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RESEARCH ARTICLE

A REVIEW ON A MICROENCAPSULATION OF FISH OIL TO IMPROVE OXIDATIVE STABILITY

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ABSTRACT

Fish oil is the lipid fraction extracted from fish and fish by-products. Currently, the production of fish oil is becoming more demanding as there is a sizeable and growing world market demand for high quality fish oils. The most important constituents of fish oils are the omega-3 fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These fatty acids are precursors of eicosanoids that helps to alleviate inflammation in the body and also have other health benefits. Lipid oxidation products are known to be health hazards because they are associated with aging, membrane damage, heart disease and cancer. However, fortification of foods with these nutraceuticals is confined because of extreme sensitivity of PUFA to oxidation and consequent formation of toxic hydroperoxides during the manufacture and storage. This article reviews the microencapsulation, very useful technology, of PUFA within microcarriers to retard the oxidation by minimizing the access of undesirable factors. Various techniques are being employed to form microcapsules, such as extrusion coating, fluidized-bed coating, spray drying, liposome entrapment, coacervation, inclusion complexation, centrifugal extrusion, and rotational suspension separation. Dried microencapsulated fish oil (DMFO) exists in powder form, which can easily be applied to instant powder products. There are many food products in which DMFO can easily and safely be incorporated like bread, biscuits, cakes, diet powder, fruit bars, milk powder etc.

INTRODUCTION

Fish has been recognized as an excellent food source for human beings and is preferred as a perfect diet not only due to its excellent taste and high digestibility but also because of having higher proportions of unsaturated fatty acids[5]. Fish are a rich source of polyunsaturated fatty acids (PUFAs), namely the n-3 and n-6 PUFAs, which are beneficial to human health. Fish meat and oils are good sources of unsaturated omega-3 fatty acids viz. eicosapentaenoic acid (EPA, 20:5; n-3) and docosahexaenoic acid (DHA, 22:6n-3) (Wang *et al.,* 2006; Fish Consumption Advisories, 2014). For health benefits, fish oils can be consumed through either by eating fish or by taking supplements. Oily fishes, which are fed on fish oil such as salmonids, grant as a magnificent source of these acids. Fish oils can directly be used in purified form in various foods. Therefore, daily recommended intake (0.25 to 0.50 g) of these omega-3 fatty acids (EPA and DHA respectively) can be complied (NIH Medline Plus, 2006; Fishery and Aquaculture Country Profiles: India, 2011).

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Fish that are exclusively rich in omega-3 fatty acids consist of mackerel, tuna, salmon, mullet, sardines, sturgeon, bluefish, anchovy, sardines, trout, menhaden and trout. It is claimed that about 1 gram of omega-3 fatty acids is found in about 3.5 ounces of fish (India - National Fishery Sector Overview, 2006). Fatty predatory fish, such as sharks, swordfish, tilefish, albacore tuna, are rich in omega-3 fatty acids, but as they are positioned at the top of the food chain, these fish may contain toxic substances which might get accumulated through biomagnifications. Therefore, the US Food and Drug Administration urges limiting intake of these fish species due to their high percentage of toxic contaminants such as mercury, dioxin, PCBs and chlordane (Export of marine products from India, 2008). Fish oil has a wide range of application. It is widely used for curing cardiovascular diseases, blood systems. It helps to lower blood pressure and cholesterol level in the blood. Fish oil, containing omega-3 fatty acids, is considered to be advantageous in curing hypertriglyceridemia and preferably in inhibiting heart disease (Fisheries, 2007). Fish oil contains a considerable amount of PUFAs which are very susceptible to oxidation and other possible side reactions and cause deterioration of oil. This problem would possibly remedied by using microencapsulated technology as it involves shielding of the core material by polymeric shell which inhibits the interaction of the core material with surrounding atmosphere. This review paper is mainly focused on fish and fish oil production, its composition and its application in food by using microencapsulation technology. In the beginning we have discussed the global production of fish, importance of fish oil, its production and compositions. Then we encountered with the problems associated with the stability of fish oil and its remedy through microencapsulation technology. It has also discussed the techniques of manufacture of microencapsulation.

Table 1. Fish production in last seven years

Year	Inland fish production		Marine fish production		Total fish production		Fish seeds produced
	Inland (lakh tonnes)	Growth rate (%)	Marine (lakh tonnes)	Growth rate (%)	Total production (lakh tonnes)	Growth rate (%)	In million fry
2004-05	35.26	1.96	27.79	-5.53	63.05	-1.48	20790.64
2005-06	37.56	6.52	28.16	1.33	65.72	4.23	21988.30
2006-07	38.45	2.37	30.24	7.39	68.69	4.52	23647.95
2007-08	42.07	9.41	29.20	-3.44	71.27	3.76	24143.57
2008-09	46.38	10.24	29.78	1.99	76.16	6.87	32177.21
2009-10	48.94	5.52	31.04	4.23	79.98	5.02	29313.17
2010-11	49.81	1.77	32.50	4.70	82.31	2.91	34110.83
2011-12	52.95	6.30	33.75	3.85	86.66	5.28	36566.43
2012-13	56.32	6.36	34.30	1.63	90.62	4.57	38196.01

Table 2. Top Five Countries contribution in global fish production for the year 2009

Name of Country	Capture	Culture	Total Production (in tonnes)	% Share
Total world	8,89,18,040	5,56,80,738	14,45,98,778	
China	1,49,19,596	3,47,79,870	4,96,99,466	34.37
India	40,53,241	37,91,920	78,45,161	5.43
Peru	69,14,452	-	69,14,452	4.78
Indonesia	50,99,355	17,33,434	68,32,789	4.73
Vietnam	22,43,100	25,56,200	47,99,300	3.32

Overview of fish production

Fishing is one of the dominant industries in coastal states of India which is taking up over 14 million people of the country. Since 1947, fish production has been raised by more than tenfold. According to the US Food and Agriculture Organization (FAO), the fish production in India, during 1990 to 2010, was doubled (Kumar, 1992). In India, there are 3827 fishing villages situated over 8,118 kilometers of the marine coastline with 1,914 traditional fish centers. Additionally, India's freshwater resources consist of rivers and canals (197,024 km), ponds and tanks (235 million hectares), oxbow lakes and derelict waters (1.3 million hectares), brackish water (1.24 million hectares) and estuaries (0.29 million hectares) (Sharma *et al*., 2010). The total marine fish output, in India, consists of about 65 important species in which oceanic and midwater species, in 2004, contributed about 52% of the total marine fish. Globally, India is a major supplier of fish. The country exported over 600,000 metric tons of fish, to some 90 countries, earning over $1.8 billion in 2006 (Ugoala *et al*., 2009). The fish production, in India, has risen at higher rate than that of vegetables and other food items during 1990 to 2007 (Moghadasian and Mohammed, 2008). Table 1 and 2 show the details of the fish production over last seven years in India as well as in foreign countries.

Importance of fish oil

Fish oil, nut oil and some plants are major sources of omega-3 fatty acids. There are three types of omega-3 fatty acids obtained from mentioned sources in which fish oil gives eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) whereas nuts, such as English walnuts, and vegetable oils, such as canola, soybean, flaxseed, linseed, and olive oils, are major sources of alpha-linolenic acid (ALA) (Freemantle *et al.,* 2006; Gao *et al.,* 2012). Researchers avert that, based on strong evidence, the consumption of required amounts of DHA and EPA either through dietary fish or fish oil supplements. These helps to lower cholesterol; reduce cardiovascular diseases such as heart attack, abnormal heart rhythms and strokes; prevent hardening of the arteries and lower blood pressure (Chaiyasit *et al.,* 2007; Evangelos *et al.,* 2012). However, high doses of these may have harmful effects on human health such as an increased risk of bleeding.

Production of fish oil

Over a quarter of wild fish that can be caught sustainably is unappetizing for direct human consumption – typically small bony and oily fish such as capelin, sand eel, anchovy, horse mackerel, pilchard and menhaden. This valuable resource is fished under carefully controlled limits set by government agencies based on stock assessments. These limits are effectively policed by government agencies in most of the countries producing fish oil. Global fish oil production has remained between 1 and 1.25 million tons for many years demonstrating the sustainable nature of the fisheries. To allow producers to demonstrate their responsible production, IFFO is developing an independently audited standard based on the FAO Code of Responsible Fishing. Wild caught fish plus trimmings from edible fish (around 25% of total) makes the overall production of fish oil around one million tones. The major fish oil producing countries are shown in Figure 1. Production of fish oil in the future is not expected to change. More oily fish are expected to be processed for direct human use, thus reducing that available for fishmeal and fish oil production. However, growing by-product production from farmed fish will maintain the total production. The fat content of fish is unique in its quantities of long chain omega-3 fatty

Table 3. Fish oil Jan-Mar 2011

	Jan-Dec		Jan-March	
'000 tonnes	2009	2010	2010	2011
Peru/Chile	256	402	21	49
Denmark/Norway	80	254	22	18
Iceland/North Atlantic	35	130	11	17
Total	371	785	54	84

Source: Globefish

Table 4. Fish body oil production by top 15 countries 1998-2009

'000 tonnes	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
Peru	123	515	593	300	221	205	352	287	286	337	275	282
Chile	7	188	171	145	146	130	138	145	155	187	167	152
USA	101	130	87	118	96	80	81	75	65	75	86	75
Denmark	136	129	98	80	86	71	68	93	67	57	55	72
Japan	58	68	70	63	64	61	68	67	68	60	62	64
Iceland	88	102	95	96	70	104	49	63	54	51	72	62
Morocco	18	14	18	31	20	29	25	31	31	20	29	44
Norway	98	69	83	56	72	52	37	30	40	44	37	42
Mexico	6	7	7	7	8	9	12	13	26	21	35	33
Vietnam								5	7	15	26	20
Turkey	5	5	5	6	9	14	14	14	15	16	17	17
Spain	20	20	20	21	20	21	22	23	23	23	16	15
China	7	10	24	27	27	30	13	12	12	12	12	14
Ecuador							14	10	10	9	11	13
UK	10	12	10	10	11	11	12	12	12	14	14	10

Source- IFFO Fishmeal and Fish Oil Statistical Yearbook 2010. Rounded up or down

acids (LC omega-3s). Because of their degree of unsaturation they help fluidity in membranes at low temperatures. These acids are produced by marine algae which are consumed first by zoo- plankton and then by fish (Lavie *et al*., 2009). Global production of fish oil is shown in table 3 and 4.

Composition of fish oils

At room temperature, fish oils are liquid but generally solidify below 158–108^{o} C. Different types of fish have different compositions. Most of the inedible fish are thalassic – that is, swim and shoal in the upper layers of the sea. These species generally store oil in the body rather than the liver. The species caught in America – South (Peru and Chile) and North (USA) have a high content of LC omega-3 fatty acids which can be up to 35% of the total fat in the fish. They are mainly eicosapentaenoic and docosahexaenoic acids (EPA and DHA) with some docosapentaenoic acid (DPA) at around 10%. The European fish species such as capelin, herring, sand eel and sprat are intermediate; have between 18% and 25% LC omega-3 fatty acids (Table 1). The demersal fish store oil in the liver, e. g. cod and halibut and have a low content of LC omega-3s (15% to 20%). The more unsaturated fish oils with a higher content of LC omega-3 fatty acids also have a higher content of saturated fatty acids such as myristic and palmitic. The unsaturated oils are susceptible to oxidation. For storage, all fish oils have to be out of contact with air, pro-oxidant metals, especially those high in iron and copper, and preferably treated with an antioxidant, such as butylated hydroxy-toluene (BHT) (Lavie *et al.,* 2009; Stansby,1967).

Storage Problem

Oils containing high percentage of unsaturated fatty acids are susceptible to oxidation by consuming atmospheric oxygen and undergo degradation due to which quality of oil, in terms of flavor and odor, is suffered (Boran *et al.,* 2006) and since fish oils are rich in polyunsaturated fatty acids including EPA and DHA, the rate of oxidation which means rate of deterioration of fish oil is considerably higher than that of other oils (Frankel, 2004). Oxidation of lipids produces rancid odours and flavours and also decreases nutritional quality of oil (Suja *et al.,* 2004). Lipid oxidation products are known to be health hazards because they are associated with aging, membrane damage, heart disease and cancer (Jackson and Lee, 1991). However, fortification of foods with these nutraceuticals is confined because of the extreme sensitivity of PUFA to oxidation and consequent formation of toxic hydroperoxides during the manufacture and storage. Encapsulation of PUFA within microcarriers has appeared as a useful technology to retard the oxidation by minimizing the access of undesirable factors.

Fundamentals of Microencapsulation

Microencapsulation is a processing method in which little quantities of solid, liquid and gaseous materials are packed into a wall material; which forms microcapsules (Terminology for biorelated polymers and application, 2012). (Fig. 1) It has been observed that these microcapsules can release their contents at controlled rates over a long period of time. Microencapsulation can also help overcome the main problems of food fortification with ω-3 PUFA, the unpleasant "fishy" flavor of fish oil and the oxidation of polyunsaturated fatty acids that has a negative influence on food acceptability (Gutcho, 1976). The structure formed by the microencapsulating agent around the microencapsulated compound (core) is called a "wall"; this wall protects the core compound from biological degradation and enhances its stability. Because of the direct effect of the wall on microencapsulation efficiency, microencapsulation stability, and protection efficiency of the core compound, the selection of the wall material is very important in the microencapsulation process. The wall material of a microcapsule produced by spray drying has to be highly soluble. It is also desirable that the concentrated solution of the wall material has a low viscosity. The stability of the microencapsulated substance is influenced by the composition of the wall. Choosing a particular wall material depends on many factors such as solubility, viscosity, glass or melting transition, forming and emulsifying properties.

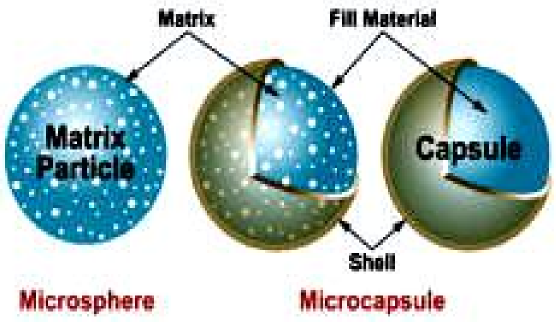

Figure 1. Graphic representation of a microencapsulated compound

Carbohydrates, especially sugars like glucose and sucrose and polysaccharides like starch, maltodextrins, pectin, alginate and chitosan, have been successfully used as wall materials (Arshady, 1999; Hanewald *et al*., 1968). However, carbohydrates cannot be used in wall systems without the presence of a surface-active constituent because they generally have no emulsifying properties. The incorporation of carbohydrates in a wall matrix has been shown to improve the drying properties of the wall by enhancing the formation of a dry crust around the droplets of the microencapsulated compound. High concentrations of low molecular weight sugars may not be suitable for spray drying due to the formation of sticky powders and caramelization (Anandaraman and Reineccius,1986; Chien *et al*.,1991).

Materials for Microencapsulation

Core Materials

The core material is a material to be coated within coating material or shell. It may be liquid or solid depending on its composition, physical and chemical properties. The liquid core material can be found in dispersed or dissolved form. The composition of core materials may include drugs or active constituents, proteins, peptides, volatile oils etc (Collins and Deasy, 1990; Dortune *et al.,* 1998).

Coating/ Shell Materials

Coating materials are used to coat the core material with desired thickness. A film formed by coating material should be cohesive with the core material. It should be compatible and inert with the core material. It should possess strength, flexibility, impermeability, optical properties and stability which are desired coating properties (Arshady *et al.,* 1989).

Techniques to Manufacture Microcapsules

Physical methods

Air-suspension coating

Air suspension coating is also known as Fluidized-Bed technology. It is limited to encapsulation of solid core materials only. Air-suspension coating of particles gives better control and flexibility. In an upward-moving air stream, the particles are coated. Solid particles to be encapsulated are suspended on air jet. Then they are allowed to coat with the help of spray of liquid coating material. The capsules are then solidified either by cooling or by solvent vaporization.

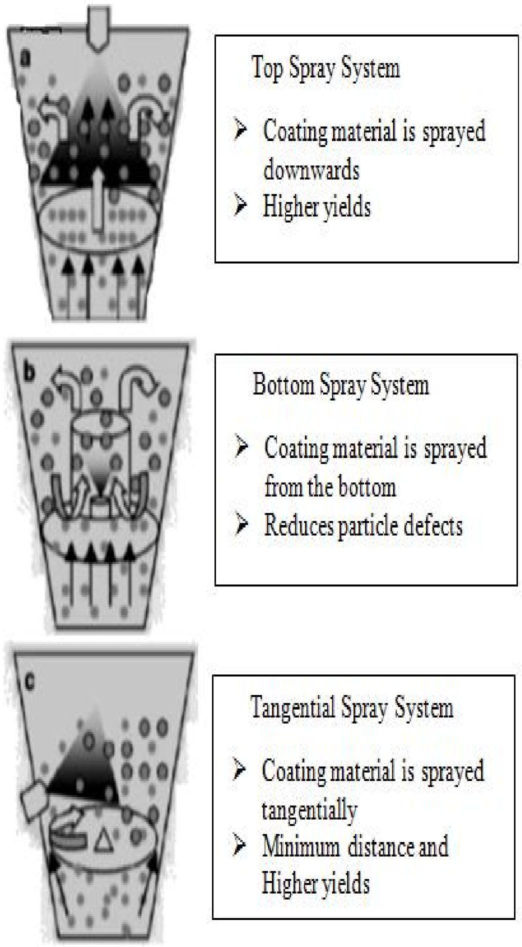

Figure 2. Air Suspension Coating

These processes are repeated until capsule gets desired shell thickness. There are different types of fluid-bed coaters, as shown in figure 2, such as top spray, bottom spray and tangential spray. A wide variety of coating materials can be obtained by this method. Further, the main advantage of this process is that coating materials can be applied in a variety of forms like solvent solutions, aqueous solution, emulsion, dispersions (Widder *et al.,*1982; Arshady *et al.,*1990).

Non Aqueous phase separation

Non Aqueous phase separation is the inverse of the aqueous phase process in that the continuous wall containing phase is organic or hydrophobic in nature, and the core material is usually water- immiscible (hydrophilic). In the process the core liquid is suspended in droplet form by stirring in polymer solution (wall material). Phasing out and core wrapping of the wall material is caused by the addition of a liquid (non-solvent) that is miscible with the organic solvent but immiscible with both the core particle and particle and the polymeric wall material (Burgess and Carless, 1985; Okoda *et al.*, 1985; Takenaka *et al.,* 1980). Coacervation-phase separation. A coacervate is a miniature spherical droplet of ordered organic molecules especially lipid molecules. It is bound together with the help of hydrophobic forces from surrounding liquid. These droplets are around 1-100 µm in size. The process of formation of coacervate, generally, includes three steps carried under continuous agitation.

- Development of three immiscible phases: This step involves the formation of three immiscible phases by dispersing a core material into the coating material and then by applying one of the phase separation methods such as changing temperature, addition of salts or non-solvent. The three phases developed are i) liquid manufacturing vehicle phase ii) core material phase iii) coating material phase.
- Accumulation of coating material over the core material: This is done by controlled mixing of core material and coating material. The adsorption phenomena, in which polymer gets adsorbed between core material and the liquid vehicle phase, is mandatory to effective coating.

Solidification of coating: This is generally done by thermally or cross-linking technique to form a solid, self-sustained microcapsule (Nimmannit and Suwanpatr, 1996; Jian You *et al.*, 2006).

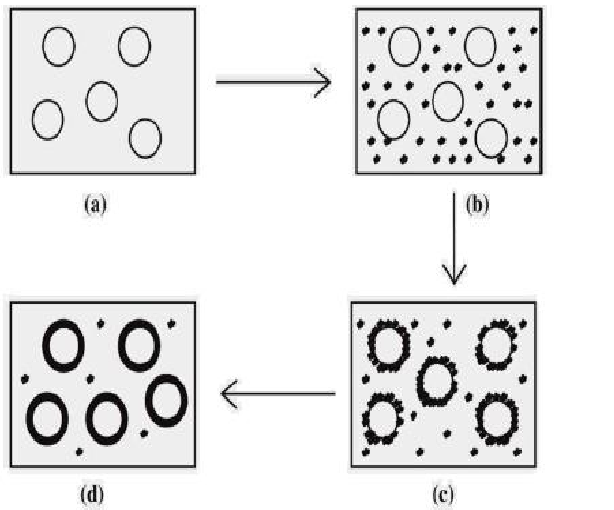

Figure 3. Schematic representation of the coacervation process. a) Core material dispersion in shell polymer solution; b) Separation of coacervate from solution; c) Coating of core material; d) Coalescence of coacervate to form continuous shell around core particles

Centrifugal extrusion

It consists of a rotating extrusion head containing nozzles at the bottom where liquids are encapsulated. This process is usually used to make capsules of larger size in range 250 µm to some millimeters. In this process, the core and coating material, which are immiscible with each other, are allowed to pass through a nozzle situated at the bottom of the extrusion. This step generates a continuous rope which naturally breaks into spherical droplets as shown in fig. 4. These droplets are then solidified through various processes like cooling, gelling bath. This process is limited to liquids as the droplets are formed by breaking up of the liquid rope (James, 2005).

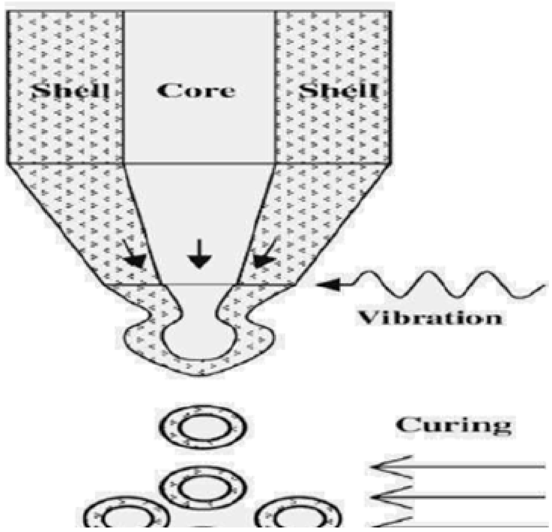

Figure 4. Centrifugal Extrusion

Pan coating

Pan coating process is extensively used in pharmaceutical industries. This is one of the oldest processes for producing capsules or tablets. In this process, core particles are spilled in the pan while the shell material is applied gradually. The core particles get mixed with a shell material and as the temperature increases, the shell material gets softened and covers the core particle which is then solidified to make capsule (Klinkesorn *et al.*, 2005).

Spray–drying

Spray drying is economical, low cost process in which dried particulates are continuously formed by spraying feed, in the fluid state, into a hot drying chamber. The core material is dispersed into a coating material to prepare an emulsion. The prepared emulsion is reduced to into a spray of droplets by passing the emulsion through a rotating disc into hot compartment of drying chamber. The viscosity of the emulsion would be about 300mPa.s. In the hot chamber, the water from the emulsion gets evaporated, giving dried capsules[43]. The ability to handle labile materials is the main advantage of this technique. Spray congealing process is very similar to spray drying process. The major differences between these two methods are that spray congealing method involves dispersion of core material into melted coating material rather than coating material solution. Further, the coating is solidified by spraying the mixture into cool air stream instead of hot chamber. Waxes, fatty acids, alcohols are generally used in spray congealing method (Mulqueen *et al.*, 1999; Gharsallaoui *et al.*, 2007). The particle size, in spray congealing method, is a function of feed rate, feed viscosity, the reducing wheel velocity (Shu *et al.*, 2006; Yamakawa *et al.*, 1992).

Chemical process

Solvent Evaporation

Solvent evaporation method is one of the most extensively used techniques for microencapsulation production. The core material is dissolved in water. It may also contain stabilizing agent. It is then transferred, with continuous stirring, to the organic phase containing a polymer solution in solvents such as chloroform to make water-in-oil (W/O) emulsion (Arshady, 1990; Hausberger and Deluca, 1995; Yang *et al.,* 2001) . The multiple emulsion (W/O/W) is formulated by adding previously made water-in-oil emulsion to a large quantity of water consisting of suitable surfactants. Continuous stirring is, then, provided to multiple emulsion formulation till most of the organic solvents get evaporated leaving behind microcapsules which are then washed and dried (Yang *et al.*, 2000; Ogawa *et al.*, 1988). The core materials are either water soluble or water insoluble (Janssen and Nijenhuis, 1992).

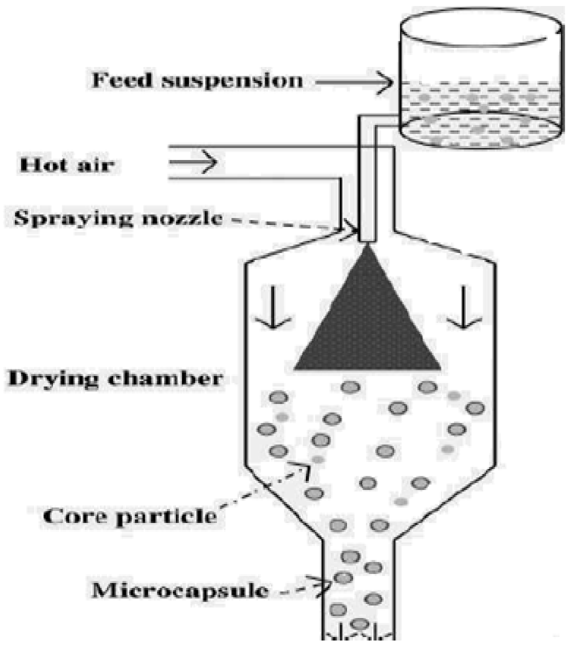

Figure 5. Schematic representation of solvent evaporation process

Polymerization

Interfacial polymerization

In interfacial polymerization, two reactants interact at an interface of two immiscible liquid and react at higher rate. It is based on Classical Schotten-Baumann reaction, in which, acid chloride reacts with a reagent consisting of an active hydrogen atom like alcohols, amines, polyurethane etc. In this process, the emulsion is formulated after dissolving a reactive monomer in the aqueous solution containing a core material solution. Once the emulsification is formed, the other monomer is dissolved into the emulsion. These monomers are polymerized immediately at the interface. The alkaline solution is added to neutralize byproducts formed during the reaction. The advantage of this process is that it is a rapid process and produces microcapsules of uniform size (Janssen and Nijenhuis, 1992).

In-situ polymerization

It is a normal polymerization in which capsules are produced on micro and nanometer range. It is generally of three types:

- Bulk polymerization: In this process, initiator is dissolved in monomer and reaction mixture is then heated to trigger polymerization. The products obtained from bulk polymerization are very pure as no chemical additives or solvents are involved except initiator.
- Suspension polymerization: It is economical process of polymerization. In this process, monomers are suspended on water in the form of fine droplets. Each monomer droplet consists of initiator and acts as an independent nucleus of the polymerization reaction. Products obtained are in the form of spherical beads with uniform droplet size.
- Emulsion polymerization: It is same as suspension polymerization except the fact that initiators are dissolved in an aqueous phase containing surfactant at high concentration. By this process, high molecular weight polymers are formed rapidly.

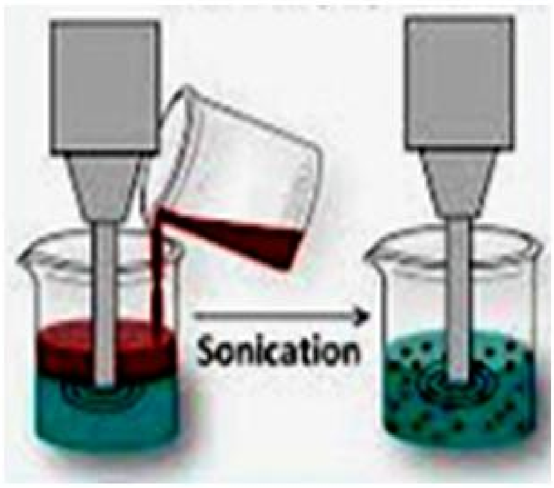

Figure 7. Emulsion Polymerization

Conclusion

The presented investigation shows that the acceptable oxidation stability can be achieved for dried microencapsulated fish oil produced by different encapsulating techniques. Several different techniques for microencapsulation of fish oil are currently used in the food industry. For successful applications, minimization of non-encapsulated oil is a key factor, which can be achieved by combining different techniques and processing steps. Different release mechanisms can be achieved depending on the shell material and the encapsulation technology used. Based on the necessities of the target application selection of the appropriate technology should be defined.

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