biochemistry, the term lecithin is usually used as a synonym for pure phosphatidylcholine - a phospholipid which is the main component of the phosphate fraction obtained from egg yolk (in Greek lekithos - λεκιθος, from which the name of the compound was derives) or from soy and rice beans, from which it is extracted by mechanical or chemical means using hexane. Lecithin is marketed in high purity as a food supplement for medical use (Mertins, 2004).

Lecithin is commercially used as an emulsifier and lubricant in various economic activities such as food or pharmaceutical industry. For example, it is used as an emulsifier in chocolate and in the production of coating for foods. Lecithin is considered as a non-toxic surfactant which is well tolerated by the organism, since it is an integral part of cell membranes and can be completely metabolized. It was classified in the United States by the Food and Drug Administration as generally recognized as safe product for human consumption. Also, it is recognized as a food additive by the European Union under the number EE322 (Zambelli and Moreira, 2009).

Another advantage of soy lecithin mainly arising is that it can be used as a vitamin stabilizer food ingredient to protect the vitamins A and E against oxidation, and as a source of choline, inositol and other growth promoter components (Meyers, 1990).

Thus, soy lecithin and rice, both of which are important by-products of soy oil (SO), and rice bran oil (OFA) respectively, the main difference of rice and soya lecithin is related to the composition and characteristics, for rice lecithin can replace soya lecithin in food and industrial Mr. Adriana Rodrigues Machado
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products; compared to rice lecithin is more rich in phospholipids, which soybean lecithin and also marketed at a lower cost (Orlandelli, 2008).

This review study aimed to show the importance of rice and soy lecithin in its diverse applications as liposomes in different areas.

DEVELOPMENT

Obtaining the lecithin

Lecithin is obtained by hydration and separation from the crude oil according to Figures 1 and 2, which show the flowcharts for obtaining the rice and soy lecithin.

Physico-chemical characterization of soy lecithin and rice

Peroxide index, insoluble in acetone (AOCS, 1993) and refractive index using the AOCS Cc 7-25 (AOCS, 1993) method and a: for physico-chemical characterization of lecithin, soy and rice as some specific analyzes are performed Abbé refractometer. Reading takes place on a scale which directly yields the absolute refractive index at 40°C. Assim atom pH which can be determined by potentiometer Digimed - DM-20 at a temperature of 20°C by the method of Institute Adolfo Lutz (2008) acidity index, according to the volumetric method by titration with 0.1 N NaOH, and the results expressed as % citric acid, the second method of Institute Adolfo Lutz (2008).

Purification of rice and soy lecithin

There are several methods of purifying the crude lecithin, but the most common are: Purification of crude lecithin by extraction and column chromatography to obtain the phosphatidylcholine (Mertins et al., 2008; Machado et al., 2013).

Phosphatidylcholine

Phosphatidylcholine (soy lecithin/rice) is a natural phospholipid of molar mass equal to 780 g/mol and = 0.05 g/cm³ (Reis, 2010; apud Willard et al., 1998). It is a part of the molecular structure of biological membranes and is also present in blood plasma as a constituent of lipoproteins. It is biocompatible, biodegradable, and has cleansing action. The structure of phosphatidylcholine (Figure 3) is formed by two long hydrocarbon chains, one saturated and one unsaturated constituting the non-polar or hydrophobic portion of the molecule. The polar or hydrophilic portion is formed by glycerol, phosphate group, and choline (Mertins, 2004).

Phosphatidylcholine is obtained from a by-product in the manufacturing process of soybean oil and rice bran oil. This raw material comprises a mixture of a large number of fatty acids, lipids, proteins, phospholipids, and pigments of different molecular structures with phosphatidylcholine between 10 and 20%. Its laboratory
Figure 2. Flowchart for obtaining soy lecithin.

and industrial purification is performed by column chromatography. The most accessible processes use columns of silica or alumina and mixtures of chloroform: methanol as eluent (Mertins, 2004). Phosphatidylcholine today is widely used in the pharmaceutical and cosmetic industries and as an emulsifier in the production of liposomes as well as in food and paint industries as a stabilizer and emulsifier (Maron et al., 2007).

Liposomes

The first description of the colloidal behavior of phospholipids such as lecithin, among others, as well as the formation of lipid vesicles occurred in 1961 by Bangham and colleagues. It was in this study Bangham and Horne (1964) described the formation of these phospholipid vesicles in dilute aqueous solutions and it was found that "Liposomes are artificial vesicles of smaller spherical shape that can be produced from natural phospholipids and non-toxic cholesterol (Cruz et al., 2009).

Thus, some of the nanostructures used for encapsulation are called liposomes (Figure 4), which are microscopic vesicles composed of one or more concentric lipid bilayers separated by aqueous medium. They are widespread in the medical and pharmaceutical industry. In the food industry they are used as flavor encapsulators, taste masking, antioxidants, and nutrient
The liposomes can incorporate various types of substances regardless of their molecular weight, solubility or electric charge, they can encapsulate hydrophilic and/or lipophilic substances, where the hydrophilic ones are in the aqueous compartment and the lipophilic ones are inserted or adsorbed into the membrane. Since liposomes are biodegradable, biocompatible and non-immunogenic, they are highly versatile for research, therapeutics, and analytical applications (Edwards and Baeumner, 2006; New, 1990; Puisieux et al., 1995). These vesicles consist primarily of phospholipids (either synthetic or natural ones), sterols, and an antioxidant (Vemuri and Rhodes, 1995).

The lipids that most be used in the formulations of liposomes are those with a cylindrical shape as phosphatidylcholines, phosphatidyl serine, phosphatidyl glycerol, and sphingomyelin, which are likely to form a stable bilayer in aqueous solution. Phosphatidylcholines are the most used in studies of liposome formulation, since they have great stability against variations in pH or salt concentration in the medium.

Phospholipids are characterized by a phase transition temperature (Tc), in which the membrane goes from a gel phase, where the lipid hydrocarbon chain is in the ordered state, to a liquid-crystal phase where the molecules are in more free motion and the grouped hydrophilic radicals become completely hydrated. The length and saturation of lipid chain influence the Tc value. Thus, membranes composed of different lipid may show different levels of fluidity at the same temperature (Frézard et al., 2005; Lasic, 1998).

The permeability of liposomes is relatively low when the temperature is lower than the liposome Tc, and this is measured by the flow rate at which the solution exits the aqueous compartment through the bilayer. However, this property depends on the nature of the solute and on membrane fluidity (Frézard, 1999).

Liposomes were developed to improve the biodistribution of compounds at specific locations in the body. Thus, they became recognized as carriers of biologically active compounds, with the ability to enhance and/or modify the activity of the compounds with which they are associated. This effect depends on the chemical composition and the phospholipid structure (Gómez-Henz and Fernandez-Romero, 2005).

Liposomes can be produced in a range of sizes, from a few nanometers to several micrometers (Figure 3). They are distinguished by the following categories: small unilamellar vesicles (SUVs), the smallest in the size range, with diameters ranging from 20 to 80 nm and the membrane formed by a single phospholipid bilayer; large unilamellar vesicles (LUVs), intermediate in the size range, with a diameter ranging from 80 nm to 1 μm and the membrane formed by a single phospholipid bilayer; small multilamellar vesicles (MLVs), with an average diameter between 400 nm and a few micrometers and the membrane formed by a phospholipid bilayer concentrically arranged (Mertins, 2008), and giant unilamellar vesicles (GUVs), larger than 1 μm, sometimes reaching tens of microns (Figure 3), comparable to the size of a eukaryotic cell (Lasic, 1995; Santos, 2002).

Liposomes may carry lipophilic substances within their lipid bilayer, thus allowing an increase in the transport of compounds to specific cells or tissues and enhancing the potency and/or reducing the toxicity of the encapsulated agent.

Liposome rating

Liposomes can be classified in terms of composition and intracellular delivery mechanism into five types: conventional liposomes, pH sensitive liposomes, cationic liposomes; Immunoliposomes and long-circulating liposomes.

Otherwise, the vesicle size is a critical parameter for...
determining circulating half-life of the liposomes, and size and number of bilayers influence the degree of drug encapsulation within liposomes. Thus, the liposomes were typically classified according to their size and number of bilayers (Sharma Sharma, 1997 cited by Laouini et al., 2012.): The small unilamellar vesicles (SUV): 20-100 nm; large unilamellar vesicles (LUV): > 100 nm; The giant unilamellar vesicles (Head): > 1000 nm; oligolamellar vesicle (OLV). 100-500 nm and multilamellar vesicles (MLV) > 500 nm (Bordi et al., 2006 cited by Laouini et al., 2012).

Techniques for the preparation of liposomes
The type of liposome is essentially constrained by their method of preparation which, several techniques can be used:

a) Hydration of the lipid film: As classical preparation, phospholipids are dissolved in an organic solvent, a flask glass. The solvent is evaporated under constant rotation of the balloon so as to produce a thin film phospholipids in inner wall of the balloon; subsequently, water or buffer hydration is added to the film. Agitation, ultrasonic agitation, sonication and heating can be applied at this stage to assist in the formation of double layers that will be self-assemble into liposomes encapsulating water or buffer inside process (Mertins, 2008).

b) Method of preparation of liposomes based on the reverse phase evaporation: The phospholipids are dissolved in organic solvent, then adding an aqueous portion causes formation of two phases; phospholipids tend to deposit on the interface water/organic solvent polarity by interactions of the polar extremities with water and interactions between apolaridade hydrocarbon chains and the organic solvent. The mixture is subjected to sonication; formation of reverse micelles where water droplets are surrounded by phospholipids and the mixture becomes calm. The organic solvent is evaporated and the reverse micelles concentrated. Finally, the stirring liposomas are formed, and the system becomes a milky liquid and an aqueous portion may be added to accelerate the formation of structures (Mertins, 2008). The advantage of this method is the high encapsulation efficiency.

c) Method of preparation of liposomes DRVs type, based on the dehydration and rehydration process: This method consists of mixing a suspension of small empty liposomes (prepared in water), freeze-dried after mixing. The preparation of this rehydration under specific conditions of temperature (> Tt) and lipid concentration leads to obtaining liposomes with a high encapsulation rate, referred to as DRVs ("dehydration rehydration vesicles") and allow a high rate of encapsulation (Frézard et al., 2005).

The classic method of lipid film hydration for production of nanosized liposomes is still used in research because of its simplicity and low cost (Mertins, 2004). Atomization, lyophilization, agitation, sonication and freeze-thaw extrusion were applied to standardize the structures, as complementary techniques (Figures 4 and 5).

Chemical stability of liposomes
The stability of liposomes can be affected by chemical, physical and biological processes (Batista et al., 2007). The chemical instability depends on the composition of the liposomes, which involves the prevention of the ester hydrolysis and the oxidation of the unsaturation located in the lipid chain (Batista et al., 2007).

Physical instability: Some of the major processes that cause instability of the liposomes are the aggregation and fusion of vesicles and the leakage of encapsulated material.
**Biological instability:** Liposomes must move and retain the material long enough for effective access and interaction with the target, typically in the blood, in the capillary walls and sometimes in the cells of extravascular areas (Batista et al., 2007).

**Use of liposomes in different fields**

Liposomes are widely used in the fields of medicine, pharmaceutics and food science. In the pharmaceutical field, they are used in the treatment of cancer, the development of vaccines, gene therapy, and the therapy of infectious and parasitic diseases. Besides, a new composition refers to the so-called transfersomes or ultradeformable or elastic liposomes, which are formed by phospholipids and surfactants (Santana et al., 2012).

The latter provides the membrane elasticity, which facilitates the conduction of these structures through the skin, including some cases reaching the deep layers. Some liposomal products approved by the Food and Drug Administration (FDA) and already marketed include: Ambisome (liposomal amphotericin B), indicated for the treatment of systemic fungal infections and for the treatment of leishmaniasis; Daunoxome (liposomal daunorubicin) for the treatment of Kaposi’s sarcoma; Doxil (stealthy liposomal daunorubicin) for the treatment of Kaposi’s sarcoma, ovarian cancer and breast cancer; Depocyt (liposomal cytarabine) for the treatment of meningitis, and Visudyne (liposomal verteporfin) for the macular degeneration with laser treatment (Santana et al., 2012).

In the area of food science, vitamins and minerals typically are added to foods in order to fortify them. However, they may give a strange taste, react with other ingredients or change the color of the product. These drawbacks can be avoided if such additions are made in encapsulated products. Encapsulation protects and enhances the stability of vitamins in some extremes of humidity and temperature as well as enables controlled release in the intestinal tract (Janovsky, 1993). There are several works using this process, such as: liposomal encapsulation of ciprosina (proteases used as rennet) to avoid loss during cheese processing (Picón et al., 1994); beta-carotene incorporation into liposomes through hydration of dried phospholipid particles produced by atomization; assessment of pediocin antimicrobial activity encapsulated against strains of *Listeria* (Mello et al., 2009); assessment of the encapsulation efficiency of liposomes containing nanometer size spirulina (Machado et al., 2011a); liposome preparation for protein source encapsulation (Machado et al., 2011b); importance of soy lecithin in the preparation of liposomes for commercial casein encapsulation (Machado et al., 2011b), and liposomal nanoencapsulation of phenolic compounds (Assis et. al., 2012b).

**Liposomal nanoencapsulation of food**

Nanoencapsulation is the incorporation, absorption or scattering of combinations of solid, liquid or gaseous bioactive compounds in small vesicles with a diameter at nanometer scale. The combinations of incorporated bioactive compounds can be protected against degradation and improve stability and solubility (such as solubilization of hydrophilic components in hydrophobic matrices and vice versa) (Assis et al., 2012a; Klaypradit and Huang, 2008; Jafari et al., 2008). Nanoliposomes are vesicles composed of phospholipid bilayers trapping materials in their aqueous compartments. Its unique properties have triggered numerous applications in various...
scientific and technological fields. Nanoliposomes can provide controlled release of various bioactive agents, including food and nutraceutical ingredients, in the right place at the right time, so they increase the efficiency and cellular uptake of the encapsulated material. Reactive, sensitive or volatile additives (vitamins, enzymes, antioxidants, weight loss etc.) can be transformed into stable ingredients using nanoliposomes (Mozafari, 2007).

Encapsulation of Spirulina platensis strain LEB 18 as a source of proteins in liposomes using the method of hydration of the lipid film, sonication or homogenization associated as complementary treatments, was efficient for the preparation of nano liposomes. The homogenization process yielded particles with average size larger and morphologically more uniform when compared to the sonication process. The purified phosphatidylcholine proved viable as wall material for the formation of Spirulina platensis strain liposomes LEB 18 as the material obtained was presented at the nanoscale and visually encapsulated (Machado, 2012).

Morais et al. (2003) used the method of reverse phase evaporation for the preparation of liposomes hydrolyzed casein. Although there are several studies of the use of soybean lecithin as wall material, in a comparative manner, the process of encapsulation by liposomes using soybean lecithin as rice behave similar way, both the classic method of preparing liposomes, as the method by reverse phase evaporation. An advantage of rice lecithin and its superiority as the phospholipid content compared to soybean lecithin.

Characterization of liposomes prepared with lecithin

Liposomes in their physicochemical characterization has the average size specified, observing the average diameter of the particles and their polydispersity indices (PDI) by light scattering technique in the Zetasizer equipment (Castro and Lima, 2006). The Zeta potential is defined as the surface potential of the particles. It is determined by electrophoretic mobility measurements that correspond to the speed of the particles in suspension, the larger the surface, the greater is the load speed of particles moving toward the oppositely charged electrode (Assis, 2007).

Toxicity of nanoencapsulated food

Miller and Senjen (2010) warned about the increasing use of nanotechnology in food production, by means of nanoparticles, nanocapsules and nanoemulsions in processing and packaging food, without proper regulation. Although nanotechnology may provide improvements in industrial performance, nutritional quality and food packaging efficiency, it can also bring risks to human health and the environment. Examples include nanoparticles of silver, titanium dioxide and zinc oxide which are used in nutritional supplements and in packages showing high toxicity to cells.

Given the potential risks associated with nanotechnology uses in agriculture and food, Miller and Senjen (2010) advocated a moratorium on the development of food products, packaging and agrochemicals until the specific security of nanotechnology is discussed and regulated under the following aspects: (a) the regulation of nanomaterials as new substances; (b) the expansion of the definition of nanomaterials; (c) transparency in the evaluation on the security of nanomaterials; (d) product labeling, and (e) greater involvement of society in the discussions of security and the sustainability of agricultural and food production.

However, the impact on the environment and human health is still controversial, mainly due to the lack of toxicological studies. As a consequence, assessment processes and risk management for such nanomaterials are hampered.

Conclusions

This study highlights the importance of soy and rice lecithin, both crude and purified (phosphatidylcholine) ones, as feasible for the encapsulation of various materials, from medical and pharmaceutical to food ones. Liposomes currently are seen as a promising source for the development of several technologies. However, its state of the art is at a level that still requires caution. Several studies on the subject are in progress and each day brings new publications, which makes their study very dynamic.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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