

MICROENCAPSULATION TECHNIQUES AND ITS APPLICATIONS IN FISH NUTRITION

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Microencapsulation

Microencapsulation is a process in which tiny particles or droplets are surrounded by a coating to give small capsules, of many useful properties. In general, it is used to incorporate food ingredients, enzymes, cells or other materials on a micro metric scale. Microencapsulation can also be used to enclose solids, liquids, or gases inside a micrometric wall made of hard or soft soluble film, in order to reduce dosing frequency and prevent the degradation of pharmaceuticals.^[1] In a relatively simple form, a microcapsule is a small sphere with a uniform wall around it. The material inside the microcapsule is referred to as the core, internal phase, or fill, whereas the wall is sometimes called a shell, coating, or membrane. Some materials like lipids and polymers, such as alginate, may be used as a mixture to trap the material of interest inside. Most microcapsules have pores with diameters between a few micrometers and a few millimeters. The coating materials generally used for coating are:

- Ethyl cellulose
- Polyvinyl alcohol
- Gelatin
- Sodium alginate

The technique of microencapsulation depends on the physical and chemical properties of the material to be encapsulated.

- **Micro-encapsulation is a process in which tiny particles or droplets** are surrounded by a coating to give small capsules many useful properties. Microencapsulation is a process by which solids, liquids or even gases may be enclosed in microscopic particles by formation of thin coatings of wall material around the substances. It is defined as a technology of packaging solids, liquids or gaseous materials in miniature, sealed capsules that can release their contents at controlled rates under the influences of specific conditions.

Micro-encapsulation may also be defined as the process of surrounding or enveloping one substance within another substance on a very small scale, yielding capsules ranging from less than one micron to several hundred microns in size.

Size range of the microcapsules

The potential size range of the microcapsules produced is enormous, with typical diameters being between 2 and 2000 μm . Capsule walls are typically 0.5-150 μm thick, although walls measuring less than 0.5 μm can be achieved. The proportion of core material in the capsule is usually between 20 and 95% by mass.

Core material & coating material

- The substance that is encapsulated may be called the core material, the active ingredient or agent, fill, payload, nucleus, or internal phase.
- ☐ The material encapsulating the core is referred to as the coating, membrane, shell, or wall material .

Microcapsules

Microcapsules may have one wall or multiple shells arranged in of varying thicknesses around the core. Particles having diameter between 3 - 800 μm are known as micro particles or microcapsules or microspheres. Particles larger than 1000 μm are known as Macro particles . The material inside the microcapsule is referred to as the core, internal phase, or fill, whereas the wall is sometimes called a shell, coating, or membrane. The potential size range of the microcapsules produced is enormous, with typical diameters being between 2 and 2000 μm . Capsule walls are typically 0.5-150 μm thick, although walls measuring less than 0.5 μm can be achieved. The proportion of core material in the capsule is usually between 20 and 95% by mass. There are over 50 different known wall materials; both natural and synthetic polymers can be used to form the microcapsules. These include the natural polymers gelatin, gum arabic, carrageenan and alginate, and synthetic polymers such as ethylcellulose.

Generally Micro particles consist of two components

- a) Core material
- b) Coat or wall or shell material

Reasons For Microencapsulation

For sustained or prolonged drug release.

For masking taste and odor of many drugs to improve patient compliance.

For converting liquid drugs in a free flowing powder.

To reduce toxicity and GI irritation

Incompatibility among the drugs can be prevented by microencapsulation.

The drugs, which are sensitive to oxygen, moisture or light, can be stabilized by microencapsulation

ROLE OF POLYMERS

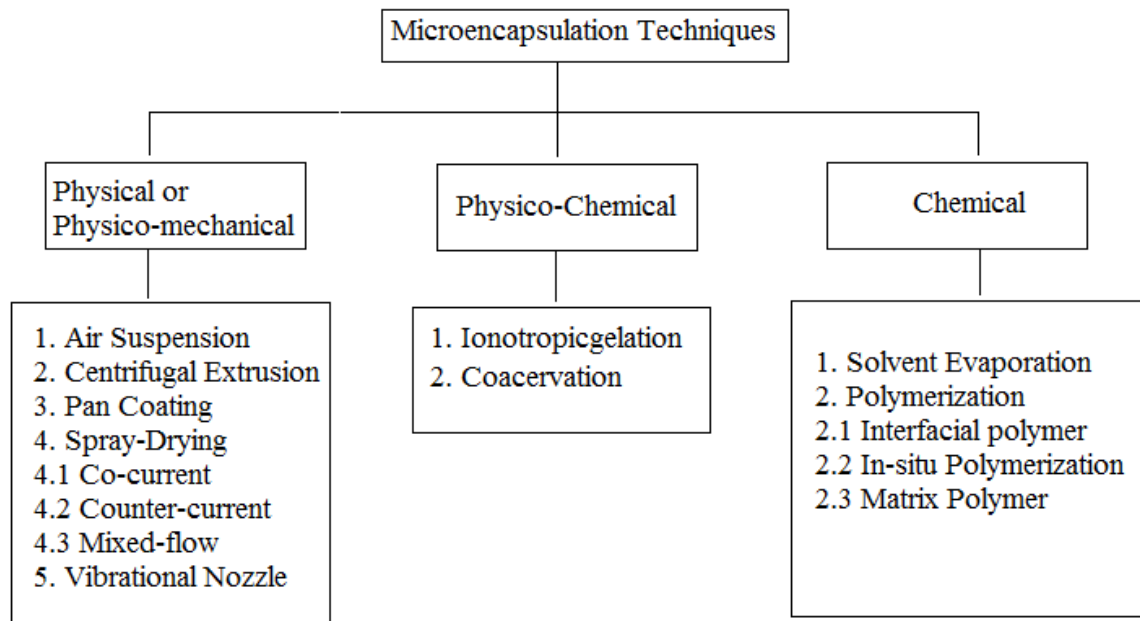
Polymers are substances of high molecular weight made up by repeating monomer units. Polymer molecules may be linear or branched, and separate linear or branched chains may be joined by crosslinks. Polymers are used widely as adjuvants, coating materials and, a components of controlled and site- specific drug delivery systems. Polymer should be chemically compatible, non-reactive with the core material.

It should provide the desired coating properties such as:

- Strength
- Flexibility,
- Impermeability,

Optical properties and stability

Microencapsulation Techniques.



Physical Processes

A dual fluid stream of liquid core and shell materials is pumped through concentric tubes and forms droplets under the influence of vibration. A membrane of wall material is formed across a circular orifice at the end of the nozzle and the core material flows into the membrane, causing the extrusion of a rod of material. Droplets break away from the rod and The shell is then hardened by chemical cross linking, cooling, or solvent evaporation. Solid capsules are removed by filtration or other mechanical means and the immiscible carrier fluid, after passing through the filter, is reheated and recycled.

This process is capable of producing capsules ranging from 400- 2000 μ m in diameter solid, particulate core materials are dispersed in a supporting air stream. The coating material is sprayed on the air suspended particles. Within the coating chamber, particles are suspended on an upward moving air stream. The design of the chamber and its

operating parameters effect a recirculating flow of the particles through the coating zone portion of the chamber, where a coating material, usually a polymer solution, is spray applied to the moving particles.

During each pass through the coating zone, the core material receives an increment of coating material. The cyclic process is repeated, perhaps several hundred times during processing, depending on:

- the purpose of microencapsulation
- the coating thickness desired
- Until the core material particles are thoroughly encapsulated

Physical or Physico-mechanical methods

Air Suspension Apparatus

Wurster technology is characterized by the location of a spray nozzle at the bottom of a fluidized bed of solid particles. Air stream is designed to induce a cyclic flow of the particles past the spray nozzle. The technology can be used to encapsulate solid materials with diameters ranging from near 50 μ m to several centimeters. Wurster Process can be used to encapsulate vitamins, minerals, and functional food ingredients.

Processing variables for efficient, effective encapsulation by air suspension techniques:

1. Density, surface area, melting point, solubility, friability, volatility, Crystallinity, and flow-ability of the core material.

2.Coating material concentration (or melting point if not a solution).

3.Coating material application rate.

4.Volume of air required to support and fluidizes the core material.

5.Amount of coating material required.

6.Inlet and outlet operating temperatures

Spray Coating

Coating technologies have been used for many years to prepare pharmaceutical capsules in the traditional macro scale. The particles are tumbled in a pan or other device while the coating material is applied producing capsules we have all used at one time or another. Building on this technique Air-Suspension Coating/Fluidised Bed Coating (a more specific version is called Wurster Coating) of particles by solutions or melts gives better control and flexibility for microcapsule production. Particles of the active ingredient, spheres or granules are coated while suspended in an upward-moving air stream. They are supported by a perforated plate having different patterns of holes inside and outside a cylindrical insert. Just sufficient air is permitted to rise through the outer annular space to fluidize the settling particles. Most of the rising air (usually heated) flows inside the cylinder, causing the particles to rise rapidly. At the top, as the air stream diverges and slows, they settle back onto the outer bed and move downward to repeat the cycle. The particles pass through the inner cylinder many times in a few minutes.

In the food industry the Wurster process has been used to encapsulate/coat vitamins, minerals and functional food ingredients for example to mask an undesirable flavour or improve stability and shelf life. The coating possibilities are relatively unlimited for example it is possible to coat a hydrophilic active ingredient with a hydrophobic polymer, or a hydrophobic active ingredient with a hydrophilic wall material at various thicknesses for process optimisation. With irregular shaped crystals it is likely that the coated particles would also be irregular in shape, although trials would have to be completed to be certain. Particles can be prepared with potential diameters of less than 50microns upwards.

Fluidized Bed

Fluidized bed technology is a very efficient way to apply a uniform layer of shell materials onto solid particles. It is one of the few technologies capable of coating particles with different kinds of shell material like (polysaccharides, proteins, emulsifiers, fats, complex formulations, powder coatings, yeast cell extract etc.). Typical fluidized bed system can efficiently process particles from 100µm to a few millimeters.

Centrifugal extrusion

The centrifugal extrusion process is a liquid co-extrusion process utilising nozzles consisting of concentric orifices located on the outer circumference of a rotating cylinder. A liquid core material is pumped through the inner orifice and a liquid wall material through the outer orifice forming a co-extruded rod of core material surrounded by the wall material. As the device rotates, the extruded rod breaks into droplets which form capsules. The rotational speed affects the capsule size. Wall materials include

gelatin, alginate, carageenan, starch, cellulose derivatives, gum arabic, fats and waxes or polyethylene glycol. Flavour oils are easily encapsulated using this methodology.

MULTIORIFIC-CENTRIFUGAL PROCESS

The Southwest Research Institute (SWRI) has developed a mechanical process for producing microcapsules that utilizes centrifugal forces to hurl a core material particle through an enveloping microencapsulation membrane thereby effecting mechanical microencapsulation. Processing variables include the rotational speed of the cylinder, the flow rate of the core and coating materials, the concentration and viscosity and surface tension of the core material. The encapsulated product can be supplied as slurry in the hardening media or as a dry powder.

Spinning Disk

Suspensions of core particles in liquid shell material are poured into a rotating disc. Due to the spinning action of the disc, the core particles become coated with the shell material. The coated particles are then cast from the edge of the disc by centrifugal force. After that the shell material is solidified by external means (usually cooling). This technology is rapid, cost-effective, relatively simple and has high production efficiencies.

Annular Jet

The technique involves two concentric jets, the inner containing the active ingredient and the outer generally molten wall material which solidifies when exiting the jet. The dual fluid stream naturally breaks into droplets which form the basis of the

microcapsule, which depending on configuration can be matrix particles or core-shell in formation

Vibrational Nozzle

Vibrational nozzle to help control the droplet size giving a more uniform product with lower microcapsule sizes down to sub-micron diameters. The solidification can be initiated by any common gelation system (e.g. sol-gel processing, melt) or by using an external additional binder system, (e.g. in a slurry). The process works very well for generating droplets between 100–5,000 μm

Spray Drying

Spray Drying is the most commonly used encapsulation method in the food industry. The process is economical and flexible uses equipment that is readily available, and produces particles of good quality.

The process involves three basic steps:

Preparation of a dispersion or emulsion to be processed

Homogenization of the dispersion and

Atomization of the mass into the drying chamber.

Spray dried ingredients typically have a very small particle size (generally less than 100 μm) which makes them highly soluble. Typical shell materials include gum acacia, maltodextrins, hydrophobically modified starch and mixtures. Other polysaccharides like

alginate, carboxymethylcellulose and guar gum. Proteins like whey proteins, soy proteins, sodium caseinate can be used as the wall material in spray drying.

Spray-Drying & spray-congealing :

Microencapsulation by spray-drying is a low-cost commercial process which is mostly used for the encapsulation of fragrances, oils and flavors.

Steps:

- 1- Core particles are dispersed in a polymer solution and sprayed into a hot chamber.
 - 2- The shell material solidifies onto the core particles as the solvent evaporates.
- The microcapsules obtained are of polynuclear or matrix type.

Spray Cooling/ Chilling...Contd

Spray cooling/chilling is the least expensive encapsulation technology. It is used for the encapsulation of organic and inorganic salts, textural ingredients, enzymes, flavors and other functional ingredients. Spray cooling/chilling is typically referred to as 'matrix' encapsulation because the particles are more adequately described as aggregates of active ingredient particles buried in the fat matrix.

Pan coating

Oldest industrial procedures for forming small, coated particles or tablets. The particles are tumbled in a pan or other device while the coating material is applied slowly. Solid particles greater than 600 microns in size are generally considered essential for effective coating. It is used for preparation of controlled release beads. Coating is

applied as solution by automated spray to desired solid core material in coating pan. Usually warm air is passed over the coated material as the coating are being applied in the coating pan.

EXTRUSION:

This method was first patented in 1957. The advantage of extrusion is that it completely surrounds the core material with wall material. The process involves forcing a core material dispersed in a coating material mass through a series of dies, into a bath of dehydrating liquid. When contact with the liquid is made, the coating material case hardens to entrap the core material. The extruded filaments are separated from the liquid bath, dried. This process is particularly useful for heat labile substances such as flavours, vitamin C and colours.

Melt Extrusion

In melt-extrusion process forcing the core material, which is dispersed in a melt coating material carriers through a series of die to form sheets, ropes or threads of different dimensions. Encapsulant ingredients like flavours can be added either at the feed port of the extruder or to the molten mass in the final zone using specialized pump systems.

CO EXTRUSION

A dual fluid stream of liquid core and shell materials is pumped through concentric tubes and forms droplets under the influence of vibration. The shell is then hardened by chemical cross linkings, cooling, or solvent evaporation.

- Different types of extrusion nozzles have been developed in order to optimize the process

Physico-chemical methods

Ionotropic gelation

Ionotropic gelation involves simply the interaction of an ionic polymer with oppositely charge ion to initiate cross linking. Eg:Barium chloride and Verapamil hydrochloride causes gastric irritation on sudden release. It is usually administered as conventional tablets containing 40-120 mg, 3 times a day. Due to its ready solubility in water and shorter half-life.

COACERVATION PHASE SEPARATION

Microencapsulation by coacervation phase separation is generally attributed to The National Cash Register (NCR) Corporation and the patents of B.K. Green et al. In simple coacervation a desolvation agent is added for phase separation, whereas complex coacervation involves complexation between two oppositely charged polymers.

The process consists of three steps:

Formation of three immiscible phases;

Solvent, a core material phase, a coating material phase.

Deposition of the coating material on the core material.

Rigidizing the coating usually by thermal, cross linking or desolvation techniques to form a microcapsule.

CHEMICAL TECHNIQUES

POLYMERIZATION

A relatively new microencapsulation method utilizes polymerization techniques to form protective microcapsule coatings. The methods involve the reaction of monomeric units located at the interface existing between a core material substance and a continuous phase in which the core material is dispersed.

1) Interfacial polymer

In Interfacial polymerization, the two reactants in a polycondensation meet at an interface and react rapidly.

2) In-situ polymerization

In a few microencapsulation processes, the direct polymerization of a single monomer is carried out on the particle surface.

Matrix polymer

In a number of processes, a core material is imbedded in a polymeric matrix during formation of the particles. National Lead Corporation- utilizing polymerization techniques

Liposome Microencapsulation

A liposome can be defined as an artificial lipid vesicle. Liposomes are usually made of phosphatidylcholine (lipid) molecules although mixtures of phospholipids can also be employed to make liposomes. A unique property of liposomes is the targeted delivery

of their content in specific parts of the food stuffs. Liposomes can also be used to deliver the encapsulated ingredient at a specific and well-defined temperature

SOLVENT EVAPORATION

Solvent evaporation techniques are carried out in a liquid manufacturing vehicle (O/W emulsion) which is prepared by agitation of two immiscible liquids. A core material (drug) to be microencapsulated is dissolved or dispersed in the coating polymer solution. With agitation, the core – coating material mixture is dispersed in the liquid manufacturing vehicle phase to obtain appropriate size microcapsules. Agitation of system is continued until the solvent partitions into the aqueous phase and is removed by evaporation. This process results in hardened microspheres which contain the active moiety. Various process variables include methods of forming dispersions, Evaporation rate of the solvent for the coating polymer, temperature cycles and agitation rates.

ELECTROSTATIC DEPOSITION

This method is suitable for both solid and liquid droplets. Core and coating material are imparted electric charges by means of high voltage. Core is charged and placed in coating chamber. Coating material is charged in solution when it leaves the atomizer device prior to spray as a mist. Since both are oppositely charged coating material gets deposited on core due to electrostatic attraction.

VACCUM DEPOSITION

This is not a popular technique. Coating material is vapourised in chamber in which core material is present. Coating material gets deposited on core particles. Core particles are

moved on conveyor system and they encounter hot vapours of coating material Which gets deposited on them

FISH NUTRITION

Fishes require different types of live diet for their better growth, efficient breeding and survival. The live diets (live foods organisms) contain all the nutrients, such as: proteins, lipids, carbohydrates, vitamins, minerals, aminoacids and fatty acids. These diets are costly to produce, variable in quality and constitute a potential source of contamination with viruses and pathogenic bacteria. The microencapsulated diets for fish are highly complex as the microparticles must contain proteins, lipids, carbohydrates, vitamins and electrolytes.

Microencapsulation offers many advantages, such as:

- Compounds can be incorporated at sizes suitable for ingestion,
- Compounds can be preserved for long periods in the microcapsules,
- And the nutrients are protected and are released by digestive processes and become available for digestion and absorption .
- Microencapsulation is a useful tool to improve the delivery of bioactive compounds into foods, particularly
- Probiotics,
- Minerals,
- Vitamins,
- Phytosterols,
- Fatty acids,
- And Antioxidants.

(Claude PChampagnePatrickFustier,2007)

Proteins

Proteins are essential dietary components for carnivorous and desirable protein sources for omnivorous fish. Different proteins extracted from animal (albumin, casein, fish protein hydrolysates) have been used as protein sources and as material for microencapsulation of different compounds. Plant-based protein sources have been receiving considerable attention as a partial or even complete replacement for fishmeal in aquafeed

Lipids

Different fish species are dependent on fish oil as a major dietary lipid source .The fatty acids from different sources (fish, vegetables, microbial oils) are extremely susceptible to oxidative deterioration. The oxidative stability of n-3 fatty acids is significantly enhanced after Microencapsulation. In the area of n-3 fatty acids, the Microencapsulation is an important strategy that has been used to stabilize the deliver bioactive and to protect n-3 fatty acids after they have been isolated from their source.

Carbohydrates

The carbohydrates may be regarded as nonessential dietary nutrients for fish, but their inclusion in fish diets is warranted because they are an inexpensive source of valuable dietary energy for noncarnivorous fish, they can spare the more valuable protein for growth instead of energy provision and they serve as good materials for microencapsulation. Carbohydrates have been applied together with proteins and lipids as materials for microencapsulation of biological substances. The carbohydrates that

have been used as wall materials for microencapsulation are pectins, starch, chitosan, maltodextrine, gums and modified cellulose .

Pectin is an ideal wall material for microencapsulation applications. Due to its relatively lower cost, it can be used as a substitute for expensive wall materials.

Starch is used in microencapsulating biological compounds due to its potential to prevent degradation in the upper gastrointestinal tract and to provide prolonged or targeted release. Chitosan and maltodextrine are suitable candidate for use in nutrient release systems in aquaculture, some authors used chitosan to coat the granules consisting of potato starch and other dietary components for feeding larvae of some fish species.

Probiotics and plant extracts

The probiotic is any microbial cell provided via the diet or rearing water that benefits the host fish, fish farmer or fish consumer. Most probiotics used in A belong to the genus Bacillus, to the photosynthetic bacteria or to the yeast, although other genera or species. In aquaculture, the probiotic have been shown to have significant positive points, such as: improvement of growth performance, pathogens inhibition and disease control in A, improvement in nutrient digestion and in water quality, increase stress tolerance of the host fish, effect on reproduction of aquatic species . In aquaculture, microencapsulation is an available vector of probiotics administration for incorporation and protecting the probiotics into fish diet.

The immobilization of probiotics using microencapsulation technique may improve the survival of probiotics in fish feed, both during processing and storage, and during

digestion. The application of plant extracts, as bioactive fish feed additive may be facilitated by the protection through encapsulation technology.

Oils and Fat:Fish Oils

Microencapsulation extends the shelf-life of fish oil in powder form increasing its versatility as a nutritional ingredient in food formulations . Fish oil powder produced by microencapsulating fish oil with micellar casein using homogenisation and spray drying had acceptable taste and modest shelf-life of 31 weeks at 4°C. Fish oil powder successfully incorporated into a fish feeds, at levels to satisfy the recommended intake of omega-3 polyunsaturated acids

Vitamins

Many of the vitamins are relatively unstable and their ability to maintain activity in fish foods depends on pH and reactions to heat, light, oxygen, oxidizing agents and enzymes. Lipid soluble vitamins such as vitamin A, b-carotene and vitamins D, E, K are much easier to encapsulate than water soluble ingredients. Using microencapsulated vitamins reduces loss during storage.

Minerals

Undesirable interactions between unprotected mineral salts and components in ingredients can lead to precipitation, colour and flavour problems. Microencapsulated iron ingredients can prevent off flavour development and maintain bioavailability.

Food industry:

Encapsulating calcium salt (calcium lactate) in a lecithin liposome it was possible to fortify 100g soya milk with up to 110 mg Calcium. The soya milk remained stable at 4°C for at least 1 week.

(Boccio *et al.* 1997)

Flavour Encapsulation

Flavours consist of tens to hundreds of aromatic, volatile organic compounds. Microencapsulation can protect flavours, it can extend shelf life and stability, control flavour release and provide liquid flavours with a granular form. The objectionable tastes and aroma of popular nutritional ingredients like soy extracts, bitter herbs and omega-3 oils, can be masked by microencapsulation. Microencapsulation can also be used to help to increase the particle size of a flavour ingredient.

Enzymes

Microencapsulation of β -galactosidase in liposomes can be used to act in vivo but protect from acting on lactose during storage. Emulsifiers can be used as effective coating material to microencapsulate lactase. Liposomes containing enzymes reduce the ripening time by 30-50% as well as improve texture and flavour. (Kwak *et al.*, 2001).

Antioxidants

Ascorbic acid by entrapping it in a liposome together with vitamin E is used for protection of emulsion-type foods. Ascorbic acid with Vitamin E has synergistic antioxidant effect. (Reineccius, 1995).

ADVANTAGES

A Single microencapsulated feed particle can contain an optimal formulation of key nutrients for growth, eg: high levels of protein and polyunsaturated fatty acids including docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), alongside disease control agents. The microencapsulated diet could be tailored to specific species, or specific geographies where certain nutrients are lacking. Decrease the production costs

and commercial risk in the case of contamination. Maximal particle capture can be ensured by customizing microparticle size and buoyancy. Size can be tailored to fishes preferences for maximum retention efficiency, and buoyancy can be optimized to ensure particles remain within reach of feeding fishes. Preingestive nutrient loss can be minimized by using an encapsulant that retains particle nutrients until they are released by the digestive processes. Storage of high quality feeds for long time periods is made possible by the stable nature of microparticles .

EVALUATION OF MICROCAPSULES

Percentage Yield

The total amount of microcapsules obtained was weighed and the percentage yield calculated taking into consideration the weight of the drug and polymer .

- $\text{Percentage yield} = \text{Amount of microcapsule obtained} / \text{Theoretical Amount} \times 100$

Particle size analysis

For size distribution analysis, different sizes in a batch were separated by sieving by using a set of standard sieves. The amounts retained on different sieves were weighed .

Encapsulation efficiency

Encapsulation efficiency was calculated using the formula:

- $\text{Encapsulation efficiency} = \text{Actual Drug Content} / \text{Theoretical Drug Content} \times 100$

Conclusion

Over the past few decades, various techniques have been evaluated for their capacity to partially replacement live food in fish nutrition. Microencapsulation technology may be a good alternative to partially or complete replacement of live diets used to culture

larvae of different species in aquaculture. Microencapsulation offers alternative methods for the development of functional food products. Its suitability depends on the product, the need for protection of food components and timed release of nutraceuticals. It can provide novel solutions to problems encountered in the development of healthy properties of foods.

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Signature of the student

**Signature of the Chairman,
Advisory Committee**