DEVELOPMENT OF OMEGA 3 FATTY ACID ENRICHED STABLE FUNCTIONAL FOODS: CHALLENGES AND OPTIONS

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ABSTRACT
The consumer interest in health-promoting foods is almost dramatically increasing because of the increased health consciousness of the modern people. Functional foods are fortified, enriched foods that contain specific health improving nutrients and prevent certain nutrition based disorders. Functional components are naturally present in certain foods (e.g. fruits, vegetables etc.,) and enrichment can be done in some cases to make it functional. Polyunsaturated omega 3 fatty acid (PUFA), a functional component present in fish such as sardine, salmon etc. and in flaxseed is generally recognized as being beneficial to the health. Omega-3 PUFA is rich in double bonds and unsaturated in nature, this attribute makes them highly susceptible to lipid oxidation and unfit for incorporation into long shelf life foods. Hence, it is necessary to find out a suitable delivery mechanisms to develop omega 3 rich functional food products. Microencapsulation allows a bioactive compound to be trapped or encapsulated inside a tiny sphere known as microsphere or microcapsule and thus protecting the core compound. The present article paper provides a literature review of different microencapsulation techniques that can be possibly used for the encapsulation of omega 3 fatty acid for developing stable omega 3 rich food products.

Key words: Functional Foods, Omega 3 Fatty Acid, Lipid Oxidation, Microencapsulation

INTRODUCTION
There is a widespread recognition that diet plays an important role in the incidence of many diseases. This is due to the lack of micronutrients such as vitamins and minerals in the daily consumption of food. The statement “Let food be thy medicine and medicine be thy food,” espoused by Hippocrates nearly 2,500 years ago, is receiving renewed interest. In particular, there has been an explosion of consumer interest in the health enhancing role of specific foods or physiologically-active food components, so-called functional foods (Hasler, 1998). The term functional foods was first introduced in Japan in the mid-1980s and refers to food which contains ingredients that aid specific bodily functions in addition to being nutritious. The Institute of Medicine Food and Nutrition Board defined functional foods as “any food or food ingredient that may provide a health benefit beyond the traditional nutrients it contains”. Hence, functional foods generally contains or enriched with specific or multiple micronutrients (e.g. n-3 fatty acid, antioxidants, proteins, vitamins and minerals) which are considered essential for human health. Some foods are considered to be functional in their natural form when the scientific information about their health qualities can be used to proclaim benefits. Several fruits, vegetables, grains, fish, meat and dairy products contain natural components that deliver benefits for human health. Examples include lycopene in tomato, omega 3 fatty acids in salmon or saponins in soy. Although the vast number of naturally occurring health-enhancing substances are of plant origin, there are a number of physiologically-active components in animal products that deserve attention for their potential role in optimal health and one of its kind is the omega 3 fatty acid found in fish oil. 

Omega 3 fatty acid - source and its components
The omega-3 fatty acids (also known as n-3 fatty acids) are a group of naturally occurring lipids, which are present in high concentrations in certain fish and plants such as flax seed oil, perilla oil and others (Cunnane et al., 1995). The term omega-3 ("n-3","ω-3") signifies that the first double bond exists at the third carbon-carbon bond from the terminal methyl end (ω) of the carbon chain. There are three most
common omega 3 fatty acids viz. Alpha linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). ALA is present in certain vegetable oils (flaxseed and canola), whereas EPA and DHA are present in fish and in more concentrated amounts in fish oil (Mantzioris et al., 2000). They are found in varying ratios in fish, particularly in cold water and pelagic species such as menhaden, mackerel, oil sardine and salmon (Stensby, 1969).

Table 1: Functional components, its sources and potential effects

<table>
<thead>
<tr>
<th>Functional Components</th>
<th>Source</th>
<th>Potential benefits</th>
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<tbody>
<tr>
<td><strong>Carotenoids</strong></td>
<td>Fruits, Vegetables, Greens.</td>
<td>Neutralize free radicals, which may cause damage to cells</td>
</tr>
<tr>
<td>Alpha carotene,</td>
<td></td>
<td>Reduce the risk of macular degeneration</td>
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<tr>
<td>Beta carotene,</td>
<td></td>
<td></td>
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<tr>
<td>Lutein</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Dietary fiber</strong></td>
<td>Wheat bran, Oats, Barley.</td>
<td>Reduce risk of breast or colon cancer, cardiovascular disease.</td>
</tr>
<tr>
<td>Insoluble fibre,</td>
<td></td>
<td>Lower LDL and total cholesterol</td>
</tr>
<tr>
<td>Beta glucan</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Omega 3 Fatty acids</strong></td>
<td>Salmon and other fish oils, flaxseeds</td>
<td>Reduce risk of cardiovascular disease. Improve mental, visual functions</td>
</tr>
<tr>
<td>DHA/EPA, CLA</td>
<td></td>
<td>Improve body composition. Decrease risk of certain cancers</td>
</tr>
<tr>
<td><strong>Phenolics</strong></td>
<td>Fruits, Citrus</td>
<td>Neutralize free radicals; reduce risk of cancer</td>
</tr>
<tr>
<td>Anthocyanidins,</td>
<td></td>
<td>Neutralize free radicals; reduce risk of cancer</td>
</tr>
<tr>
<td>Flavonones</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Prebiotics / Probiotics</strong></td>
<td>Jerusalem artichokes, Yogurt, Other dairy</td>
<td>Improve quality of intestinal microflora; gastrointestinal health</td>
</tr>
<tr>
<td>Fructo-oligosaccharides</td>
<td></td>
<td>Improve quality of intestinal microflora; gastrointestinal health</td>
</tr>
<tr>
<td>Lactobacillus</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Soy Phytoestrogens</strong></td>
<td>Soybeans, Jerusalem artichokes, shallots, onion powder</td>
<td>Menopause symptoms, such as hot flashes</td>
</tr>
<tr>
<td>Isoflavones,</td>
<td></td>
<td>Protect against heart disease and lower LDL</td>
</tr>
<tr>
<td>Fructo-oligosaccharides</td>
<td></td>
<td>Improve quality of intestinal microflora; gastrointestinal health</td>
</tr>
</tbody>
</table>

The origin of the omega-3 fatty acids found in these species of fishes is the chloroplasts of marine phytoplankton and algae (Stamey, 2010; Cohen, 1995). The EPA and DHA are then passed through the food chain, and ultimately to humans. The omega-3 fatty acids are considered 'essential', because the humans must ingest omega-3 fatty acids from their diet, since the omega-3 structure cannot be synthesized in humans. Fish oils are a main source of omega 3 fatty acids, the consumption of which yields higher concentrations of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) (Brown et al., 1990; Cobiac et al., 1991). In that EPA is a 20 hydrocarbon chain with five double bonds whereas DHA is a 22 hydrocarbon chain with six double bonds. The major difference among omega-3 fatty acids is the length of the carbon chain and the number of double bonds it possess.

**Omega 3 fatty acid as a functional food ingredient**

More recent studies assessed that omega 3 polyunsaturated fatty acid supplementation could be helpful against many inflammatory diseases (Meydani et al., 1993). Their anti-inflammatory effects is mainly
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related to its competition as substrates for cyclooxygenase (COX) and lipooxygenase (LOX) leading to the formation of less active prostaglandins and leukotrienes (James et al., 2000; Ziboh et al., 2000). The beneficial anti-inflammatory effects of high-dose fish oil in the reduction of joint pain, improvement in dry eyes and macular degeneration, reduction in infarction from coronary artherosclerosis is well documented (Adkisson et al., 1992; Belch et al., 1988). The consumption of fish and fish oil supplementation raise the blood levels of DHA and slowed down the progression of abnormal thickening of artery walls due to fatty deposits and prevents artherosclerosis (Erkkila et al., 2006; Bemelmans et al., 2002). The inclusion of functional foods containing omega 3 fatty acid in the normal diet have been supported by clinical evidence of reduced cardiovascular disease (Siscovick et al., 2000), reduced ultra violet radiation induced erythemal sensitivity (Rhodes et al., 1994) and breast cancer prevention. Controlled studies showed that ibuprofen and omega-3 fatty acid demonstrating equivalent effect in reducing arthritic pain. Dietary supplements ranging 1–8g per day of n-3 PUFA have been reportedly beneficial in the treatment of inflammatory bowl disease, eczema, psoriasis and rheumatomid arthritis and it reflects the necessity of its use as a functional food. The study conducted by Gustafsson et al. (1996) investigated the effects of the consumption of seafood products additionally enriched with omega-3 fatty acids. The diets including the omega-3-enriched seafood provided 3.0 g of omega-3 PUFA per day, compared with 0.3 g per day in the control group. Results showed a significant 25% reduction in plasma triacylglycerol levels, a significant 3.7% fall in systolic blood pressure and a 19% reduction in insulin secretion in the volunteers who consumed the fortified diet compared to the control diet. To match the amounts of omega-3 fatty acids provided in the test diet of the study (3.0 g per day), volunteers would have been required to consume at least 10 portions of oil-rich fish per week.

Table 2: Guidelines for EPA and DHA intake by different organizations (Kelley et al., 2006)

<table>
<thead>
<tr>
<th>Recommendations for EPA and DHA Intake</th>
<th>Recommendation</th>
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<tbody>
<tr>
<td>American Heart Association</td>
<td>1.5-3g/day</td>
</tr>
<tr>
<td>British Nutrition Foundation Task Force</td>
<td>1.0-1.5g/day</td>
</tr>
<tr>
<td>UK Department of Health</td>
<td>0.5g/day</td>
</tr>
<tr>
<td>World Health Organization</td>
<td>0.7g/day</td>
</tr>
<tr>
<td>Institutes of Medicine Dietary Reference Intakes</td>
<td>1.1-1.6g/day</td>
</tr>
</tbody>
</table>

Delivering long-chain omega-3 polyunsaturated fatty acids in foods

In several countries average fish intake is far below the recommended minimum of two fish servings per week (i.e. approximately 200.0 mg EPA and DHA per day) (Kolanowski et al., 2006). Besides changing of eating and culinary habits, the increase of omega-3 PUFA level in a diet may be achieved by intake of fish oil supplements or food enriched with fish oil (Shahidi et al., 1998; Wallace et al., 2000). Fortification of commonly consumed food products with n-3 LCPUFAs is considered an innovative way of providing the health benefits to people without major alteration in their dietary habits (Garg et al., 2006). There is a restriction in the level of oil fortified in different products such as bakery, dairy and other frozen foods as fish oil is highly susceptible to oxidation during storage. (Willumsen, 2006). Fatty acid present in fish oil would undergo oxidative deterioration leading to degradation of the original long carbon chain to yield highly reactive intermediate lipid radicals and ultimately potentially unhealthy small molecules. It is found that hydroperoxides which is the primary product of lipid oxidation are considered to be toxic for human health (Orada, 1988). As the number of double bonds in a fatty acid increases so does the rate of oxidation. This makes polyunsaturated lipids such as omega-3 fats highly susceptible to oxidation (Keogh et al., 2001).

Therefore, the effort to incorporate fish oil directly into food formulations always a challenging task mainly because the “fishy” flavours revealed from final food products (Kelly, 2000). Therefore, various
attempts were taken to protect the long-chain polyunsaturated fatty acids (LCPUFA) from oxidation, in order to harness the beneficial effect of omega-3 fatty acids. One way of protecting these oils in foods is by encapsulating them in a matrix that acts as a barrier, reducing the contact between the unsaturated fatty acids in the oil and oxidizing agents, such as light, heat, and metal ions (Ye et al., 2009). Therefore, fish oils may be encapsulated to increase storage stability and to enable supplementation in foods (Drusch et al., 2006).

Figure 1: Different techniques used for Microencapsulation.

**Microencapsulation of Omega 3**

Microencapsulation is a process in which small particles of the active and/or sensitive component known as the core are packaged within an encapsulating matrix (Desai and Park, 2005). The stability of the microencapsulated ingredient depends on how well the core has been stabilized and also the robustness of the carrier matrix that plays a major part in keeping these ingredients stable during various processing conditions. The technique used for microencapsulation must be very effective in preventing the formation of even very low levels of oxidation by-products (Barrow et al., 2007, Drusch and Berg, 2008). Microencapsulation by spray-drying has been applied in the food industry and is still the predominating technology as it is rather inexpensive and straightforward (Gouin et al., 2004). This monograph gives detailed account on various microencapsulation techniques available to the food industry to facilitate incorporation of n-3 LCPUFAs into foods as a function of their complexity and production capacity.

**Physical process**

The mechanical methods of microencapsulation are as follows,

*Spray Drying:*

Spray drying is a low-cost microencapsulation technology and the most commonly used in the food industry. This technique has been widely used for drying heat-sensitive foods, pharmaceuticals and other substances, because of the rapid evaporation of the applied solvent from the droplets. Spray drying is used not only for drying various food products (e.g., dairy products such as milk) but also for specific microencapsulation of various food ingredients, such as flavourings, colouring substances, fats and oils. Spray drying involves atomization of the feed (dispersion or emulsion) using a pressure nozzle or a centrifugal wheel into hot medium (typically air), resulting in rapid evaporation of water (Vega and Roos, 2006, Zuidam and Shimon, 2010). It involves the transformation of a feed from a liquid form (solution, dispersion or paste) into a powder by spraying the feed into a hot drying medium. It is a continuous operating process involving a combination of several stages, namely atomization, mixing of spray and hot air, evaporation and dry product separation (Ashady, 1993; Re, 1998). Depending upon the feed composition, dryer design, and operating parameters, spray drying can result in powder particles or agglomerate type conformations (Beindorff and Zuidam, 2010). The powder particles obtained after spray drying of an oil-in-water emulsion generally have a matrix-type structure in which the oil droplets are embedded in a continuous phase of wall material and carrier material (Jafari et al., 2008). There are
several materials used for coat formation during microencapsulation by spray drying. The coatings are generally food-grade hydrocolloids such as gelatin, plant gums, modified starch, dextrin or non-gelling proteins (Kanawija, 1992). Most commonly used wall materials for microencapsulation by spray drying include gums (gum arabic, locust bean gum), lipids (wax, palm fat), proteins (gelatin, milk proteins, soy protein), polysaccharides (starch, xanthan, pullulan, guar gum, alginate), and mono-, di-, and oligosaccharides (hydrolyzed starch, lactose), as well as cellulose and its derivatives (carboxymethylcellulose, methylcellulose) (Drusch et al., 2007). Maltodextrin, glucose syrup, modified starch, sugars, and/or modified cellulose can be used as carrier material (Beindorff and Zuidam, 2010).

Under appropriate handling and storage conditions, the physicochemical properties of spray dried powders are dictated by the composition of the particle surface. Properties like flowability and redispersion are directly associated with the amount of free oil on the surface of the powder. Most importantly, in the case of powders containing fish oil, any free oil is available for oxidation and may lead to off-flavor development (Drusch and Berg, 2008). To prevent the surface oil oxidation, fluidized bed coating subsequent to spray drying has been used to provide a second coating on the surface of the dried particles by using materials such as starches, waxes, maltodextrin, and gums (Drusch and Mannino, 2009). Modification to nozzle and spray-drier design has also led to the highly efficient coating capabilities by simultaneously allowing feed, hot air, and a second coating material in the drier (Drusch and Mannino, 2009). The disadvantage of this technology is the high temperature conditions necessary for drying and access to air. Moreover, parts of the product during drying may adhere to the surface of the capsules, which presents potential for oxidation and changes in the flavor balance of the finished food products (Kanawija, 1992).

Complex coacervation:
In a system with two colloids, the separation of the colloids having opposite charges has been named complex coacervation. This method can be used to deposit multiple layers of polymers on the core material (Drusch and Mannino, 2009). It is defined as the partial desolvation of a homogeneous polymer solution into a polymer-rich phase (coacervate) and the poor polymer phase (coacervation medium) (de Jong, 1949). Currently, two methods for coacervation are available, namely simple and complex processes. In simple coacervation, a desolvation agent is added for phase separation, whereas complex coacervation involves complexation between two oppositely charged polymers. The three basic steps in complex coacervation are: (i) formation of three immiscible phases; (ii) deposition of the coating; and (iii)
rigidization of the coating. The process begins with the formation of an emulsion with a solution of a positively charged polymer, such as gelatin type A (isoelectric point of 9) or chitosan and fish oil. A second solution made with a negatively charged polymer, such as sodium polyphosphate, gelatin type B, gum arabic, alginate, or carboxymethylcellulose, is added to the emulsion. The pH of the emulsion is adjusted; resulting in the coacervates with the polymers precipitating on the core, giving it enhanced stability.

The first step is the dispersion of core material in a solution of the coating polymer. The coating material phase, an immiscible polymer in liquid state, is formed by (i) changing temperature of polymer solution, e.g. ethyl cellulose in cyclohexane12 (N-acetyl P-amino phenol as core), (ii) addition of salt, e.g. addition of sodium sulphate solution to gelatine solution in vitamin encapsulation, (iii) addition of non-solvent, e.g. addition of isopropyl ether to methyl ethyl ketone solution of cellulose acetate butyrate, (iv) addition of incompatible polymer to the polymer solution, e.g. addition of polybutadiene to the solution of ethylcellulose in toluene, (v) inducing polymer–polymer interaction, e.g. interaction of gum arabic and gelatine at their iso-electric point. The second step includes deposition of liquid polymer upon the core material. Finally, the prepared microcapsules are stabilized by cross-linking, desolvation or thermal treatment. Cross-linking is the formation of chemical links between molecular chains to form a three-dimensional network of connected molecules. The strategy of covalent crosslinking is used in several other technologies of commercial and scientific interest to control and enhance the properties of the resulting polymer system or interface, such as thermostets and coatings (Wicks et al., 1999). The size of the microparticles is typically 1–5 μm. These microcapsules can be agglomerated by agitation while cooling, which may be followed by addition of a cross-linking agent (glutaraldehyde or transglutaminase) to strengthen the shell. This is followed by washing with water and spray drying to remove moisture. The final powder is free flowing with a particle size of 10–100 μm and may have oil loading of 40% to 90% (Beindorff and Zuidam, 2010; Drusch and Mannino, 2009; Yan et al., 2004).

The main advantage of complex coacervation over simple spray drying is the higher oil content and the relatively low amount of surface oil. Surface oil content in the produced coacervates is about 0.2% of the total oil, as compared with 0.2% to 1.0% in spray-dried powders (Barrow et al., 2007). Higher amounts of surface oil are thought to be directly proportional to unencapsulated oil on the surface available for oxidation, given that sample concentration of oxidation initiators are available in the environment (Jafari et al., 2008). The main disadvantage of complex coacervation is the use of gelatin in the process, which makes it difficult to acquire halal or kosher status because it is obtained from pig skin (Barrow et al., 2007, Beindorff and Zuidam, 2010). Other sources of gelatin (beef, poultry, and fish) are available but are not very cost effective (Beindorff and Zuidam, 2010). The process is also thought to be quite complex, as meticulous control over processing parameters is required throughout the process and can be rather expensive. Also, the use of gluteraldehyde as a cross-linking agent has strict legislation in some countries, and therefore its concentration has to be carefully monitored (Desai and Park, 2005).

Spray congealing:

Spray congealing can be done by spray-drying equipment where protective coating will be applied as a melt. Core material is dispersed in a coating material melt rather than a coating solution. The suspension mixture is then cooled, and the resulting solidified microcapsules are separated from the oil. Coating solidification is accomplished by spraying the hot mixture into cool air stream. Waxes, fatty acids and alcohols, polymers which are solids at room temperature but meltable at reasonable temperature, are applicable to spray congealing. Insulin has been microencapsulated in polyanhydride by using this technique. In the polymer precipitation process, an aqueous solution of the polymer containing the drug is dropped into a stirred solution, which acts as the precipitating medium. Here, the polymer droplets precipitate immediately and are thus converted into the drug loaded microcapsules. Enzymes have been encapsulated in conjugated phenolic polymers by using this technique (Maschke et al., 2006).

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Figure 3: Schematic representation of the coacervation process. a) Core material dispersion in solution of shell polymer; b) separation of coacervate from solution; c) coating of core material by microdroplets of coacervate; d) coalescence of coacervate to form continuous shell around core particles.

Fluidized-bed technology:
The liquid coating is sprayed onto the particles and the rapid evaporation helps in the formation of an outer layer on the particles. The thickness and formulations of the coating can be obtained as desired. Different types of fluid-bed coaters include top spray, bottom spray and tangential spray. In the top spray system the coating material is sprayed downwards on to the fluid bed such that as the solid or porous particles move to the coating region they become encapsulated. Increased encapsulation efficiency and the prevention of cluster formation are achieved by opposing flows of the coating materials and the particles. Dripping of the coated particles depends on the formulation of the coating material. Top spray fluid-bed coaters produce higher yields of encapsulated particles than either bottom or tangential sprays. The bottom spray is also known as ‘Wurster’s coater’ in recognition of its development. This technique uses a coating chamber that has a cylindrical nozzle and a perforated bottom plate. The cylindrical nozzle is used for spraying the coating material. As the particles move upwards through the perforated bottom plate and pass the nozzle area, they are encapsulated by the coating material. The coating material adheres to the particle surface by evaporation of the solvent or cooling of the encapsulated particle. This process is continued until the desired thickness and weight is obtained. Although it is a time-consuming process, the multilayer coating procedure helps in reducing particle defects. The tangential spray consists of a rotating disc at the bottom of the coating chamber, with the same diameter as the chamber. During the process the disc is raised to create a gap between the edge of the chamber and the disc. The tangential nozzle is placed above the rotating disc through which the coating material is released. The particles move through the gap into the spraying zone and are encapsulated. As they travel a minimum distance there is a higher yield of encapsulated particles (Srivastava et al., 2010).

Solvent evaporation:
Solvent evaporation techniques have become more useful method as compared to other methods. Controlled particle sizes in the nano to micrometer range can be achieved by this method, but there is a need of careful selection of encapsulation materials and various conditions in order to achieve high encapsulation efficiency and a low residual solvent content. Several process variables had been identified by researchers which could affect the formulation of microsphere by solvent evaporation method such as type of solvent, volume of solvent, drug to polymer ratio, rate of solvent removal, effect of internal
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aqueous phase volume in case of solvent evaporation followed by multiple emulsion, effect of addition of buffer or salts to the internal or external phase which can affect the size of microsphere. So change in one of the above parameter causes the significant change in drugs loading & desired release rate (Tiwari and Verma, 2011). In solvent evaporation method three phases are present. They are core, coat material, liquid manufacturing vehicle (LMV). Initially coat material will be dissolved in a volatile solvent, which is not soluble in LMV phase. A core material to be encapsulated is dissolved or dispersed in the coating polymer solution. This mixture is added to the liquid manufacturing vehicle phase with agitation, the mixture is heated to evaporate the solvent for polymer. Here the coat material shrinks around the core material and encapsulates the core. Microspheres were formed after solvent evaporation and polymer precipitation. The content of the microspheres increased with increasing theoretical loading, increasing amounts of organic solvent, polymer and polymeric stabilizer and decreased with increasing stirring time, increasing pH of the continuous phase and increased volume of the internal and external aqueous phase (Rainer and Bodmeier, 1990).

Chemical process

Emulsion polymerisation:

According to this technique the monomer (alkyl acrylates) is added dropwise to the stirred aqueous polymerisation medium containing the material to be encapsulated (core material) and a suitable emulsifier (Tiarks et al., 2001). The polymerisation begins and initially produced polymer molecules precipitate in the aqueous medium to form primary nuclei. As the polymerisation proceeds, these nuclei grow gradually and simultaneously entrap the core material to form the final microcapsules. In addition to the entrapment of active materials during microcapsule formation, content loading can also be accomplished by incubation of cyanoacrylate nanocapsules (empty nanocapsules) with the dissolved or finely dispersed drug.

Suspension cross linking:

Suspension cross linking is the method of choice for the preparation of protein and polysaccharide microcapsules. Microcapsule formation by this technique involves dispersion of an aqueous solution of the polymer containing core material in an immiscible organic solvent (suspension/dispersion medium) in the form of small droplets. The suspension medium contains a suitable stabilizer to maintain the individuality of the droplet/microcapsules. The droplets are subsequently hardened by covalent cross linking and are directly converted to the corresponding microcapsules. The cross linking process is accomplished either thermally (at >500 °C) or by the use of a cross linking agent (formaldehyde, terephthaloyl chloride, etc). Suspension cross linking is a versatile method and can be adopted for microencapsulation of soluble, insoluble, liquid or solid materials, and for the production of both micro and nanocapsules. Albumin nanocapsules containing doxorubicin and magnetite particles have been synthesised by using this technique (Arshady, 1989; Katti, 1999).

In situ polymerization

The capsule shell formation usually takes place because of polymerization monomers added to the encapsulation reactor. In this process no reactive agents are added to the core material, polymerization occurs exclusively in the continuous phase and on the continuous phase side of the interface formed by the dispersed core material and continuous phase. Initially a low molecular weight prepolymer will be formed, as time goes on the prepolymer grows in size, it deposits on the surface of the dispersed core material there by generating a solid capsule shell (e.g. encapsulation of various water-immiscible liquids with shells formed by the reaction at acidic pH of urea with formaldehyde in aqueous media. The Carboxyl-functionalized magnetic microspheres were prepared by in situ polymerization of styrene and methyacrylic acid at 85°C in the presence of nano-Fe₃O₄ in styrene, using lauryl peroxide as an initiator (Wang et al., 2004).

Alginate-based microspheres

Alginates-based microspheres are widely used in the pharmaceutical and medical fields. More recently, alginate have been used to encapsulate biomaterials, such as probiotic bacteria, functional oils, and those
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in other controlled delivery applications. Derived from brown algae, alginates are generally regarded as nontoxic and biocompatible. Alginates are block copolymers composed of \( \beta \)-D-mannuronic acid and linked with 1–4 linkages. Calcium is commonly used to start ionic interaction between \( \alpha \)-L-guluronic acid residue to form chains of two or more units, causing gel formation. Alginates, being ionic biopolymers, exhibit pH and charge sensitivity that offers encapsulation opportunities. There are several methods used to produce microspheres, depending on the release of calcium ions to cause gelation. One such method involves the preparation of a water-in-oil emulsion in which the calcium ions are present in the water phase. Calcium alginate solution is then added to this emulsion, which causes the alginate to deposit on the surface of the droplets because of phase inversion. Another method involves the use of an oil-in-water emulsion made with alginate, an emulsifier, and a second surfactant sprayed into calcium chloride solution. These particles are dried after being collected and washed to remove excess oil on the surface (Drusch and Mannino, 2009). Further coating, hot air drying, and cross linking may be applied to enhance the barrier properties of the alginate microspheres. Being relatively inexpensive, readily available, and safe to use in food products allows alginates to be common in the food and pharmaceutical industries. At the lab scale, microcapsule or bead systems are relatively easy to prepare using various available biopolymers, often resulting in high loading of core material in the final product. However, scaling up has proven to be very difficult and expensive because of the process being limited by small-sized batches. In addition, the final matrix of the bead systems tends to be extremely porous, allowing diffusion of water in and out of the matrix.

Other processes

Mechanical processes, such as extrusion, are frequently used to stabilize sensitive core materials, such as flavours, in a glassy carbohydrate matrix with the primary objective of protecting the core from oxidation. These matrices are known to have very good barrier properties, and extruded particles are less porous than spray-dried particles, as no water is required. Submerged coextrusion, a variation of the conventional extrusion process, involves the oil droplets being dropped simultaneously with a shell material (gelatin or other polysaccharides and a plasticizer) through a concentric vibrating nozzle in a stream of cooling oil. The cooling oil causes the hardening of the outer shell of the microparticles, which are then carried through for filtration and harvest. A second coating may also be applied to aid formulation and handling. A method described in a patent gives details of submerged coextrusion used for microencapsulation of fish oil. In this process, gelatin was used as wall material, and glycerol was used as plasticizer. A wall material solution was first prepared by mixing gelatin (25% w/w) and water. Glycerol was added to this solution and heated to 80°C and subsequently cooled to 45°C. A coextrusion nozzle was used to feed the two streams simultaneously, i.e., fish oil was sent through the inner nozzle and gelatin solution was sent through the outer nozzle. The nozzle was connected to an oscillator for controlled vibration production, which aids in droplet breakage. The nozzle head was submerged in cooled vegetable oil at 14°C, which caused the gelatin to set on contact. The formed microcapsules were filtered and centrifuged to remove excess oil. This was followed by air drying. A second coating was applied to aid formulation and handling.

Melt injection is another mechanical process used to microencapsulate fish oil by using sugars around the core droplet to create a glass barrier that is impermeable to oxygen. The process involves blending of fish oil with antioxidants, emulsifiers, and water and then heating them to 100°C to solubilize all the sugars. For size reduction, this mixture is passed through a filter followed by dropping into a pool of cold solvent that creates solid microparticles. The solvent also aids in removing any unencapsulated oil. One of the main disadvantages of the melt injection process is the vulnerability of the microparticle to fluctuation of humidity, which leads to loss of particle quality. Also, the oil loading is limited to 10% to 20%. However, this process yields very stable particles that have a long shelf life if the storage temperature and humidity are well controlled.

The encapsulation of fish oil using calcium carbonate starts with the use of an anionic surfactant (such as sodium dodecyl sulfate) for emulsification of the oil. Calcium carbonate is abundantly found as the
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inorganic component in the shells of marine life and limestone. Being relatively cheap and environmentally benign in nature, calcium carbonate has been shown to form a microcapsule wall using a relatively simple technique. It is also suggested to improve rigidity of the shell and provide resistance to deformation due to heat. The calcium carbonate particles, when added to the emulsion while stirring, are electrostatically adsorbed on the surface of the oil droplets. The resulting solution could be spray dried or freeze dried. Calcium carbonate dissolves at low pH once the capsules reach the stomach. The oil loading of the capsules is similar to spray-dried powders; however, to make up for the costly processing, the capsules usually have high temperature and pressure resistance. A number of encapsulation processes using cyclodextrins have been described. These processes involve the dissolution of cyclodextrin in water and then further mixing of the core material in the solution. Crystallized molecules are then separated and dried into powder form (Desai and Park, 2005). The main difference in these processes is the amount of water added to dissolve the cyclodextrin molecules (Madene et al., 2006). The process that uses kneading of cyclodextrin with minimal amounts of water has been suggested as being the most commercially practical out of the three, as very little water has to be removed by drying (Madene et al., 2006). The main function of cyclodextrins is believed to be in reducing off-flavor in n-3 LCPUFAs by means of complexation Fish oil coated γ-cyclodextrins has been shown to resist oxidation for up to 24h upon storage at 100°C. One of the main disadvantages of the process is the low amount of oil loading, which is usually between 5% and 15%. The use of cyclodextrins in food application is limited because of regulatory requirements in a number of countries and the high cost of the ingredient itself.

Release mechanism

Microencapsulated oils enriched in food could be biologically useful only when it is biologically available. The bioavailability of microencapsulated fish oil as delivered by spray-dried emulsions has been shown to be high in animal and human studies (Wallace et al., 2000) However, spray-dried emulsions tend to have high surface oils levels, low oil content, and poor stability, making their use limited commercially. Different release mechanisms of encapsulated materials provide controlled, sustained or targeted release of core material. Generally there are three different mechanisms by which the core material is released from a microcapsule - mechanical rupture of the capsule wall, dissolution or melting of the wall, and diffusion through the wall. Less common release mechanisms include ablation (slow erosion of the shell) and biodegradation.

CONCLUSION

Current knowledge relating to oxidation mechanisms and microencapsulation techniques has allowed the design of functional foods enriched with n-3 LCPUFAs. Strategies involving the use of novel microencapsulation techniques along with the use of a combination of antioxidants and improved packing have helped increase the stability of such products. However, product development has been limited to short to medium shelf-life products because of oxidative deterioration and lack of stability during long-term storage. However, it must be emphasized that the n-3 LCPUFA enriched foods should be stable and convenient, have acceptable taste/flavor, and be without heavy price premiums in order to have an effective population-wide increase in consumption of these bioactive fatty acids. There is no doubt that the inclusion of n-3 LCPUFAs into food products can be achieved, but novel approaches may be required to achieve the target outcomes. A multidisciplinary effort between food manufacturers, food technologists, food scientists, and packaging technologists may be required. With the ongoing developments, the n-3 LCPUFA market is certain to grow in the future, which is good news for consumers, who are bound to reap the health benefits.

REFERENCES


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