Microencapsulation of Fish Nutrients for High Value Product Development

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The Millennium Development Goals (MDGs) India Country Report 2015 outlines India's progress and the challenges in achieving the goals and targets set at the United Nations Millennium Summit in September 2000 by 189 heads of States, including India, to adopt measures—to fight against poverty, hunger, illiteracy, gender inequality, disease and environmental degradation. To quote from the report " though there are impressive achievements in several sectors, all the MDGs are unlikely to be met". The most worrying aspect which should concern us is that the largest undernourished population in the world call India their home. The proportion of underweight children under 5 declined from 52% in 1990 to 33% by 2015, but is still far from the target of reducing it by half.

In India, nutrient deficient diets are a fact of everyday life for millions. It is a matter of grave concern that India is doing dismally on the nutrition front; according to the World Economic Forum, its Global Competitive Index with respect to infant mortality rate is a dismal 114/140. One of the reasons for the large prevalence of undernourished and underweight children with stunted growth in India is the multi-micronutrient deficiencies that these children suffer from. It is our strong perception that even smallest of right kind of interventions may go a long way in improving this statistic. Current approaches to address malnutrition have serious limitations. Interestingly, fish is probably the most affordable source to provide almost 40 essential nutrients. one of the ways of ensuring the right nutrition reaches the malnourished population is by fortification of food with nutrients. Commonly consumed local foods can be used as vehicles for fortification of fish fat and protein that are known to be unique nutrients: fish fat being rich in eicosapentaenoic acid and docosahexaenoic acid and fish protein being rich in all essential amino acids.

Increasing dietary consumption of fish oil rich in ω3 PUFAs and fish protein hydrolysates/peptides may improve overall health and well-being and formulating food products to contain recommended levels of ω3 PUFAs and protein hydrolysates/peptides is of interest to the food industry. Fish oil is a good source of EPA and DHA and has value as a food ingredient but low stability of PUFAs and off flavors and odors associated with fish oil limit its use as an ingredient. PUFAs are highly susceptible to oxidation and

intervention strategies are necessary to preserve the integrity of PUFAs and PUFA enriched products throughout processing and storage. Fish protein hydrolysates and peptides are also considered a category of promising functional food ingredients. But utilization of protein hydrolysates and peptides can be impeded by their low bioavailability, bitter taste, hygroscopicity and undesirable interaction with the food matrix.

Microencapsulation by spray drying is one method for preserving PUFAs that may also mask undesirable flavors and odors and reduce food matrix incompatibility issues. Although much research has been conducted on materials for preparing fish oil microcapsules by spray drying, there is still an interest in alternative materials and a need for a improved understanding of the relationships between emulsion characteristics, drying conditions, and wall materials, and their impact on microcapsules properties, in particular oxidative stability. Encapsulation as a delivery mechanism can also be used for fish protein hydrolysates/peptides to overcome these challenges for improving the bioavailability and organoleptic properties of the peptides. Proteins, polysaccharides and lipids are the three carrier systems that have been utilized in peptide and fish oil encapsulation. The protein and polysaccharide systems mainly aim at masking the bitter taste and reducing the hygroscopicity of protein hydrolysates, whereas the lipid-based systems are intended for use in enhancing the bioavailability and biostability of encapsulated peptides. A spray drying technique is largely used to achieve microencapsulation in both fish oil and protein and polysaccharide systems while, for lipids, liposomes are prepared by a film hydration technique. Achieving adequate encapsulation efficiency through cost effective techniques is indispensable for encapsulation to be applicable to bioactive nutrients-based product commercialization. Furthermore, the design of high quality functional foods requires detailed understanding of the release mechanism and kinetics, gastrointestinal stability, bioavailability and physiological bioactivity of the encapsulated nutrients. Biostability and bioavailability are pivotal for achieving physiological benefits as the nutrients need to reach their targets intact in order to exert their bioactivity.

1. Introduction

- Encapsulation has been used in the food industry and for delivery of several bioactive compounds that are sensitive to environmental factors, such as polyphenols, carotenoids and omega-fatty acid.
- Bioactive food protein hydrolysates and peptides are different from other food bioactive compounds such as vitamins or polyphenols in that the chemical species within the protein hydrolysates are highly heterogeneous.

- Bioactive peptides are primarily encapsulated for the purpose of masking the bitter taste that result from exposure of taste receptors to hydrophobic amino acid residues generated from protein hydrolysis.
- Another major objective of encapsulation is the reduction of hygroscopicity to ensure textural and storage stability of protein hydrolysates and peptides.

2. Need for peptide encapsulation

- A primary challenge is the susceptibility of peptides to gastrointestinal (GIT) digestion with the risk of losing their structural integrity and function. Bioavailability is used to depict the portion of the bioactive compound that is unchanged, absorbed and that reaches the systemic circulation. It has been understood that oral ingestion of bioactive peptides will expose them to the action of at least 40 different enzymes before reaching systemic circulation. Several studies have demonstrated that most food protein-derived bioactive peptides containing more than 2–3 amino acid residues do not withstand simulated gastrointestinal enzymatic digestion.[7]
- Protecting bioactive peptides is essential in translating *in vitro* activities into bioactivities in humans. Therefore, encapsulation has become a relevant technology for fish-derived bioactive peptides.

3. Type of carrier systems for peptide encapsulation

Lipid, polysaccharide and protein-based carriers for encapsulation of bioactive compounds, protein hydrolysates and peptides are shown in Fig. 1.

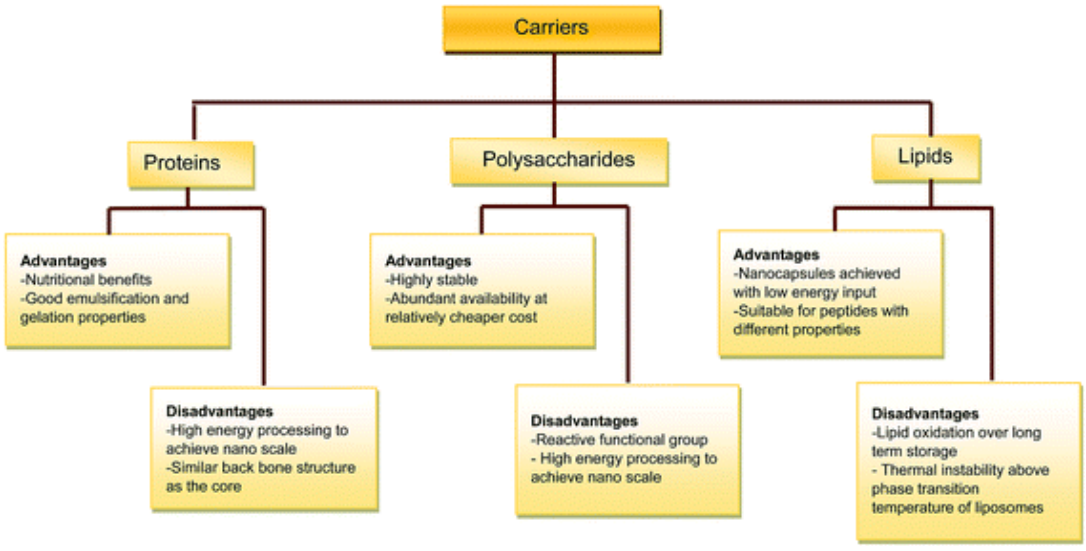

Fig. 1 Carriers used for encapsulation of protein hydrolysates and peptides.

3.1. Protein-based carriers

- Encapsulation using the protein-based matrix is the most nutritionally beneficial system. Despite the their popularity for delivering bioactive compounds such as flavonoids, vitamins and β-carotene, their use in bioactive peptide encapsulation is limited.
- Encapsulating a core substance with a chemically similar material is challenging because of structural similarity.
- Protein carriers have been shown to reduce the hygroscopicity of peptides.
- The inclination towards the use of proteins for delivery of bioactive compounds is due their functional properties such as film and gel forming ability, emulsification and solubility, in addition to their nutritional benefit as sources of essential amino acids.
- Although not extensively used as carriers for peptide encapsulation, milk proteins are well established as major sources of bioactive peptides.
- Furthermore, polysaccharides can be combined with the protein carriers to provide structural stability to the encapsulation.

3.2. Polysaccharide-based carriers

- Polysaccharides are generally ideal for use as delivery agents because they are structurally stable, abundant in nature and inexpensive. The reactive functional groups of polysaccharides make them one of the best choices as carrier matrices.
- On the other hand, under extreme conditions, such as high temperature, the polysaccharide wall is susceptible to reacting with the peptide core to form complex products (*e.g.* Maillard reaction products), which can be potentially toxic and also deplete the bioactive peptides.
- In order to circumvent this challenge, the reactive functional groups of polysaccharides have been modified by processes such as carboxymethylation to produce relatively inert carriers.
- The colossal molecular structure of polysaccharides contributes to their stability as carriers during production and processing of encapsulated products. Polysaccharides derived from plants, animals and microbial sources, such as gum arabic, chitosan, cyclodextrin and maltodextrin, have been utilised for protein and peptide encapsulation .

3.3. Lipid-based carriers

- Liposphere and liposome are two lipid-based systems that are currently used for encapsulating protein hydrolysates and peptides. The former has a fatty acid inner layer and outer layer composed of phospholipid, the hydrophilic part whereas the latter is a single or multiple concentric bilayer made of phospholipids constituting a vesicle.

- Accordingly, liposphere appear appropriate for encapsulating hydrophobic peptides that can interact with the hydrophobic inner layer of the carrier. For instance, a combination of stearic acid and phosphatidyl choline (PC) was used to encapsulate casein peptide fractions to get EE of 50–83% .
- Liposome is a more popular encapsulation carrier compared to the liposphere, which would be less preferred for food applications because of its high saturated fatty acid content, and the limited choice of substances that can be incorporated in its highly hydrophobic core.
- However, liposome is compatible with a wide variety of bioactive peptides. The aqueous core appears suitable for hydrophilic peptides and other compounds, while the interior of the bilayer is compatible with hydrophobic peptides. Moreover, amphiphilic peptides can exist at the interface between the shell and core of the liposome structure, which would interact with the hydrophobic and hydrophilic amino acid residues, respectively.
- PC is the commonly used phospholipid for liposome preparation. Liposomes can have certain shortcomings in functional food application.
    - thermal instability of liposome encapsulated food peptide products can limit their incorporation in thermally processed food.
    - Liposome preparation involves the use of cholesterol to increase the stability of the lipid bilayer, which is a health concern for application in functional foods.
    - There is a risk of lipid oxidation during production, processing and storage of the products.
    - Optimum conditions need to be developed to take advantage of the lipid-based system in protein hydrolysate and peptide encapsulation considering the health and product quality challenges posed by the use of saturated and unsaturated lipids in lipospheres and liposomes, respectively.

4. Criteria for determining the quality of peptide encapsulation

4.1. Particle size

The dispersibility and solubility of the encapsulated peptide product greatly depend on the particle size.

Particle size of above 50 µm can significantly affect the solubility, dispersion and hence, the texture and feel of the food.

Encapsulation products can be either of micro or nano scale. Nanoencapsulation is advantageous because of its high surface area that can increase the solubility and bioavailability of the product and enhances delivery or release of the active molecules.

4.2. Zeta potential

Surface charge is one of the properties that convey the stability of encapsulated products. Stability enables the prediction of the behaviour of the encapsulated product in a food matrix. However, encapsulation performed for the purpose of masking the bitter taste of protein hydrolysates and peptides has not been focused on this surface property.

Liposome-based encapsulation studies report high net negative zeta potential (surface charge) due to the presence of phospholipids, which have negatively charged hydrophilic heads. A decrease in the magnitude of the zeta potential would decrease the stability of the encapsulated product.

4.3. Encapsulation efficiency

- EE can be defined as the amount of bioactive compound trapped in the core of the carrier compared to the initial amount of the bioactive material.
- EE of peptides can be assessed indirectly by removing unencapsulated portion of the protein hydrolysate by centrifuging followed by estimation of peptide concentration using Lowry assay. Membrane ultrafiltration has also been used to separate unencapsulated hydrolysate from the capsules prior to protein quantification.
- EE of over 50% increases the risk of leakage, but lower EE leads to inefficient use of the bioactive materials and also higher amount of encapsulated products would be required to attain the peptide quantities needed to exert physiological bioactivities.
- EE depends on core-wall ratio, encapsulation conditions, encapsulation technique utilized.
- Encapsulation using protein and polysaccharide carriers have higher EE compared to liposome -based peptide encapsulation, possibly since the former is controlled and involves high energy processes in encapsulating the peptides. Liposome formation is entropy-driven, spontaneous and less controlled process. In general, techniques using high shear forces, pressure and high temperature result in higher EE, while mild preparation techniques such as film hydration and ionotropic gelation result in lower EE.

5. Factors that can affect encapsulation of peptides

The chemistry of the encapsulated bioactive material fundamentally affects the EE. EE partly depends on some factors as discussed below.

5.1. Peptide charge

Encapsulation of casein-derived peptides using liposomes mostly resulted in low EE (14%), which is attributable to the phosphoserine residues in caseinophosphopeptides. PL and the phosphopeptides are highly negatively charged leading to molecular repulsion and reduced encapsulation.

5.2. Type and purity of carrier/wall material

High EE of 74–80% have been achieved for protein hydrolysates using purified PC to form the liposomal carrier. Encapsulation of a similar protein hydrolysate with crude soy lecithin resulted in low EE of 46%. EE was between 50% and 83% using liposphere-based encapsulation -PC and stearic acid. The high EE of liposphere encapsulation can be attributed to the affinity of hydrophobic peptides in the core to the hydrophobic stearic acid inner layer. Using purified carrier materials allows for reduced amount of materials needed to achieve high EE. Most polysaccharide-based encapsulation uses purified carrier materials but is not economical.

5.3. Core-to-wall ratio

Encapsulation involves the use of large amounts of wall materials than the active core compounds. EE is found to always decrease with increase in the core concentration, which can be due to overloading of the encapsulation system.
Increase in the concentration of the wall material initially leads to increase in the EE until a certain point. For instance, studies reported a maximum EE of 74.6% at 1 : 31.5 (w/w) core-to-wall ratio (PC and sea bream scale protein hydrolysate); which decreased to 67% when the ratio was slightly changed to 1 : 38.5 (w/w). Another study achieved 80% EE of fish protein hydrolysate using a much lower core-to-wall (PC) ratio of 1 : 5 (w/w), which suggests that EE depends on the nature and molecular composition of the encapsulated material.

5.4. Techniques used for encapsulation

Several techniques are in use for encapsulation of bioactive compounds such as coacervation, spray cooling, extrusion, supercritical fluid extraction, cocrystallization and inclusion. Following are the techniques currently relevant for encapsulation of food protein hydrolysates and peptides.

5.4.1. Film hydration. Liposome-based encapsulation of protein hydrolysates and peptides employs the film hydration technique. Here, phospholipids self-assemble in response to energy input in the form of heat, agitation and sonication thereby trapping the aqueous core containing the peptides. The disadvantage of liposome formation is that the uncontrolled assembly mechanism can lead to poor reproducibility and varying EE.

5.4.2. Spray drying. Protein and polysaccharide-based encapsulation employ spray drying to achieve encapsulation due to the relatively low processing cost and ease of the technique. This technique involves forming droplets and spraying at high temperature resulting in dried particles. Spray drying has been found to result in microspheres with the active material uniformly distributed in the carrier, which typically occurs when the carrier and core materials are similarly hydrophilic. This phenomenon is known to lead to high EE. The high temperature used during spray drying can lead to denaturation of protein carriers and may alter peptide structure due to their reactivity. For instance, non-enzymatic browning can occur if considerable amount of reducing sugar is present in the system.

5.4.3. Coacervation. This technique is considered effective for encapsulation since it is based on electrostatic attraction between the core and wall materials. The technique involves phase separation and deposition of coacervate phase on the core. Coacervation could achieve EE above 91.6% using similar amounts of core and wall materials. The affinity between the core and wall due to surface properties contributed to the resulting high EE. Limitation with high affinity between the core and wall is that it can be difficult to release the peptides. Also the wall material should have compatible (opposite) charge with the core to be able to coacervate. For instance, anionic polysaccharides such as gum arabic or alginate can be used to coacervate cationic peptides, and *vice versa*.

5.5. Production condition

Peptide net charge is dependent on the pH of the medium during encapsulation, and this can influence the EE due to electrostatic effects. Encapsulation with both protein and polysaccharide-based carriers have been found to occur favourably at alkaline pH 8. Maximum EE was observed at pH 10 with dilute salt ($CaCl_2$) solution while the least EE was observed at neutral pH and high salt concentration. Conversely, liposome formation has been found to result in higher EE when conducted at neutral pH.

6. Release and gastric stability of encapsulated peptides

High affinity of the core and wall materials is important for the formation of stable encapsulated peptide products that can withstand food processing and storage conditions with limited loss of the core materials.

One study evaluated the biostability of bioactive peptides encapsulated with a carboxymethylated gum and sodium alginate, and found minimal (up to 10%) and maximal (up to 60%) release of protein materials after simulated gastric and intestinal digestion phases, respectively. The released peptides at the intestinal phase can then be presented for absorption into the enterocytes and subsequently into circulation where they are still susceptible to further peptidolytic modification. Therefore, it is imperative to assess the digestion

kinetics and biostability of encapsulated peptides, and their bioavailability in different physiological sites to ensure the release of the intact bioactives at appropriate time and target location.

7. Challenges and future prospects of peptide encapsulation

- The heterogeneity of protein hydrolysates with peptides of different charge, hydrophobicity, molecular weight and surface properties makes it challenging to achieve high and uniform EE.
- Enhancing the EE is important to avoid the use of large quantities of the encapsulated protein hydrolysates in attaining the desired amount of the active material.
- Purifying the peptides from protein hydrolysates can improve the condition; however, it requires high-end processing techniques that can be uneconomical for small and medium-sized food industry. However, some techniques are showing promise for use in purifying peptides or concentrating bioactive fractions at a large scale and low cost.
- Some promising techniques currently used for the drug delivery may be used for protein hydrolysates. Proliposomes, used for drug delivery, can be used to overcome the quality issues associated with liposomes such as oxidation, aggregation and phospholipid hydrolysis.

8. Omega-3 Polyunsaturated Fatty Acids - Encapsulation and Challenges

Polyunsaturated fatty acids (PUFAs), and in particular omega-3 PUFAs, play an important role in maintaining physical and mental health. The most well known and widely researched ω3 PUFAs are eicosapentaenoic acid (EPA, 5 double bonds) and docosahexaenoic acid (DHA, 6 double bonds). Fish oil is an important source of the omega-3 polyunsaturated fatty acids (ω3 PUFAs). EPA and DHA have been associated with health benefits including prevention of cancer, cardiovascular disease, diabetes, inflammatory diseases, autoimmune disorders and depression as well as with improved brain and visual development (reviewed by Shahidi 2008). Omega-3 fatty acids naturally occur in fatty fish (such as salmon) and also in some seeds and nuts (such as flax seed, walnuts, and almonds). Despite the well established health benefits associated with long chain ω3 PUFAs and the availability of natural sources, dietary consumption of these fats remains low. Supplementing food products with ω3 PUFAs is a growing trend in the food industry. Fish oils, algae oils, and flax products are the main sources of ω3 PUFAs used for supplementation of foods. Adding such products to foods presents several challenges, mainly preventing ω3 PUFA degradation, removing or masking undesirable flavors and odors, and overcoming matrix incompatibility issues. The most significant challenge is preventing degradation of the long chain fatty acids. The high content of

unsaturated double bonds causes PUFAs to be highly susceptible to oxidative degradation. When oxidized the long chain molecules break down eventually forming small molecules including alcohols, aldehydes, and ketones. These compounds can render a product unacceptable in terms of sensory attributes. Preventing oxidation throughout ingredient storage, processing, and product storage ensures that the ω3 PUFA enriched product is providing the anticipated nutrients. Oxidation of ω3 PUFA sources is prevented through the use of controlled storage conditions (eg. packing in an inert atmosphere and chilling), through the addition of antioxidants, and by microencapsulation.

8.1 Microencapsulation

Microencapsulation of fish oil refers to surrounding or embedding the oil in a matrix typically composed of proteins or carbohydrates and can be accomplished through a variety of processing techniques. In theory, microencapsulation protects the core material against degradation by light, heat, and oxygen. Stability of encapsulated lipids depends on properties including oil distribution within the particle, particle size and surface area, particle density, wall material composition (glass transition temperatures, crystallinity, extent of interaction with the core material), moisture content, and water activity. If processing conditions and wall materials are selected appropriately, microcapsules with long term stability can be prepared. Microencapsulation also can facilitate incorporation of oily ingredients into a variety of food matrices as it transforms the lipid into a dried powder. Encapsulation also may mask undesirable flavors and odors associated with ω3 PUFA sources.

8.2 Microencapsulation of ω3 PUFA Sources by Spray Drying

Spray drying is commonly used in the food industry and has recently been widely applied to prepare ω3 PUFA microcapsules. To encapsulate lipid based materials by spray drying, an oil in-water emulsion is generated. Emulsifiers are commonly added when surface active wall materials are not used. The emulsion is fed into the spray drier where it is atomized and exposed to hot air. Rapid drying occurs and dried microcapsules are collected. Properties of the spray dried particles depend on properties of the feed emulsion, properties of the wall material(s), and drying conditions. Sources of ω3 PUFAs have been encapsulated by spray drying with proteins (whey, soy, casein, caseinates, gelatin), carbohydrates (derivitized starches, maltodextrins, glucose, corn syrup solids, pectin), and gums (gum arabic, alginate, carageenan). In general, a liquid is transformed into a powder in the microencapsulation process and the powder has better stability against light, heat, and oxidation, and is easier to incorporate into a variety of food matrices. Furthermore, microencapsulation can mask undesirable flavors or

odors, control the release rate and location of a compound, and impact bioavailability of the encapsulated material. Microcapsules can have a variety of structural types – core shell, multi core, single wall, multi wall, continuous matrix.

An ideal wall material would be one that forms a fine and stable emulsion, forms microcapsules with high encapsulation efficiency (low surface oil content) at high oil:wall ratios, produces a glassy shell capable of preventing diffusion of oxygen to the encapsulated material, and maintains structural integrity throughout long term storage. Taste-masking and antioxidant activity would also be beneficial for ω3 PUFA encapsulation. In general, the following characteristics suggest a material may be effective for encapsulation of ω3 PUFAs by spray drying: emulsifying capabilities, good film forming abilities, water solubility, low viscosity, bland flavor/sensory acceptability, barrier properties (water vapor and oxygen), low cost, and compatibility with regulatory and labeling requirements.

Carbohydrates, proteins, and gums are the most commonly used wall materials for spray drying. Among these, gum arabic, modified starches including n-octyl succinate starch and maltodextrins, whey and soy protein, gelatin, sodium caseinate, alginate, carrageenan, and pectin are most commonly used. The following properties are important factors affecting a material's performance as a spray drying encapsulation material: good film forming properties, water solubility, low viscosity, bland flavor/sensory acceptability, barrier properties (water vapor and oxygen), emulsifying ability (for lipid based ingredients), low cost, and compatibility with regulatory and labeling requirements. The most effective materials are those that will form a complete wall or shell around the core material and maintain the integrity of this shell during storage, and processing, and in the food as well, depending on the product application

8.3 Promising Materials for ω3 PUFA Encapsulation- Chitosan, High-Amylose Starch

Chitosan is a (1-4) linked copolymer of D-glucosamine and N-acetyl-D-glucosamine. It is the deacetylated form of chitin, the second most abundant naturally occurring polysaccharide. Chitosan is typically generated from waste materials (e.g. shells of marine animals such as crab and shrimp). Chitosan has been studied in food applications including antimicrobials, edible films, emulsion stabilization, and texture modification. The emulsifying properties of chitosan make it particularly attractive for applications involving encapsulation of lipid ingredients. Furthermore chitosan has been shown to exhibit antioxidant effects, mainly through interactions with metals, which could aid in preserving ω3 PUFAs. Despite

several properties that make it attractive as an encapsulating material, chitosan is limited as a spray drying component due to its high viscosity.

Starch is a polysaccharide comprised of D-glucose units. It has two fractions; amylose, a linear fraction formed from glucose units, and amylopectin, a branched fraction where branches are generated by linkages. Amylose content in starch is typically 20-30% . High-amylose starch is a unique form of starch that contains a higher percentage of amylose. High-amylose starch has been noted to be a better film former than other forms of starch, and films prepared form high-amylose starch have shown superior oxygen barrier properties. High-amylose starch also acts as a dietary fiber and has been linked to improved gut health.

8.4 Supplementation of Foods with Fish Oil and Other Omega-3 Lipid Sources

Despite the growing body of research indicating numerous health benefits associated with consumption of omega-3 PUFAs, dietary intake remains below the recommended amounts. The health benefits of omega-3 containing lipids combined with the growing functional foods market has led to considerable interest in supplementing a variety of food products with fish oil and other omega-3 fatty acid sources such as algae and flax oils. Omega-3 supplemented breads, cereals, milk, yogurts, juices, pastas, and cheeses can all be located in grocery stores.

8.5 Susceptibility to Oxidation

The polyunsaturated nature of omega-3 and omega-6 fatty acids is critical to their functioning in terms of health benefits, but this same property also renders them highly susceptible to oxidative deterioration. Oxidation reduces the nutritive quality of the lipid and produces off flavor and aroma compounds through the breakdown of lipid hydroperoxides. Polyunsaturated lipids are much more susceptible to oxidation than saturated lipids due to their high content of bis-alliylic methylene groups.

9. Conclusion

Encapsulation of bioactive food compounds is well-positioned to facilitate the design of better and efficient functional foods. This is essential in advancing the research on bioactive nutrients and to develop the market of the fish nutrients as natural health products and nutraceuticals. To achieve high EE, the choice of the carrier material used is dependent on the encapsulation and processing techniques, environment and chemistry of the nutrients, although more work is needed to delineate the impact of the latter on EE. Apart from high EE, knowledge of digestion and release kinetics, and the morphology of

encapsulated nutrients is paramount to obtaining applicable functional materials for food formulation.

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