Microencapsulation of Fish Oil – Current Perspectives

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Introduction

The current focus of the world food market is now on foods that not only satisfy hunger and provide necessary nutrients but also prevent nutrition-related diseases and improve physical and mental well-being of the consumers (Menrad, 2003; Roberfroid, 2000). This kind of foods are often referred to as functional foods. Fish oils are often categorized under functional foods because of its rich content of polyunsaturated fatty acids such as Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) that is reported to have numerous health benefits. Evidences from researches have shown the importance of omega-3 fatty acids and their role in human health such as the potential use of n−3 PUFA in psoriasis, on bowel diseases, on treatment and prevention of mental illnesses, on prevention of several types of cancer, effect on rheumatoid arthritis and cardiovascular diseases and so on.

With the increase in awareness of the health benefits associated with the fish oil consumption, there has been a growing demand for fish oil and fish oil-fortified foods. Fish oil, being rich in unsaturated fatty acids is highly susceptible to oxidation, resulting in the production of toxic hydro peroxides and other byproducts, off-flavors and hence a shorter shelf-life of the product (Nawar 1996; Watkins and German, 1998). Moreover, the high temperatures during processing and further storage can also cause rapid deterioration of these fatty acids and affect the sensory acceptance of the food also. The adverse health effects due to the consumption of foods containing oxidized fats have already been reported by many researchers. Gotoh and Watanabe (2006) reported cases of food poisoning due to consumption of instant noodles containing oxidized fats. Liver damage in rats fed with oxidized oil was reported by Totani and Burenjargal (2008). Hence a direct addition of fish oil is not advisable as it decrease the safety of fortified foods due to the formation of oxidized compounds. Hence certain measures should be adopted to protect fish oil from oxidation and also for its safe fortification in food products. Microencapsulation is a promising and advancing technology that can be used to protect fish oil against oxidation and allow the manufacturer to handle and incorporate oil in food products (Kolanowski *et al.*, 1999; Klinkesorn *et al.*, 2005). The application of encapsulation technology is briefly discussed here.

Encapsulation

Encapsulation is a rapidly expanding technology that involves the entrapment of an active compound within another polymeric substance. The active compound that will be entrapped is usually referred to as the core material and the polymeric substance surrounding it is known as the wall material or the shell material. The technology find its applications in many areas such as pharmaceutical, nutraceuticals and even in cosmetic industries (Sanguansri *et al.*, 2013). The important benefits associated with the encapsulation of bioactive compounds are as follows:

- Better handling of the bioactive components. Eg: It transforms the oil into a powder, where the small droplets of oil are surrounded by a dry matrix of proteins and/or carbohydrate coating materials (Heinzelmann *et al.*, 2000a; Keogh *et al.*, 2001).

- Protection of active components from factors that can cause oxidation and hence a prolonged shelf life.
- The microencapsulation process can also help to mask undesirable fishy odours and flavors in the final product.
- Controlled release of active components, especially in the case of drug delivery systems.
- Enhanced bioavailability and efficacy.

The microcapsules thus produced can be categorized according to their morphology such as mononuclear, polynuclear and matrix. The mononuclear microcapsules contain the wall material layer around the core and polynuclear microcapsules will be having number of cores enclosed within the wall material. In matrix type, there will be a homogenous distribution of the core material in the wall material. Since the wall material has an important role to play in many aspects such as encapsulation efficiency, stability as well as the protection of the core compound, a proper selection of the wall material is an important task.

The most commonly used wall materials include:

- Polysaccharides – Chitosan, maltodextrin, sucrose, gum Arabic, modified starch, corn syrup solids, agar, alginates, carrageenan, pectin etc.
- Proteins – Whey protein isolate, skimmed milk powder, gelatin, sodium caseinate etc.

The wall material selection depends on a number of factors such as solubility, molecular weight, glass transition temperature, diffusibility, film forming, emulsifying properties etc. Moreover, the main role of wall material is to protect the core material from factors that can affect its oxidative stability and the controlled release of core material under the desired conditions. Apart from this, the cost of wall material also has to be taken into account because the total process should be economical.

Apart from the wall materials used, another important criteria that determines the success of encapsulation is the method of encapsulation. There are a number of encapsulation technologies now being used to attain a better encapsulation process and efficiency. The technologies that are presently being used for fish oil encapsulation are discussed here.

Microencapsulation Technologies

Spray drying: One of the most commonly used microencapsulation technology is spray drying which finds wide range of applications in food and pharmaceutical industries. It is a very economical, flexible, efficient, easy to scale-up technology which produces good quality powder with low water activities that can be easily stored and transported (Ashady, 1993). The process of spray drying involves the dissolution of wall material and core material resulting in the formation of an emulsion, followed by proper homogenization, pumping of the emulsion, atomization of the emulsion and the subsequent dehydration of the atomized droplets to yield a microcapsule. The size of microcapsules formed will depend on the concentration of solid content in the dispersion content and accordingly can vary from smaller to larger particles. Apart from this, the viscosity of the emulsion, feed rpm, inlet and outlet temperatures etc. also have an influence on the particle size as well as the oxidative stability of the particles. Many researchers have used spray drying technology for the encapsulation of fish oil using different wall materials such as gelatin, caseinate and maltodextrin; casein and lactose; sodium caseinate and dextrose equivalence; highly branched cyclic dextrin and sodium caseinate; methylcellulose and hydroxypropyl methylcellulose; sodium caseinate, glucose, glucose syrup; n-octenylsuccinate derivatized starch/glucose syrup or terhalose, gum Arabic; sugar beet pectin and glucose syrup, corn syrup soilds etc. (Lin *et al.*, 1995; Keogh *et al.*, 2001; Hogan *et al.*, 2003; Kagami *et al.*, 2003; Kolanowski *et al.*, 2004; Augustin *et al.*, 2006; Drusch *et al.*, 2006; Kolanowski *et al.*, 2006).

Freeze drying: Freeze drying is widely accepted as one of the best methods for production of superior quality dried products (Calvo *et al.,* 2011). Though spray drying is the most widely accepted technology for encapsulation, a lower oxidative stability of spray dried products has also been reported. Because of the low temperature employed in freeze drying process and removal of about 97-98% moisture content and oxygen, this technology is often reported to produce good quality products than spray drying (Minemoto *et al.*, 2001). The process of freeze drying involves three processes, freezing at a lower temperature of −90 and −40 °C, followed by primary and secondary drying under low pressure. But one limitation of this technology is that it is an expensive process requiring high energy consumption and higher processing time. Moreover, certain researchers have reported spray drying as a better process than freeze drying for fish oil encapsulation (Chen *et al.*, 2013). Some others have reported that freeze drying produces microencapsulated powder with porous, irregular, and flake-like structure that can accelerate the oxidation process (Heinzelmann *et al.*, 2000b and Anwar and Kunz, 2011). Taking into consideration the limitations of both freeze drying and spray drying, it can be concluded that freeze drying can be employed to encapsulate products that are highly sensitive to heat.

Coacervation: Coacervation, also known as phase separation is the separation of two liquid phases in a colloidal solution. The phase which is rich in polymer is known as the coacervate phase and that is devoid of polymer is known as equilibrium solution. 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 (Ke-Gang *et al.*, 2005). The biopolymers that are being 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). The microcapsules thus produced are collected by centrifugation or filtration and further dried by either spray or fluidized bed drying (Carvalho *et al.*, 2015). The size of microcapsules produced depends on a number of factors such as temperature, stirring speed, viscosity and pH (Carvalho *et al.*, 2015). One limitation with this technology is that the coacervates produced are stable over a narrow range of pH and ionic strength. Fish oil has been encapsulated by complex coacervation using hydroxypropyl methylcellulose with maltodextrin; sugar beet pectin and glucose syrup; whey protein, gum Arabic etc. (Wu *et al.*, 2005; Drusch, 2007).

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). The extrusion microencapsulation includes three processes such as centrifugal extrusion (coextrusion), melt injection and melt-extrusion. Centrifugal extrusion, also known as co-extrusion is another extrusion technology that is commonly used for microencapsulation which can produce microcapsules in the size range of is 500–1000 μm. Since the particle size of the extruded powder is more, it can impact the mouth feel. Melt injection process involves dispersion of the core material in a matrix containing starch, anti-oxidants, sugars, emulsifiers and water at about 130 °C, extruded through a die or filter into a bath filled with organic solvent such as isopropanol which solidifies the sugar matrix. The microcapsules thus formed are collected by filtration or centrifugation (Valentinotti *et al.*, 2006). The melt-extrusion process and melt-injection process is almost similar, where melt-injection is a vertical screw-less process with surface-washed particles, while the melt extrusion is a horizontal screw process with particles that are not surface-washed.

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.

Inclusion complexation: In this method, a cyclic oligosaccharide cyclodextrin is used as a main encapsulant which can form complexes with fish oil and thereby conferring it better oxidative stability. The formation of an inclusion complexation was observed by mixing fish oil and gamma cyclodextrin in the presence of nitrogen at 45 °C for 24 h and the resultant product contained fish oil of 15–40% (Schmid *et al.*, 2001). Similarly, Choi *et al.* (2010) reported that fish oil encapsulated in beta cylodextrin had 84.1% encapsulation efficiency.

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 al.*, 2003). But the limitation of this technology is its high cost and low stability.

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. In one of the patented technology for double encapsulation of fish oil, corn starch was used for coating the already spray dried powder (Skelbaek and Andersen., 1994). In another method, molten hydrogenated palm wax (30% w/w) was used to coat over the already spray dried fish oil powder (Ponginebbi and Publisi, 2008).

Other emerging methods for encapsulation of fish oil

Electrospraying for ultrathin coating: The electrospraying method was used to microencapsulate DHA using zein prolamine and the nano particles thus formed were found to have increased induction period and hence a better oxidative stability. Moreover, the method doesn't seem to alter the textural characteristics of the product (Sergio *et* al., 2010).

Spray granulation and fluid bed film coating: In this method, a combination of two technologies, spray granulation and fluid bed coating is used for encapsulation (Anwar *et al.*, 2010).

Encapsulation using ultrasonic atomizer: Ultrasonic atomizers use ultrasonic energy for atomization of emulsion and produces smaller particles of uniform size distribution. This is quite different from the conventional pressure spray nozzles where there is no control over the size of particles produced (Topp and Eisenklam, 1972; Bittner and Kissel, 1999). Fish oil encapsulated using ultrasonic atomizer obtained small particle size and better emulsion stability (Klaypradit and Huang, 2008).

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 oxidative stability.

The important parameters that have to be taken into account for its characterization are described below:

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 should be less than 0.1% (w/w).

Encapsulation efficiency = (TO-SO/SO)*100

Where, TO - Total oil content; SO - Surface oil content

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

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 of oil and wall material used)*100

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.

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.

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%.

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.*, 2011). This also plays an important role in the product reconstitution since it can lead to caking and thereby reducing dispersibility.

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 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 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.

Other indices: Apart from this, many other characterizations for the encapsulated product such as Scanning Electron Microscopy measurements, FTIR, Zeta potential, water activity, colour, true density, wettability and solubility are also being used nowadays for complete analysis.

Conclusion

The demand for fish oil is increasing in the food industry because of its high content of long chain polyunsaturated omega-3 fatty acids, which itself is responsible for important physiological functions and health benefits. But because of the high degree of unsaturation and the lack of the endogenous antioxidants, it is highly susceptible to oxidation. By employing the encapsulation technology, the oxidative instability problem of fish oil can be controlled to a greater extent and offer a better and targeted release of the product. But the success of technology often depends on the type of encapsulation methodology adopted, the conditions in which encapsulation is carried out, the type of wall material used, the concentration of core material and wall material used, pH, ionic strength conditions used etc. These factors can affect the encapsulation efficiency and other physico-chemical characteristics of the product. Hence a better selection of the wall material and encapsulation methodology has to be made for the production of microcapsules having all the desired properties as well as oxidative stability.

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