# Encapsulaton as Delivery System for Marine Biomolecules for Improved Bioavailability and Stability

**[1]Lekshmi R.G. Kumar, [1]K.K. Anas, [1]C.S. Tejpal, [1]Dr.N.S. Chatterjee and [2]Dr. Suseela Mathew**

[1]Scientist, [2]Head, B&N Division, ICAR-Central Institute of Fisheries Technology, Cochin-682 029
Email: lekshmirgcof@gmail.com

## Introduction

The technological advancements in food industry along with the increase in consumer awareness about the ability of certain foods to reduce disease risk or improve health along have together contributed to the new sector known as functional foods. Functional foods are those foods enriched with functional ingredients to offer long term health benefits or to reduce the incidence of chronic diseases beyond their nutritional functions (Menrad, 2003; Roberfroid, 2000). Several bioactive compounds of significant bioactivity have been isolated from various natural sources for development of functional foods. Marine organisms are considered as one of the richest sources of bioactive molecules that finds wider applications in the biomedical, pharmaceutical and nutraceutical industries. The most commonly employed nutraceuticals for development of functional foods includes omega-3 fatty acid, phytosterol, phytochemicals, fibres and vitamins and minerals such as retinol, ascorbic acid, iron, calcium, zinc etc. (Sloan, 2002). However, direct incorporation of many of these bioactive compounds into foods is not an easier task. Some of the challenges involved in the direct incorporation of bioactive compounds into foods include poor solubility in aqueous phase, higher melting point, loss of bio-availability, loss of physico-chemical stability and interactions with other components which are present in the food matrix (McClements, 2015). For instance, omega-3 rich fish oil can not be directly incorporated into food as it will undergo oxidation very fast and will affect the quality of the final product. It has to be either converted into a stable dispersion or powder form for incorporation as a functional food ingredient. These challenges have to be adequately addressed by adopting certain intervention strategies for the development of shelf stable functional foods.

Encapsulation is one such promising technology used for retaining the bioactivity and stability of sensitive bioactive compounds.

## Encapsulation

Encapsulation is the process of entrapping a specific component (active component) within some kind of matrix or encapsulant and thereby helping in controlled release under specific conditions (Desai and Park, 2005). The active component that will be entrapped is referred to as the core material, actives, fill, internal phase or payload and the polymeric substance surrounding it is known as the wall material, capsule, membrane, carrier or shell. The technology finds it applications in many areas such as pharmaceuticals, nutraceuticals and even in cosmetic industries (Sanguansri *et. al.*, 2013). This technique was found useful in protecting sensitive ingredients from biochemical deterioration and facilitates its controlled release (Adamiec and Kalemba, 2006). The encapsulation of bioactive compounds finds several applications in the food industry such as: (i) Protection of active components from factors that can cause oxidation, (ii) Reduction of transfer rate of the active components to the outside environment. (iii) Enhanced bio-availability and efficacy, (iv) Masking undesirable odors and flavors in the final product, (v) Better handling of the bioactive components, (vi) Better storage and enhanced shelf life, and (vii) Controlled and sustained release of active components, especially in the case of drug delivery systems (Desai and Park, 2005).

Depending on the size of material produced, either microparticles or nanoparticles can be obtained. Microcapsules thus produced can again be classified into three, based on their morphology.

1. Mononuclear (core-shell) microcapsules containing the shell around the core.
2. Polynuclear capsules have many cores enclosed within the shell.
3. Matrix encapsulation in which the core material is distributed homogeneously into the shell material.

## Wall materials for encapsulation

The main role of wall material is to protect the core material from factors that can affect its oxidative stability and controlled release of core material under the desired conditions. The judicious selection of wall material is an important task as it has an important role to play in many aspects of encapsulation such as encapsulation efficiency,

stability as well as the protection of the core compound. The wall material selection depends on number of factors such as solubility, molecular weight, glass transition temperature, diffusibility, film forming, emulsifying properties etc. Apart from this, the cost of wall material also has to be taken into account because the total process should be economical.

The wall materials used for encapsulation should be safe and should not have any adverse effects on human health and well-being. It is important to test the potential toxicity of wall materials to ensure their safety. If it is meant for food applications, it should have "Generally Recognized As Safe" (GRAS) status. Moreover, it should be biodegradable, economical, efficient and form a protective barrier between the core material and the surrounding medium. It should be able to remain physically and chemically stable when exposed to various environmental stresses such as pH, ionic strength etc. while retaining its desirable functional properties (McClements, 2015). The most commonly used materials are:

1. Carbohydrate polymers (starch and cellulose and their derivatives),
2. Plant exudates and extracts (gum, galactomannans, pectins and soybean polysaccharide)
3. Marine extracts (carragenan and alginate)
4. Microbial and animal - derived polysaccharides (xanthan, gellan, dextran and chitosan)
5. Proteins (whey protein, zein, collagen, gelatin etc.)

**Methods used for encapsulation**

One important criteria that determines the success of encapsulation is the method of encapsulation. Currently, there are a number of encapsulation technologies being employed to attain a better encapsulation process and efficiency. Some of the most commonly employed technologies for microencapsulation are discussed here:

**(1) Spray Drying:** Spray drying is the most widely used encapsulation technique in the food industry. This is mostly employed for preparation of dry, stable food additives and flavors (Desai and Park, 2005). It is economical, flexible, efficient, easy to scale-up technology which produces good quality powder with low water activities that can be easily stored and transported. This is used to produce huge quantities of material in a minimal continuous processing operation

(Anandharamakrishnan *et. al.*, 2007). It can produce high quality microcapsules, usually with a size less than 40 μm, by atomizing a liquid solution or emulsion through a nozzle to a hot gas chamber where subsequent dehydration of the atomized droplets occurs to yield microcapsule. However, certain researchers have reported that it can cause oxidation of poly unsaturated fatty acids due to high temperatures employed during the process (Heinzelmann *et. al.*, 2000).

(2) **Freeze drying:** Freeze drying is widely accepted as one of the best methods for production of superior quality dried products (Calvo *et. al.*, 2011). The low temperature and removal of about 97-98% moisture content and oxygen employed in freeze drying process leads to production of superior quality products than spray drying (Minemoto *et. al.*, 2001). The technology also ensures good rehydration properties of the powdered product. However, one limitation with this technology is that it is an expensive process requiring high energy consumption and processing time. Compared to spray drying, freeze drying is upto 30–50 times more expensive. Freeze drying produces microencapsulated powder with porous, irregular, and flake-like structure that can accelerate the oxidation process (Heinzelmann *et. al.*, 2000; Anwar and Kunz, 2011). Taking into consideration the limitations of both freeze drying and spray drying, it can be concluded that the freeze drying can be employed to encapsulate products that are highly sensitive to heat.

(3) **Coacervation:** Coacervation is the phase separation of one or many hydrocolloids from the initial liquid solution and subsequent deposition of the newly formed coacervate phase around the active ingredient (Gouin, 2004). There are two kinds of coacervation - simple and complex. In case of simple coacervation, there will be only one polymer whereas in complex coacervation, the interaction between oppositely charged polymers is made use of (Wu and Xiao, 2005). Complex coacervation is an expensive method for encapsulating food ingredients (Gouin, 2004) and is commonly employed for the encapsulation of high-value, labile functional ingredients, such as polyphenol compounds. The biopolymers that are widely employed in the complex coacervation process are gelatin or whey protein and oppositely charged gum Arabic, sodium polyphosphate or carboxy methyl cellulose. This method produces microcapsules that are having better and controlled release activities along with heat resistant properties (Jun-xia *et al.*, 2011). One limitation with this technology is that the coacervates produced are stable over a narrow range of pH and ionic strength.

(4) **Extrusion:** Extrusion process involves mixing of the molten wall material with the core material which is then allowed to pass through a nozzle under high pressure to produce microcapsules of higher density and less porosity (Serfert *et. al.*, 2009). But the technology is more expensive than spray drying and moreover, the generation of high shear forces during the extrusion process affects the stability of the microcapsule (Gouin, 2004).

(5) ***In situ* polymerization:** *In situ* polymerization is commonly used for the preparation of microcapsules and functional fibers. The process doesn't include any reactants in the core material and polymerization occurs in the continuous phase itself. By adjusting pH and temperature, the wall material precipitates and distributes evenly over the surfaces of core material. Particles produced by this technology is found to have better encapsulation efficiency, good chemical, thermal and storage stability and controlled release.

(6) **Liposome entrapment:** Liposomes are microscopic, spherical lipid bilayers that can enclose a number of aqueous compartments. The most commonly used encapsulating agent in this method is phospholipids and they are bio-compatible and bio-degradable substances (Kim and Baianu, 1991). The formation of a lipid bilayer is mainly attributed to the amphiphilic nature of phospholipids. These liposomes can be used to encapsulate omega-3 fatty acids by dissolving them in phospholipid before addition of water. This mixture of phospholipid, omega-3 oil and water is then sonicated to form encapsulated products and oil encapsulated in liposomes is said to have better oxidative stability (Kubo *et. a.l.* 2003). But the limitation of this technology is its high cost and low stability.

(7) **Fluidized bed drying:** Fluidized bed drying is the method that is restricted mainly to the encapsulation of solid core materials where a coating is applied on the powder particles (Rumpler and Jacob, 1998). Hence this method cannot be used for the direct encapsulation of fish oil, instead it can be considered as a secondary method to provide an additional coating on the already microencapsulated fish oil for better oxidative stability and physico-chemical properties.

**Characterization methods for encapsulated particles**

Microencapsulated oil produced by different encapsulation technologies using wide variety of wall materials has to be characterized for studying its physico-chemical

properties and its oxidative stability. The important parameters that have to be taken into account for its characterization are described below:

(1) **Encapsulation efficiency** The encapsulation efficiency (EE) is the ratio of oil entrapped inside the wall material to the initial concentration used. For better encapsulation efficiency, the content of surface oil, that is the oil that is not entrapped inside the wall material, should be as low as possible. This is because of the fact that the surface oil content remains unprotected and can easily undergo oxidation, resulting in off-flavour generation and poor oxidative stability. For safe storage and better oxidative stability, ideally the surface oil content should be less than 0.1% (w/w).

Encapsulation efficiency = (TO-SO/SO)*100, Where, TO - Total oil content; SO-Surface oil content

(2) **Loading capacity (LC)** Loading capacity is the measure of the amount of oil encapsulated per unit mass of the encapsulation material.

LC = (Mass of encapsulated oil / Mass of wall material)*100

(3) **Percentage yield (PY)** The percentage yield (PY) is a measure of the encapsulation process to produce the encapsulated powders.

PY = (Mass of loaded encapsulation system/ Mass f oil and wall material used)*100

(4) **Particle size** The size of microcapsule has an important role in the oxidative stability of the product and its size range should be of a uniform nature in order to maintain the product consistency. The size of the microcapsules can be measured using laser scattering or particle size imaging using microscopy. For the detailed study of the morphology of microcapsules, high resolution imaging using electron microscopy or confocal laser scanning microscope (CSLM) can be used.

(5) **Bulk density and tapped density** Bulk density and tapped density are important parameters from an economic point of view. These two parameters play an important role in the packaging, storage and transport of powdered products. Based on these two parameters, the flowability and cohesiveness of the encapsulated material can be measured.

(6) **Moisture content** The moisture content of the encapsulated powder plays an important role in determining the flowability, stickiness, cohesiveness, hygroscopicity and storage life. The encapsulation method adopted is also found to have a profound influence on the moisture content. The maximum moisture content allowed for dried powders of food industry application was found to be around 3-4 %.

(7) **Hygroscopicity** Hygroscopicity is the capacity of food to contain occluded moisture and this can be affected by inherent product composition and the concentration of the carrier material used (Ferrari *et. al.*, 2012). This also plays an important role in the product reconstitution since it can lead to caking and thereby reducing dispersibility.

(8) **Oxidative Stability** The main application of encapsulation technology is to protect the bioactive material against oxidation by providing an oxygen barrier in the form of wall materials. Currently, a number of substances are being used as the wall material for encapsulation depending upon the nature of substance to be encapsulated and the type of encapsulation technology used. Oxidative stability of the encapsulated powder is measured by storing the microcapsules under a set of temperature and relative humidity for a definite period and quantifying the type and amount of oxidative products formed. There are different methods employed for the measurement of oxidative stability of the encapsulated powders. Peroxide value, TBARS, acid value, propanal and rancimat test are some of the commonly used tests to assess the oxidative stability of the product. The rancimat analysis is one of the best ways to study oxidation because of its ease of use and less time when compared to the conventional oxidative stability indices (Velasco *et.al.*, 2000).

(9) **Other Indices** Apart from this, many other characterizations are being used for the encapsulated product such as Scanning Electron Microscopy measurements, FTIR, Zeta potential, water activity, colour, true density, wettability, solubility, *in vitro* release studies for complete analysis etc.

## Conclusion

The potential of the encapsulation technique has greatly increased as the process has become one of the best alternatives to improve the stability and functional attributes of many biologically sensitive compounds. With the increasing interest in the

development and fabrication of nutraceuticals, several types of nascent and structured bioactives has been developed. For the successful delivery of these nutrients, special delivery systems have to be designed which will guarantee their release at the target site as well as the overall stability of the final formulated product. Apart from this, research should focus on the physico-chemical stability, dispersibility, bio-availability, release and safety of such microencapsulated products before their commercialization.

## References

Adamiec, J. and Kalemba, D. (2006) - Analysis of microencapsulation ability of essential oils during spray drying. *Drying Technol.*, 24(9): 1127-1132.

Anandharamakrishnan, C., Rielly, C.D. and Stapley, A.G.F. (2007) - Effects of process variables on the denaturation of whey proteins during spray drying. *Drying Technol.*, 25(5): 799-807.

Anwar, S.H. and Kunz, B. (2011) - The influence of drying methods on the stabilization of fish oil microcapsules: Comparison of spray granulation, spray drying, and freeze drying. *J. Food Engg*, 105(2): 367-378.

Calvo, P., Castaño, Á.L., Hernández, M.T. and González Gómez, D. (2011) - Effects of microcapsule constitution on the quality of microencapsulated walnut oil. *European J. Lipid Sci. & Technol.* 113(10):1273-1280.

Desai, K.G.H. and Jin Park, H. (2005) - Recent developments in microencapsulation of food ingredients. *Drying Technol.*, 23(7): 1361-1394.

Ferrari, C.C., Germer, S.P.M. and de Aguirre, J.M. (2012) - Effects of spray-drying conditions on the physicochemical properties of blackberry powder. *Drying Technol.* 30(2): 154-163.

Gouin, S. (2004) - Microencapsulation: Industrial appraisal of existing technologies and trends. *Trends in Food Sci. & Technol.*, 15(7): 330-347.

Heinzelmann, K., Franke, K., Jensen, B. and Haahr, A.M. (2000) - Protection of fish oil from oxidation by microencapsulation using freeze drying techniques. *European J. Lipid Sci. & Technol.* 102(2): 114-121.

Heinzelmann, K., Franke, K., Velasco, J. and Marquez-Ruiz, G. (2000) - Microencapsulation of fish oil by freeze-drying techniques and influence of process parameters on oxidative stability during storage. *European Food Res. & Technol.* 211(4): 234-239.

Jun-xia, X., Hai-yan, Y. and Jian, Y. (2011) - Microencapsulation of sweet orange oil by complex coacervation with soybean protein isolate/gum Arabic. *Food Chem.*, 125(4): 1267-1272.

Kim, H.H.Y. and Baianu, I.C. (1991) - Novel liposome microencapsulation techniques for food applications. *Trends in Food Sci. & Technol.*, 2: 55-61.

Kubo, K., Sekine, S. and Saito, M. (2003) - Docosahexaenoic acid-containing phosphatidyl ethanol amine in the external layer of liposomes protects docosahexaenoic acid from 2, 22 -azobis (2-aminopropane) dihydrochloride-mediated lipid peroxidation. *Arch. Biochem. Biophys.* 410(1): 141-148.

McClements, D.J. (2015) - Nanoscale nutrient delivery systems for food applications: Improving bioactive dispersibility, stability, and bioavailability. *J. Food Sci.* 80(7).

Menrad, K. (2003) - Market and marketing of functional food, *European J. Food Eng*, 56: 181–188.

Minemoto, Y., Adachi, S. and Matsuno, R. (2001) - Effect of relative humidity during storage on the autoxidation of linoleic acid encapsulated with a polysaccharide by hot-air-drying and freeze-drying. *Food Sci. & Technol. Res.*, 7(1): 91-93.

Roberfroid, M.B. (2000) - Concepts and strategy of functional food science: The European perspective. *Am. J. Clin. Nutrn*, 71: S1660–S1664.

Rumpler, K. and Jacob, M. (1998) - Continuous coating in fluidized bed. *Food Market Technol.*, 12: 41-43.

Sanguansri, L., Day, L., Shen, Z., Fagan, P., Weerakkody, R., Cheng, L.J. and Augustin, M. (2013) - Encapsulation of mixtures of tuna oil, tributyrin and resveratrol in a spray dried powder formulation. *Food & Function*, 4: 1794–1802.

Serfert, Y., Drusch, S. and Schwarz, K. (2009) - Chemical stabilization of oils rich in long-chain polyunsaturated fatty acids during homogenization, microencapsulation and storage. *Food Chem.*, 113(4): 1106-1112.

Sloan, A.E. (2002) - The top 10 functional food trends: The next generation. *Food Technol.* 56(4): 32-57.

Velasco, J., Dobarganes, M.C. and Márquez-Ruiz, G. (2000) - Application of the accelerated test rancimat to evaluate oxidative stability of dried microencapsulated oils. *Grasas y Aceites*, 51(4): 261-267.

Wu, K.G. and Xiao, Q. (2005) - Microencapsulation of fish oil by simple coacervation of hydroxypropyl methyl cellulose. *Chinese J. Chem.*, 23(11): 1569-1572.