Microencapsulation of Omega-3 fatty acids for enhancing their stability

A thesis submitted for the award of the degree of Master of Biotechnology at Flinders University of South Australia

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Declaration

I certify that this thesis does not contain material which has been accepted for the award of any degree or diploma, and to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text of this thesis or in the notes.

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Abbreviations

µl, ml	microliter, milliliter
µM, mM	micromolar, millimolar
%	percent
ANOVA	Analysis of Variance
approx.	approximately
hr	hour
$CaCl_2$	calcium chloride
Conc	concentration
HCL	Hydrochloric acid
$Fecl_2$	Ferric chloride
MDH	Methanol, Decanol, hexane

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Shweta

Abstract

Microencapsulation of omega-3 oil and biomass enriched in omega-3 fatty acid was investigated via ionic gelation and complex coacervation to enhance their stability. It was observed that gelation process, had a significant effect on the encapsulation efficiency and on bead properties. The alginate-oil emulsion (alginate 2%v/v with an oil loading 10%, v/v) resulted in an encapsulation efficiency of 95% and found to be stable. Under similar condition, when biomass was encapsulated the efficiency was 94%. Similarly, other wall materials such as maltodextrins and gelatin were used to encapsulate the omega-3 oil. The beads were spherical that resulted in improved encapsulation efficiency 96%. In complex coacervation, the optimum ratio between gelatin A, sodium alginate and pH to form a coacervate complex was found to be 1.4:0.8 and 3.6, respectively. Storage stability was significantly high when maltodextrin and gelatin wall material was used as compared to alginate beads ($p<0.05$). During 5-day oxidative stability analysis, beads and coacervates exhibited significantly high ($p<0.05$) stability.

Chapter 1

Introduction and literature review

1.1. Importance and market status of omega-3 fatty acids

Microalgae have evolved as a potential source of renewable energy and the nutraceutical industry. Long-chain polyunsaturated omega- 3 fatty acids are of particular interest because these are highly important for the growth of higher eukaryotes(Adarme-Vega et al., 2012).DHA (docosapentaenoic acid) (C22:6 n-3) and EPA (eicosatetraenoic acid) (C20:5 n-5) are the most abundant omega-3 fatty acids (Gupta et al., 2012). These are considered essential fatty acid i.e. they must be obtained through the diet as a human body cannot synthesize them (Sprague et al., 2016). There is an ample number of health benefits associated with these fatty acids such as they are essential for the brain and eye development, reduces the risk of depression, prevents cardiovascular diseases, Schizophrenia, Alzheimer's disease, and Parkinson's disease (Byreddy, 2016). These are widely used in functional foods and beverage products, infant formula, dietary supplements, and pharmaceuticals (Jónasdóttir, 2019). Due to multiple health benefits, the functional food and nutraceutical industries are more inclined towards them. It has been estimated that in 2014 the market size of omega-3 fatty acids was USD8.71 billion and it was expected that at CAGR of 13.8% it would be significantly increased to USD 18.95 billion by the end of 2020. During the forecast year of 2016-2022, the expected market is $6,955 million by 2022 with an increased CAGR of 14.9% (Prasad, 2016).

1.2 Sources of omega-3 fatty acids

Fish oil from Tuna is the primary source for EPA and DHA, but due to the over-exploitation, resource depletion and presence of harmful heavy metals such as mercury, various alternative sources for omega-3 fatty acids have been found (Ryckebosch et al., 2012). At present, marine-algae is the best source of PUFA, as they are abundantly present in nature. Compared with the other sources such as plants, and other marine sources, micro-algae, have high area productivity, high oil content, and do not depend upon the arable land and freshwater sources. They can be cultures photoautotropically or heterotrophically (Coustets et al., 2015). In photoautotrophic

cultivation, they need light for the synthesis of organic material from an organic matter. Whereas during heterotrophic cultivation they use the organic source as a primary source of cultivation. In Table 1, based on the mode of cultivation heterotrophic algae is regarded as the best source of lipid production since no light is required for their growth, less risk of contamination, high biomass yield, and lipid content (Van Wagenen et al., 2012).

Table 1: List of various Microalgal species exhibiting different lipid accumulation.

Microalgae	Bioactive compounds	Technology used	Dry biomass (g/L)	Lipid content (%)	References
Photoautotrophic					
Chlorella Vulgaris	PUFA	Open pond	4.5	14.05	(Sahu et al., 2013)
Dunaliella bardawill	PUFA	Open pond	0.8	6.45	(Sahu et al., 2013)
Nanochloropsis gaditana	PUFA (EPA)	Closed bioreactor		3.7	(Van Wagenen et al., 2012)
Nanochloropsis salina	Fatty acids	Closed	0.72	40	(Ren and Ogden, 2014)
Phaeodactylum tricornutum	fucoxanthin	Raceway	0.41	25.4	(Kim et al., 2012)
Dunaliella sp.	β - carotene	Photoreactor	0.12	22	(Coustets et al., 2015)
Spirulina maxima	GLA	Open pond	0.33	9	(Batista et al., 2013)
Amphora sp.	Fatty acids	Photobioreactor	0.16	24	(Talebi et al., 2013)

Isochrysis	PUFA (EPA)	Open pond	0.34	25-33	(Cuellar-Bermudez et al., 2015)
Tetraselmis sp	EPA	Photobioreactor	0.2	8.44	(Makri et al., 2011)
Paragymnodinium shiwhaense	PUFA (DHA)	Raceway		16.3-34.3	(Veloza et al., 2006)
Phaeodactylum tricornutum	PUFA (EPA)	Open pond	0.13	10.2	(Patil et al., 2007)
Amphidinium carterae	PUFA	Open pond	0.037	65	(Jang et al., 2017)
Heterotrophic algae					
Crypthecodinium cohnii	PUFA (DHA)	Fermentation	6.9	50	(Okuda et al., 2013)
Chlorella Vulgaris	PUFA (EPA)	Photobioreactor	53.1	60.8	(Singhasuwan et al., 2015)
Schizochytrium sp. SP3, Schizochytrium sp.DT3	DHA	Fermentation	12.23, 14.65	29, 35	(Byreddy et al., 2016)
Schizochytrium sp. DT3	DHA	Fermentation	1.98	37.7	(Gupta et al., 2015)
Thraustochytrid sp. Z105	DHA	Flask cultivation	16.72	32	(Zhou et al., 2010)
Aurantiochytrium B L10	DHA	Fed-batch cultivation	59	29	(Yang et al., 2010)
Schizochytrium sp. DT7	DHA	Fermentation	3.64	41.97	(Gupta et al., 2016)
Thraustochytrium sp. T18	DHA	fermentation	14.86	43.3	(Lowrey et al., 2016)

Aurantiochytrium Schizochytrium sp.	DHA	Fermentation	9.9	50	(Singh et al., 2016)
Thraustochytrid F24-2	DHA	fermentation	10.0	36	(Ugalde et al., 2018)

Thraustochytrids are heterotrophic fungi like organisms ubiquitously found in the marine environment. Molecular phylogenetic studies revealed that they are classified under the class labyrinthulomycetes, phylum Heterokonta within the kingdom Chromista (Chang et al., 2012). Ultra-structurally, they show a close relationship with heterokont algae because of the presence of unequal flagella. These species are regarded as a promising producer of omega 3 fatty acids including DHA and EPA (Sun et al., 2017). To enhance the biomass and oil accumulation in microalgae various substrates are used such as glucose (1.44g/l), glycerol (raw) (8.32g/L) ,sucrose xylose (0.48g/L),standard cellobiose(0.38g/L), sugar hydrolysates (1.8g/L) (Gupta et al., 2013a), agricultural wastes such as rice straw (Joe et al., 2015), sugarcane bagasse hydrolysates provides (10.45g/L) dry biomass whereas 45.15% of lipid content was obtained after the 72 h cultivation (Nguyen et al., 2018). Because of the presence of various hydrolytic enzymes, these species are considered as highly adaptive organisms (Xie et al., 2017). This diversified feature sparkled the research and explore the various biotechnological applications to produce a wide range of bioactive compounds such as DHA and EPA (Chang et al., 2011).

1.3 Microencapsulation of omega-3 and its importance

Due to the unsaturated nature of the omega-3 fatty acid, they are chemically unstable and more susceptible to oxidation and produce free radicals, unpleasant taste, and off-flavors. This will lead to effects on the shelf life and overall accessibility of the product (Chang et al., 2016). Therefore, to overcome these problems, and to improve the biological (oxidation) as well as functional (shelf life) characteristics of oils, microencapsulation technology has been extensively used. Oxidation process can be initiated by various factors such as heat, light, and enzymes, it also decreases the nutritional value of the lipid through the breakdown of lipid hydroperoxides which produces the rancid flavor (Peanparkdee et al., 2016) It is a technique which is used to protect the extracted bioactive such as PUFA oils, antioxidants, vitamins, probiotics by building a physical barrier of homogeneous or heterogeneous matrix (Zhang et al., 2012). The bioactive component/

encapsulated substance is referred to as a core, internal or payload phase whereas the biopolymers shell the core material known as wall material, matrix, shell, encapsulant, and coatings (Desai and Jin Park, 2005). Omega-3 can be encapsulated by using different techniques such as spray drying, freeze-drying, coacervation, extrusion, fluidized-bed coating, and Ionic Gelation.

1.4. Types of encapsulation

1.4.1. Spray drying

In the food industry, spray drying is extensively used because of easy scale-up, simple operation, and cost-effective (Drusch, 2007). The general process involves the spray drying of emulsion (produced by core material and biopolymer), then pumping of the emulsion and atomization of the mixture and dehydration of the droplets leads to the formation of microcapsule (Kaushik et al., 2015). Therefore, to protect omega-3 from oxidation microencapsulation has been successfully used to incorporate these oils to increase their bioavailability.

1.4.2. Extrusion

Extrusion is another extensively used technology for microencapsulation of omega 3 fatty acids. it involves the mixing of the molten carriers with bioactive compounds and passed the emulsion through a nozzle under high pressure (Gouin, 2004). Compared with spray dried microcapsule powder, extruded one usually has porous structure which leads to the oxidation of the entrapped omega-3 oils.

1.4.3. Complex coacervation

Complex coacervation is another important method in which the separation of two liquid phases in a colloidal solution. Basically, it is the phenomenon that occurs when electrostatically opposite biopolymers at specific pH come in contact with each other. It is regarded as one of the most promising methods of encapsulating omega -3 fatty acids.(Eratte et al., 2014) The phase which is rich in the polymer is called the coacervate phase and the phase which is devoid of polymer is known as the equilibrium phase (Barrow et al., 2013). In simple coacervation only one polymer is used whereas in complex coacervation oppositely charged polymers are used. Complex coacervation is more advantageous in terms of high oil payload (>50%, w/w) and low surface free

oil content (<0.5%, w/w). Moreover, the particle size of the spray dried microcapsule can be tailored in the ranges from 1 to 1000μm (Zhang et al., 2012).

Figure 1: Due to copyright the image has been removed

1.4.4 Ionic Gelation

Ionic gelation is the other cost-effective technique for encapsulated long-chain polyunsaturated fatty acids. sodium alginate is the most commonly used polysaccharide in the technique (Lin et al., 2016). It consists of β-1,4 linked D- mannuronic acid and guluronic acid. When this polysaccharide combines with the divalent crosslinker such as calcium chloride, bead formation occurs (Bannikova et al., 2018). According to (Lin et al., 2016), the utilization of the alginate to encapsulate PUFA with the aid of calcium ions significantly improves its thermal stability.

Figure 2: Due to copyright, image has been removed.

1.5. Types of wall materials

The selection of wall material plays an important role as it influences the stability, entrapment efficiency, and protection of the encapsulated product (Desai and Jin Park, 2005). Synthetic polymers such as polystyrene, polyamide, and polyacrylate have extensively used in the medicinal and pharmaceutical industry whereas biopolymers such as carbohydrates and proteins are widely used in the food industry (Chang et al., 2016). Different matrices have been used to encapsulate the fatty acids such as maltodextrin: gum Arabic (60:40), chitosan, gelatin., pectin and sodium alginate in Table 2. These carbohydrates combine with the protein such as animal-based protein (Milk), milk proteins such as casein and whey proteins, lactose (de Jesús Bonilla-Ahumada et al., 2018), and plant-based proteins(PPI, SPI) For various bioactive (PUFA oils, antioxidants, probiotics, and vitamins) milk proteins are considered to be the natural vehicles. Because of the presence of several properties such as the ability to bind with small molecules, excellent gel formation abilities, useful for entrapment and release, their biocompatibility and biodegradability, and shielding properties make them useful in the process of microencapsulation. The biocompatibility of milk proteins is usually very high except for a small fraction of the population, who are allergic to these proteins (Livney, 2010). Studies revealed that people who are allergic to milk found that 60% of them allergic to casein. But during the denaturation process, the heating and drying process reduces the allergenicity to various proteins. The digestive biodegradability of

milk proteins shows that it is easily digestible (Katz et al., 2009). It offers low viscosity and good protection against oxidation (Tonon et al., 2011). Another biopolymer used in microencapsulation is the maltodextrins which are the depolymerized starches having less than 20 DE (Dextrose Equivalent). According to previous studies, DE value affects oxidative properties and hygroscopicity. According to (Wang et al., 2014) DE value between 10 – 20 is good for the production of microcapsules. This wall material is highly soluble, low affinity to hydrophobic materials, low viscosity at high concentrations. In addition to high maltodextrins with high DE increases the encapsulated stability oil by building dense wall material around it (Jafari et al., 2007). But because of the less interfacial and low emulsifying properties, they are used as secondary wall material (Gharsallaoui et al., 2007) Gelatin-encapsulated algae can be recommended for functional food ingredient for human consumption. (de Jesús Bonilla-Ahumada et al., 2018).

1.5. Physical properties of microcapsules

There are various physical properties of microcapsule such as water content, particle size, and entrapment efficiency. Particle size, entrapment efficiency and stability of the encapsulated oil (Koç et al., 2015). Depending upon the nozzle size and pressure microcapsule can be 10-50µm or large up to 2-3nm. The desirable particle size of the microcapsule is below 40µm (Kaushik et al., 2015). Different variables such as drying temperature, air pressure determines the quality, as well as the moisture content of the microcapsule, which plays an important role in the stability of the microcapsule (Chang and Nickerson, 2018). Surface oil is another important category which is defined as the presence of oil on the surface of dried microcapsules. Entrapment efficiency is determined by the percentage of encapsulated oil within the wall matrix to the percentage of oil used in the formation (Chang et al., 2016). Higher efficiency indicates less amount of oil on the surface of the dried microcapsule represented in Table 2 (Kaushik et al., 2015). By providing the oxygen barrier in the form of wall materials, oxidation of omega -3 oil can be prevented. Under the different parameters such as light and temperature, the oxidative stability of the oil can be measured. Peroxides, aldehydes and conjugated dienes can be measured in the oil samples (Kaushik et al., 2015). The various methods and outcomes of different oxidation methods are listed in Table 3.

Table 2: **Microencapsulation of PUFA by using various wall materials and methods. SPI (Soy Protein Isolates), MD (Maltodextrins), PPI (Pea Protein Isolates), SC (sodium caseinate), OSA (ocetinylsuucinate starch), and LPI (Lentil Protein Isolate).**

Core material	Wall material	Technology used	Particle size (μm)	Moisture content (%)	Surface oil (%)	Entrapment efficiency (%)	Reference
β – Carotene	Sodium alginate	Microfluidization	285	-	-	-	(Zhang et al., 2016b)
Canola oil	Lentil protein isolate, MD, sodium alginate	Spray drying	59	0.36	2.4	88	(Chang et al., 2016)
Chlamydomonas reinhardtii	Hybrid beads	-	-	-	-	81.5%	(Zhang et al., 2016a)
Fish oil	Chitosan, MD	Spray drying	0.30-0.34	-	-	97	(Ghasemi and Abbasi, 2014)
Fish oil	B-lactoglobulin, pectin, glucose syrup	Spray drying	>2			99	(Tamm et al., 2016a)
Fish oil	Hydroxypropyl	Simple coacervation	-	-	-	-	(Wu and Xiao, 2005)

	methylcellulose						
Flaxseed oil	Whey protein isolate	Spray drying	-	75	-	-	(Partanen et al., 2008)
Flaxseed oil	CPI, LPI, MD	Spray drying	21.6-26	3.6-4.1	1.05-2.64	84-90	(Can Karaca et al., 2013)
Flaxseed oil	Microbeads	Extrusion	50	-	-	-	(Kuhn et al., 2019)
Kenaf seed oil	SC, MD	Spray drying	0.13	-	-	97	(Ng et al., 2013)
Oil from *Schizochytrium sp*.	SPI, Gum Arabic, MD	Spray drying	10.05	2.5	-	93.03	(Chen et al., 2016)
Oil from *Schizochytrium sp*.	Gelatin-gum Arabic with transglutaminase	Complex coacervation	30.7	-	-	-	(Zhang et al., 2012)
Olive oil	SPI, PPI, MD, OSA	Spray drying	8.7	75	-	83-87	(Zhao and Tang, 2016)
Propolis	Soy, pea and rice protein	Spray dryer	-	-	-	70%	(Jansen-Alves et al., 2018)
Rapeseed oil	PPI, glucose syrup	Spray drying	0.5-7.83	-	-	94-95.05	(Tamm et al., 2016b)

Soy protein	SPI, lactose	Spray drying	10	-	-	91-99	(Tang and Li, 2013)
Tetrasel mis chuii	Gelatin, gum Arabic	Spray drying	3.5	-	-	84%	(de Jesús Bonilla-Ahumada et al., 2018)

1.6. Oxidative stability of encapsulated omega-3 oil.

The primary purpose of encapsulating omega fatty acids-rich oils is to protect those oils against oxidative degradation by building a barrier around the oil droplets(Chang and Nickerson, 2018). Basically three steps includes in the lipid oxidation : initiation, propagation, and termination (Chen et al., 2016) During initiation, the abstraction of hydrogen from polyunsaturated fatty acids (PUFA) produces the alkyl radical, on which the free radical is delocalized on the carbon chain, and upon molecular rearrangement, to form conjugated double bonds (secondary product). Subsequently, as a chain reaction, the alkyl radical reacts with triplet state oxygen to produce a peroxyl radical, which has high energy to promote the abstraction of hydrogen from another PUFA(Carneiro et al., 2013). Therefore, the addition of hydrogen on the peroxyl radical produces hydroperoxide, and this step is known as propagation. In termination, the combination of two radicals (e.g., the combination of peroxyl radicals and alkoxyl radical, and the combination between alkyl radicals) occurs to form non-radical species or the radical reacts with a chain-breaking antioxidant (e.g., vitamin E) to form a relatively stable radical (FE NE MA et al., 2007). In practice, primary lipid oxidation products (produced by the initiation and propagation steps of lipid oxidation, e.g., hydroperoxides, conjugated dienes and trienes) and secondary lipid oxidation products (produced by the decomposition of primary lipid oxidation products via β-scission reaction, e.g., aldehydes, carbonyls, and ketones) are monitored to determine the oxidative reaction (Mozuraityte et al., 2017) and the methods are summarized in (Table 3). In general, different types of wall materials and microencapsulation techniques offer the different protective effects on the core materials, based on the various ability of wall materials/structure to inhibit the oxygen transfer.

Table 3: Different methods of measurement of oxidation of omega-3 fatty acids.

Method	Principle	Outcomes	References
Peroxide value	Spectrophotometric method (500nm)	Measurement of primary oxidative products such as hydroperoxides.	(Mozuraityte et al., 2017)
Conjugated dienes	Spectrophotometric method (232-234nm)	Measurement of secondary oxidative products such as aldehydes.	(Mozuraityte et al., 2017)
Propanol measurement	Static headspace gas chromatography	Measurement of secondary oxidative products such as propanal.	(Gheysen et al., 2018)

Based on above, following aims were investigated:

Aim/s:

- I: Fermentation of the *Thraustochytrid* strains for achieving high biomass yield and polyunsaturated fatty acid (Omega-3) production
- II: To maximize stability, omega-3 enriched biomass will be encapsulated to meet the challenges of successfully incorporating and delivering functional ingredients to the wide range of food types.

Hypotheses: The following hypotheses were tested in the research.

- Stabilization of the biomass/oil by Ionic Gelation by sung alginate
- Stabilization of omega-3 oil by ionic gelation using a different matrix
- Stabilization of omega-3 oil by complex coacervation
- High oxidative stability of the encapsulated oil/ biomass

Chapter 2

2. Materials and Methods

2.1. Chemicals

All the chemicals used in this study were of analytical grade. Medium components such as glucose, yeast extract, peptone (Sigma-Aldrich, USA) sea salt (Instant Ocean, France) and MiliQ water were used for biomass production. Chloroform (from Merck), and methanol (from Sigma-Aldrich, USA) were used in the lipid extraction. Omega -3 oil (rich in DHA and EPA sourced from the marine source) procured from Ethical Nutrients (Priceline pharmacy, Australia) Sodium alginate, Gelatin type A (from Sigma-Aldrich, USA) calcium chloride (from Sigma-Aldrich, USA), maltodextrins DE 10 (Melbourne Food Depot, Australia) and Glacial Acetic acid, HPLC grade (from Scharlau) were used for microencapsulation of lipids.

2.2 *Thraustochytrid* biomass cultivation

Thraustochytrium S2 strain was sourced from in house collection of the Centre for Marine Bioproducts, Medical Biotechnology College of Medicine and Public Health, Flinders University. This strain was isolated by Dr. Adarsh Gupta (Post-Doctoral researcher working with Associate Professor Munish Puri). Thraustochytrid strain (S2) used in this study was maintained on GYP medium containing (g/L) glucose 10, Yeast Extract 1, Peptone 1, agar 10 and 50% of artificial seawater and miliQ water at 25℃ Seed medium (50ml) contains glucose 10, Yeast Extract 1, Peptone 1 was inoculated from agar plates and grown for 2 days in a shake flask at 25°C, at 150rpm (Gupta et al., 2013b). After 2 days, inoculum (5%) was used to inoculate the production media which contains glucose 10, Yeast Extract 1, Peptone 1 and incubated under the same conditions for 7 days.

2.2.1. Biomass analysis

Fermentation was carried in a 50 ml medium contained in a 250 ml Erlenmeyer flask for 7 days. The samples (5 ml) were withdrawn from the fermentation medium at regular intervals of time (24 h). The sample was centrifuged (4500rpm,10min) and the supernatant was removed for glucose consumption analysis. The remaining pellet was freeze-dried and weighed to find the final weight of the biomass. Freeze-dried biomass was stored at -20°C for further use.

2.2.2. Lipid extraction

Freeze-dried cells (10mg) were taken in a 1.5ml centrifuge tube to which 600µl of chloroform: methanol (2:1) is added. The tube was vortexed for 2 min before centrifugation at 10,000rpm for 10 min. The extraction was repeated three times. The supernatant was collected in glass vials (after filtration with 0.22µm filter). Collected solvent supernatant was dried in Dry Block Heater *(Ratek instruments)* at 50°C and the lipid weight was measured gravimetrically based on a protocol (Gupta et al., 2016).

2.3. Encapsulation by Ionic gelation using alginate

2.3.1. Effect of alginate-calcium concenteration on bead shape

Sodium alginate (1.0-2.5%) was dissolved in distilled water to produce a homogenous solution. To disengage bubbles These solutions were left standing for 24h. at continuous stirring conditions for disengaging the bubbles (Chan, 2011).A calcium chloride solution of different conc i.e. (100mM, 150mM, 200Mm) was prepared for optimizing the encapsulation process.

2.3.2. Preparation of alginate-biomass/oil emulsion

To alginate solution dried biomass (5-10% w/v, containing 37% of oil) was added. The alginate biomass emulsion was thoroughly mixed at 300rpm for 30min. The biomass was gradually added to the alginate solution during mixing until the desired concentration was obtained.

2.3.3. Encapsulation of biomass/oil

The alginate-biomass emulsion (50ml) was extruded through a syringe (23G) into calcium chloride solution(150mM) there was a formation of alginate beads. To prevent the sticking of bead, gelation was carried on a magnetic stirrer. The beads were hardened for 30 min.

2.3.4. Determination of encapsulation efficiency

Encapsulation efficiency was determined with slight modifications (Chatterjee and Judeh, 2015). The beads were separated from calcium chloride solution with a sieve. Hexane(20ml) was added to the $Cacl_2$ solution to extract the oil which was not encapsulated. The absorbance of hexane was determined at 280nm. The unencapsulated oil/biomass content was calculated from the standard curve. To determine the surface oil the beads were transferred into hexane(20ml) solution.The solution was kept for 10 min at shaking conditions. The absorbance of hexane was determined at 280nm.

The encapsulation efficiency (EE) can be calculated by-

EE (%) = Initial oil content-(unloading oil-surface oil) / initial oil content x 100

2.3.5. Freeze-drying of beads

Beads were frozen at −20 °C overnight and were freeze-dried the temperature of the ice condenser was set at −50 °C and the vacuum pressure was set to 0.04 bar. The frozen samples were dried for 30 h and the dried product was collected, pulverized and stored in an airtight container for further use.

2.4. Encapsulation using gelatin, maltodextrins and sodium alginate

Encapsulation of omega-3 oil was investigated using various polymers. The gelatin and maltodextrins were dissolved in water (2:5). The resulting solution was heated at 50 °C to obtain a clear solution (Xia et al., 2019). This solution was homogenized (14000 rpm, Ultra-Turrax T25) to which omega-3 oil (10%, v/v) was added gradually. For preparing a fine emulsion, homogenization was extended to 20 min. To this emulsion, sodium alginate (2%, w/v) was added and homogenized to achieve uniform mixing. This emulsion was extruded dropwise in a calcium chloride solution (100 mM), and resulting beads were monitored for stability. The amount of oil released was calculated from the standard curve.

2.4.1. Storage stability

Freshly prepared beads were dispersed in the 0.1M phosphate buffer (pH 7.5) at 4℃ for assessing encapsulation efficiency over a period of time. While the rest of the beads were freeze-dried and stored in airtight containers to check oxidative stability.

2.5. Microencapsulation by coacervation

Different parameters were considered to optimize the complex coacervation process which includes ratios of protein: polysaccharide, the effect of homogenization time, the effect of pH, and stirring speed during cooling (Wang et al., 2014).

2.5.1 Effect of gelatin-to-alginate ratio

Different concentrations of gelatin and sodium alginate were used to optimize the process. Gelatin (8% w/v) with alginate (4%w/v), and less than alginate (1%) was used with the same gelatin concentration.

2.5.2. Effect of homogenizing time on emulsion

Gelatin with oil emulsion was homogenized at 15000rpm at different intervals of time i.e. 5, 10,20,25 and30min to get the < 3micron oil droplets in order to achieve good microencapsulation efficiency. All the images after regular time intervals were observed on the microscope (Nikon Eclipse Ts2R).

2.5.3. Effect of pH

As coacervation is pH-dependent process, upon adding glacial acetic acid (5%v/v), different microscopic images were observed under the microscope during the process of coacervation. Different pH range was used (4.9 to 3.6) to optimize the pH at which anionic and cationic polymers interact with each other and forms a coating around the droplets in the form of coacervates.

2.5.4. Effect of stirring speed during the cooling process

After the formation of liquid capsules, the mixture was cooled at 4°C under continuous stirring at various speeds such as 600rpm and 450rpm. The mixture was cooled for about 3h so that the microcapsules were solidified.

2.5.5. Microencapsulation of Omega-3 oil by complex coacervation

Firstly, Gelatin type A(20g) dissolved in 230g of water (Xia et al., 2017). The solution was heated at 60°c until gelatin gets fully dissolved. To the above solution omega-3(10%) oil was added and homogenized at 15000rpm for 30min. During homogenization, microscopic images were observed. After homogenization tap water (5ml) was added until the oil droplets observed on the

surface of the emulsion. A known amount of sodium alginate (1% w/v) was added to the emulsion dropwise. The emulsion was heated at 60°C and this temperature was maintained throughout the experiment. The pH of the mixture was brought down to 3.6 by adding glacial acetic acid (5%v/v). The system was then cooled to 5-10°Cto harden the microcapsules. After cooling samples were freeze-dried for further processing.

2.5.6. Drying of coacervate microcapsules

Liquid microcapsules were frozen at −20 °C overnight and were freeze-dried the temperature of the ice condenser was set at −50 °C and the vacuum pressure was set to 0.04 bar. The frozen samples were dried for 30 h and the dried product was collected, pulverized and stored in an airtight container for further test.

2.5.7. Determination of encapsulation efficiency (%) of the coacervate powder

Microencapsulation efficiency can be determined by calculating the surface oil (the oil on the surface of coacervates) and total oil (both encapsulated oil and surface oil). Surface oil can be calculated by washing method (Xia et al., 2017) with minor modifications. 20mg of coacervate powder add 200µl of hexane, vortex the mixture for min. centrifuge the mixture at 12,000rpm for 15minso that to extract the surface oil. Weigh the empty glass vial, add the mixture to the vial and kept the solvent in a fume hood for complete evaporation of the solvent. After evaporation, measure the oil content gravimetrically.

Total oil was determined by treating the powder with 4N HCL (0.6ml), vortex the mixture for1 min. Add hexane to the prepared mixture, vortex for 1 min. Put the mixture under continuous shaking conditions i.e. 130rpm and at ambient temperature for 15h to extract the total oil completely. The mixture was centrifuged at 12,00rpm for 15 min. weigh the glass vial, put the mixture and nitrogen evaporation was done under the fume hood. Total oil content was determined gravimetrically.

The surface oil (SO) (%), total oil (TO) (%), and encapsulation efficiency (EE) (%) can be calculated by the following equation-

$$SO = W_S / W_m \times 100\ \%$$

$$TO = W_t / W_m \times 100\%$$

$$EE = W_t - W_s / W_t \times 100\%$$

where W_t(g) and W_s(g) are the mass values (g) of the total oil and surface oil W_m is the mass (g) value of the microcapsules taken.

2.6. Oxidative stability

2.6.1 Stability test using the oven test method

Freeze-dried microcapsule powder (1g) in an airtight container placed in an oven at 60°c for 5 days and samples were collected periodically. Along with the encapsulated oil, unencapsulated oil was kept in the same conditions as a control. For stability test total oil was extracted every other day to check the formation of hydroperoxides in the sample (Scheffler et al., 2009).

Peroxide value test was conducted using the International Dairy Federation (IDF) standard method. Briefly, mix 2 volume parts of 1-decanol with 1 volume part of hexane. Add 3 volume parts of methanol to the mixture and mix them thoroughly.

2.6.2. Preparation of standard curve for ferrous ion solution.

Iron powder (0.5g) dissolved in 50ml of 10N HCL and add 2ml of 30% of hydrogen peroxide. The solution will be heated in a boiling water bath for 5 min to remove the excess of hydrogen peroxide. After 5 min let the solution cool down at room temperature and diluted with 500ml of deionized water. The iron chloride solution containing 1g/l of Fe. The resulted ferric ion solution will further be diluted with methanol/decanol/n-hexane mixture to obtain a final concentration of 750, 500, 250 and 125µglml. Add 50µl of ammonium thiocyanate to the resultant solution. Measure the absorbance at 510nm.

2.6.3. Preparation of iron chloride solution

Add 0.132M barium chloride solution to 50ml of water. This solution will be slowly added to the iron sulfate solution. 2ml of 10N HCL added to the resulting solution. Barium sulfate precipitate will be filtered to give a clear iron solution. The solution will be stored in a dark bottle.

2.6.4. Preparation of ammonium thiocyanate solution

Dissolved approx. 30g of ammonium thiocyanate in water and diluted with water to 100ml.

2.6.5. Measurement of lipid hydroperoxide

Peroxide value of the sample was measured by withdrawing the sample from the oven extract the total oil and dissolve the oil in 5 ml of MDH solution, 50µl of ammonium thiocyanate solution and 50µl of ferrous ion solution. Samples were vortexed and held for 5 min to complete the reaction and the absorbance was measured at 500nm.

Peroxide value expressed as milliequivalents of peroxide per kg of the sample,

Peroxide value can be calculated by-

Peroxide value (PV) = $(As - Ab) \times M / 55.82 \times M_o \times 2$

Where, As is the absorbance of the sample

Ab is the absorbance of the blank

M is the slope obtained from the standard curve

55.84 is the atomic weight of iron

M_o is the mass of sample (g)

Statistical Analysis

The results presented were the averages and standard deviation calculated from replicate measurements. An analysis of variance (ANOVA) where ($P<0.05$) was used to assess the difference between samples at a significance level of 95%.

3. Results and discussion

3.1. Biomass growth profile of *Thraustochytrids*

Higher biomass (3.1g/L) and lipid (37%, 0.35g) was obtained when S2 strain was grown in a fermentation medium containing 1% of glucose in a fermentation medium for 7-days (Fig. 3).This result was in accordance with the (Gupta et al., 2016), where 10g/L of glucose enhances the production of the *thraustochytrid* biomass and lipid.

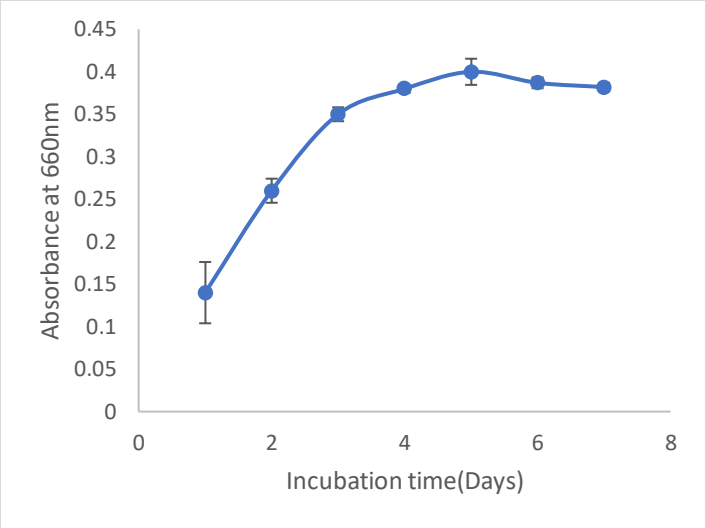

Figure 3: Growth Profile of *thraustochytrid* S2 strain grown in fermented medium at 25°C.

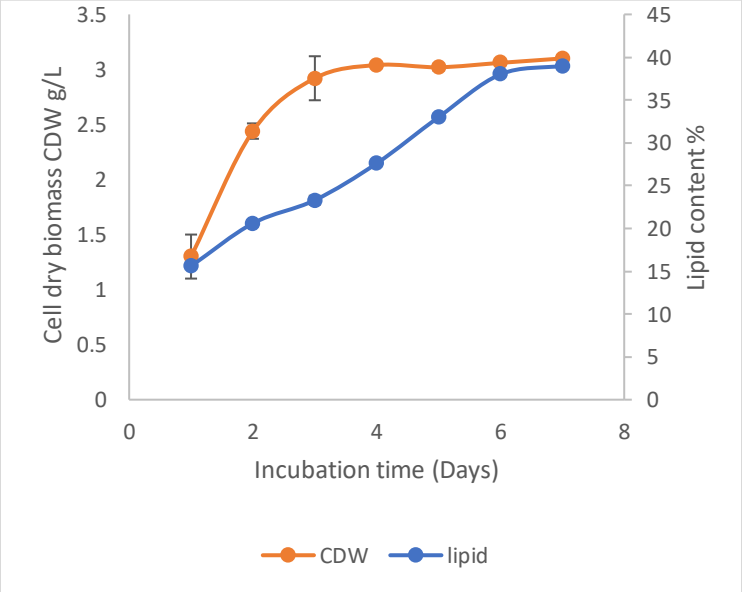

Figure 4: Lipid vs Cell Dry Weight of *thraustochytrid* S2 strain.

3.2. Encapsulation of algal biomass by Ionic gelation

3.2.1 Optimization of the alginate concentration

When alginate (2%, w/v) was extruded dropwise into calcium chloride (100Mm) round beads were formed. The concentration of alginate solution could affect the shape of calcium-alginate beads (Fig. 4). Upon using higher alginate concentration (2.5% w/v) more thick and round beads were formed. Higher concentration led to greater average weight and size of the alginate beads in (Table

3). This is because higher alginate concentration which increases the viscosity and the bead weight and size (Fig 4). At (2% w/v) Alginate concentration and at 100mM calcium chloride, round beads were formed. In the present study alginate (2% w/v) was used for encapsulation of bioactive compounds. Lower alginate concentration could not attain round shape and therefore tear -like beads were formed (Li et al., 2013).

3.2.3. Optimization of the calcium-chloride concentration

Three different concentrations of calcium chloride (100mM, 150mM, and 200mM were used (Table 2). Calcium chloride solution (100mM and 150mM) gave similar results (Fig 8). This divalent molecule act as a crosslinker, when the calcium ions bound with the carboxylic groups of the alginate, egg-box like structure formed. If the concentration of calcium chloride solution is high, then the excessive calcium ions which are not bound to the carboxylic group (Lin et al., 2016). After 30min gelling time, the beads extracted from the solution and in the present study of alginate concentration (2% w/v) and 100mM of calcium chloride were optimized for encapsulation of biomass. Similarly, the same optimized conditions used by other researchers for the encapsulation of omega -3 oil.

3.2.4. Encapsulation efficiency

An encapsulation efficiency of 94% was observed which is in agreement with literature finding (de Jesús Bonilla-Ahumada et al., 2018). Similarly, upon using omega-3 oil (10%v/v), 95% of encapsulation efficiency was observed. Non-encapsulated oil (was observed on the beads and in the gelling bath). The fraction of non-encapsulated oil is also found in the gelation bath i.e. calcium chloride solution and on the surface of wet beads which was in agreement with literature findings (Chan, 2011).

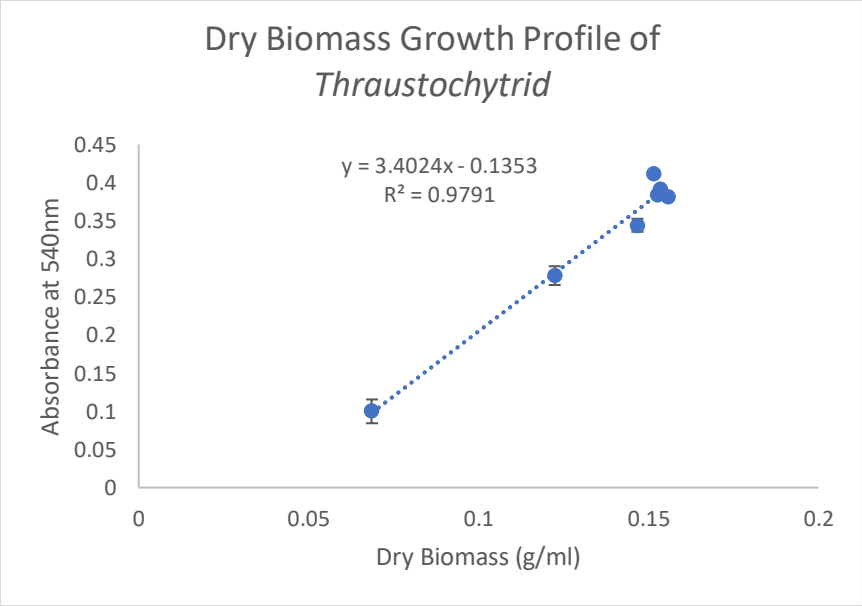

Figure 5 Standard curve for dry biomass of *Thraustochytrid*

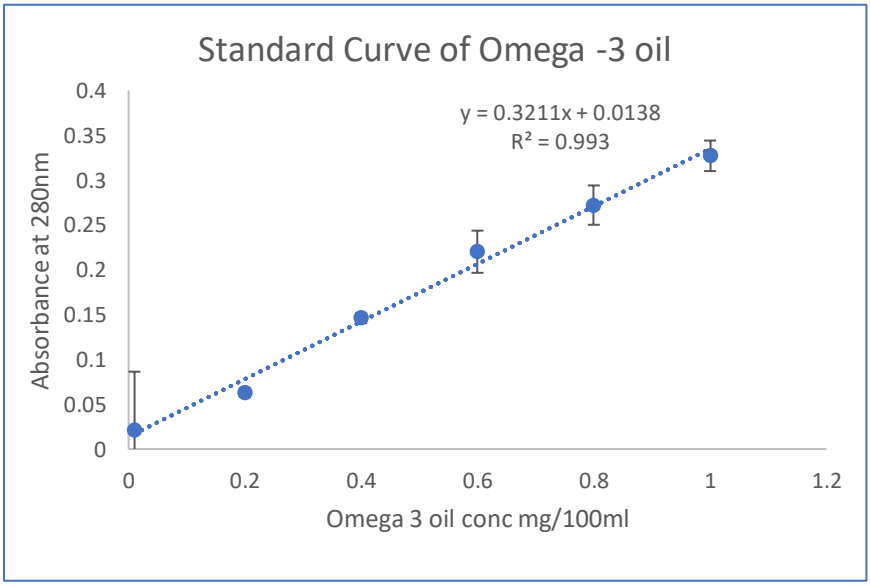

Figure 6: Standard curve for omega-3 oil.

3.2.5 Storage stability

The encapsulation efficiency of the beads was checked after every 24h. Some beads got fragmented in 24h (Table 4). According to (Zhang et al., 2015) when the bioactive compound was retained within the hydrogel i.e. Alginate and when they were exposed to certain pH conditions-

do experience physical changes i.e. shrinkage or swelling of the gel was observed. Anionic hydrogel mainly shrinks when the pH is less than the pKa of the ionizable groups due to the electrostatic repulsion between the charged molecules (Peppas and Khare, 1993). Fragmentation or degradation of the beads may occur due to the surface erosion, bulk erosion and may be due to the cross-links between the biopolymers (Zhang et al., 2015)

Table 4: Encapsulation efficiency (EE) of biomass and omega-3 oil.

Days	**Biomass loading alginate beads (%)**	**EE (%) Sodium alginate Beads in phosphate buffer**	**Oil Loading (%)**	**EE (%) Sodium alginate Beads in phosphate buffer**	**EE (%) Alginate+Maltodextrins+gelatin Oil Beads in phosphate buffer**
1	10	94.5 ±0.08	10	95 ± 0.09	96±0.3
2	10	Beads fragmented	10	Beads fragmented	94±0.06
3	10	-	10	-	90±0.05
4	10	-	10	-	87±0.62
5	10	-	10	-	82±0.04
6	10	-	10	-	80±0.05
7	10	-	10	-	77±0.08

Table 5. Evaluation of Preliminary experimental conditions for preparing Alginate beads (Control).

Calcium chloride	**Alginate**	**Size**	**Weight (g)**	**Weight (g)**
(mM)	**(%w/v)**	**Diameter(mm)**	**Before Freeze Drying**	**After Freeze Drying**
100mM	**1**	**2.32±0.24**	**2.74±0.44**	**0.072±0.009**

	1.5	**2.51±0.221**	**2.08±0.722**	**0.096±0.311**
	2	**2.61±0.272**	**3.76±0.026**	**0.130±0.006**
	2.5	**2.97±0.188**	**3.52±0.84**	**0.148±0.22**
	1	**2.25±0.341**	**1.84±0.44**	**0.068±0.01**
	1.5	**2.99±0.043**	**3.56±0.62**	**0.131±0.00**
	2	**2.81±0.255**	**4.59±0.1**	**0.181±0.00**
150mM	**2.5**	**2.79±0.332**	**4.38±0.28**	**0.211±0.023**
	1	**2.42±0.295**	**1.66±0.35**	**0.070±0.02**
	1.5	**2.55±0.221**	**3.24±0.04**	**0.151±0.02**
	2	**3.02±0.102**	**3.88±0.09**	**0.162±0.00**
200mM	**2.5**	**3.02±0.163**	**4.08±0.46**	**0.272±0.008**

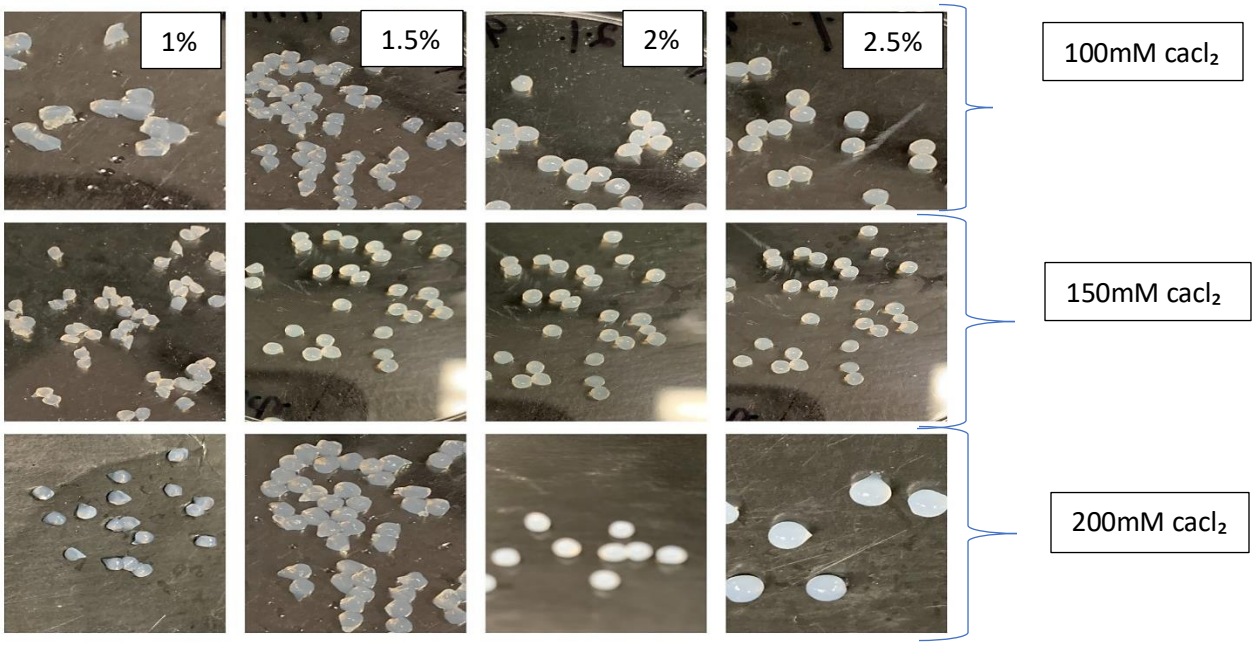

Figure 7: **Beads at various concentrations of sodium alginate without biomass. Images were captured by NIKON microscope imager.**

Table 6: Encapsulated beads prepared with various parameters showing different characteristics including weight(g), size(mm), and encapsulation efficiency (%).

Sodium alginate (% w/v)	Calcium chloride(mM)	Wet biomass (%)	Average weight of beads(g)	Average size(mm)	Encapsulation efficiency (%)
1.5	150mM	10%	3.546±0.401	2.5±0.05	92.5±0.707
		5%	2.611±0.564	2.45±0.04	82.5±3.53

2	**150mM**	**10%**	**6.0418±0.058**	**3.0±0.06**	**90.1±0.141**
		5%	**6.1955±0.162**	**2.8±0.09**	**84.95±0.07**
1.5	**100mM**	**10%**	**6.351±0.076**	**3.2±0.05**	**89.5±0.707**
		5%	**2.888±0.047**	**2.32±0.06**	**88±0.026**
2	**100mM**	**10%**	**4.613±0.155**	**2.4±0.02**	**94±0.565**
		5%	**3.042±0.024**	**2.9±0.07**	**83.85±0.777**

3.3. Encapsulation of omega-3 oil by using maltodextrins, gelatin, and sodium alginate.

During storage stability, alginate beads fragmented due to the pH change described in (3.2.5). To enhance the storage stability of the oil loaded alginate beads, two wall materials such as Gelatin and maltodextrins were incorporated in the experiment in a ratio (2:5) based on literature findings. Encapsulation efficiency was checked after every 24h up to 7 days (Table 4). Fragmentation of beads was not observed which indicates that the use of more than one wall material facilitated masking of the encapsulated oil. Although gelatin is insoluble in cold water and hydrated when stirred into the cold water. The swollen gelatin beads could hold up to 5 to10 times of water molecules (Meng and Cloutier, 2014). Hence this temperature can be considered as a sol-gel transition temperature when the gel crosslinked and forms a thermostable gel. When the temperature raised, the solution again changes into the colloidal solution (Schrieber and Gareis, 2007).

3.4 Encapsulation by Complex Coacervation

To improve upon leakage and stability of the omega-3 oil, complex coacervation was investigated. Following parameters (protein: polysaccharide ratios, and pH) were optimized during encapsulation process.

3.4.1. Gelatin to sodium alginate ratio

The highest encapsulation efficiency (91%) of omega-3 oil was achieved when mixing gelatin (8%) with sodium alginate (0.95%) was investigated. Further increasing the colloid (polysaccharide) concentration, there was more formation of ionic aggregates than the complex coacervates. According to (Siow and Ong, 2013), the addition of one of the biopolymer imbalances the charges in the coacervation system. This may result in the reduction of the attraction between the two colloids and effects microencapsulation efficiency. Therefore, the optimized ration of gelatin: alginate was 1.4:0.8.

3.4.2. Optimization of homogenizing time

On the addition of oil to gelatin, the emulsion was formed. The resulting emulsion was observed under the microscope after regular intervals of time (5-30mins) (Fig 9). The emulsion was homogenized until the size of the oil droplet become <3microns achieved. Lesser the size of the oil droplet better will be the encapsulation efficiency of the process (Eratte et al., 2014). Therefore 30min homogenizing time was optimized for the given process.

3.4.3. Optimization of pH for complex coacervation

The interaction between the gelatin and sodium alginate is expected to be electrostatic in nature. Because the electrostatic complexes would be formed in the pH window where both the colloids are in opposite charges. Furthermore, the ratio of these biopolymers played an important role because at a specific ratio and at certain pH coacervates are formed. Based on the observed data the optimized ratio for gelatin and alginate (1.4:0.8) and at this ratio the optimized pH was 3.6. At this pH, coacervation occurs, where coacervates migrate to the surface of the oil droplet and thus formed a coacervate layer (Xia et al., 2017). It was observed that at pH 3.6 aggregation of the droplets takes place (Fig 10). This may be due to the reason that the gelatin becomes positively charges below its isoelectric point and forms a complex with the anionic complex i.e. sodium alginate (Eratte et al., 2014). Maximum coacervates were formed by using 5% of glacial acetic acid. Hence this pH implies that the complexation between two biopolymers was highest at this pH 3.6 (Fig 12).

Furthermore, when the liquid microcapsules were cooled and stirred at 600rpm, no coacervates where found. Due to faster stirring speed, the free coacervates were not able to bind to the oil droplets, hence appropriate capsules were formed (Fig 10). However, upon lowering stirring speed i.e. 400rpm the free coacervates in the continuous phase began to absorb on the oil droplets. After freeze-drying, the encapsulation parameters were calculated where encapsulation efficiency coacervate was 91% whereas total oil loading was 70% and surface oil was 6%. (Table 7).

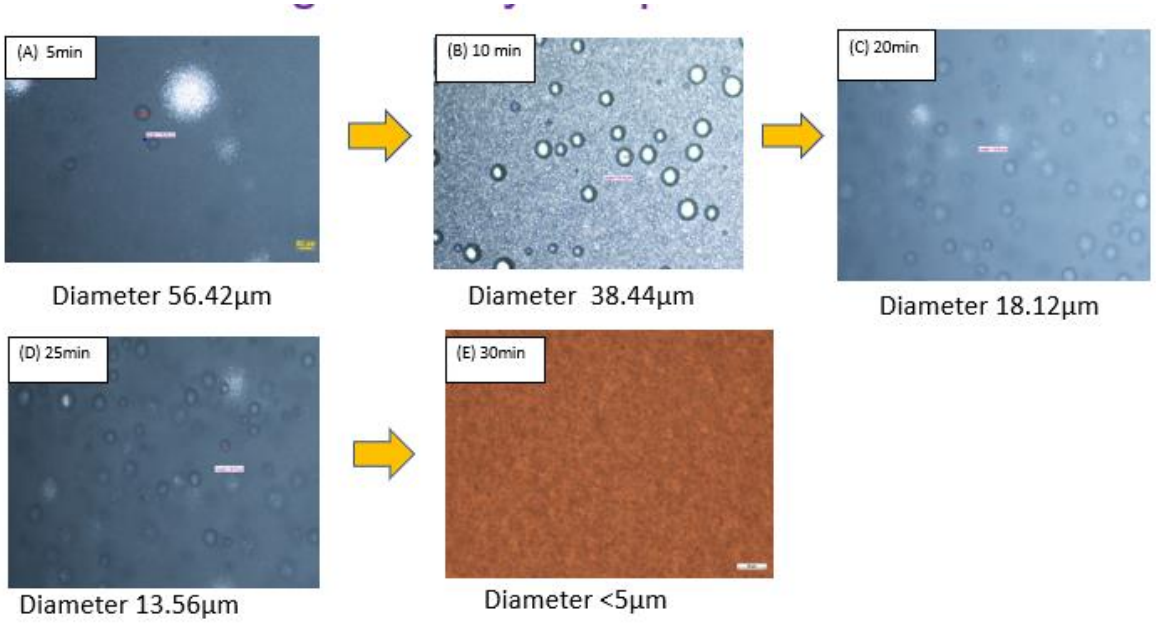

Figure 8: Microscopic images (A-E) represents the homogenization time at different time intervals at 15,000rpm.

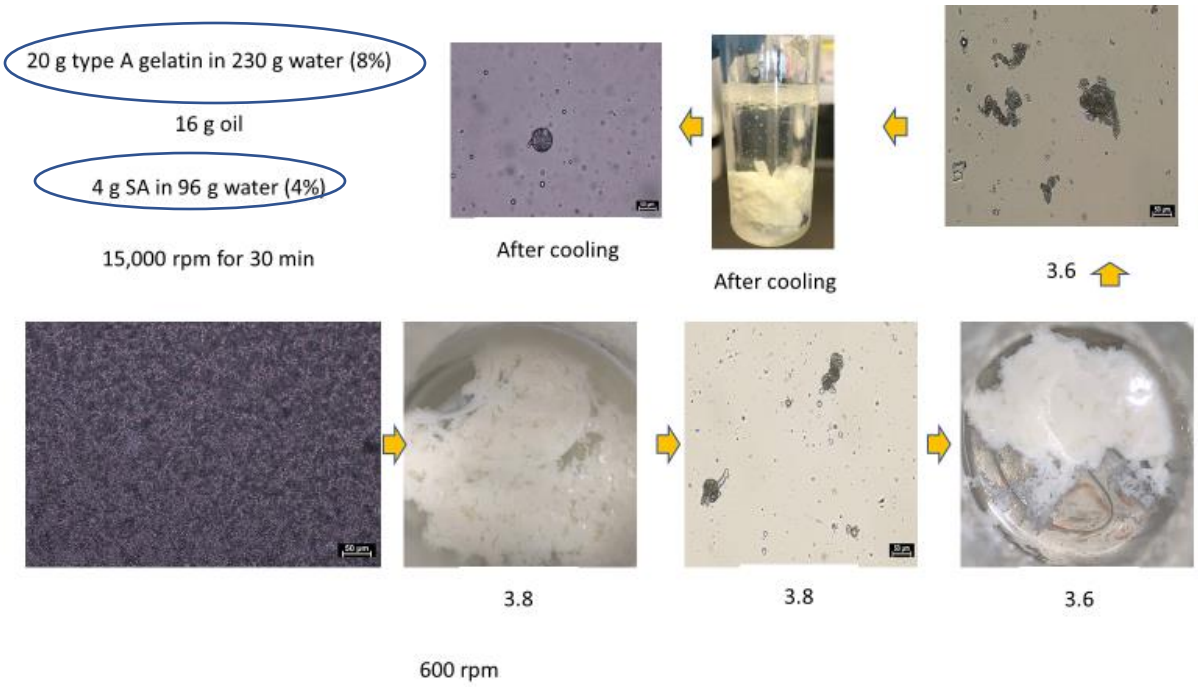

Figure 9. Microscopic images of optimization of complex coacervation process for encapsulation of omega-3 oil

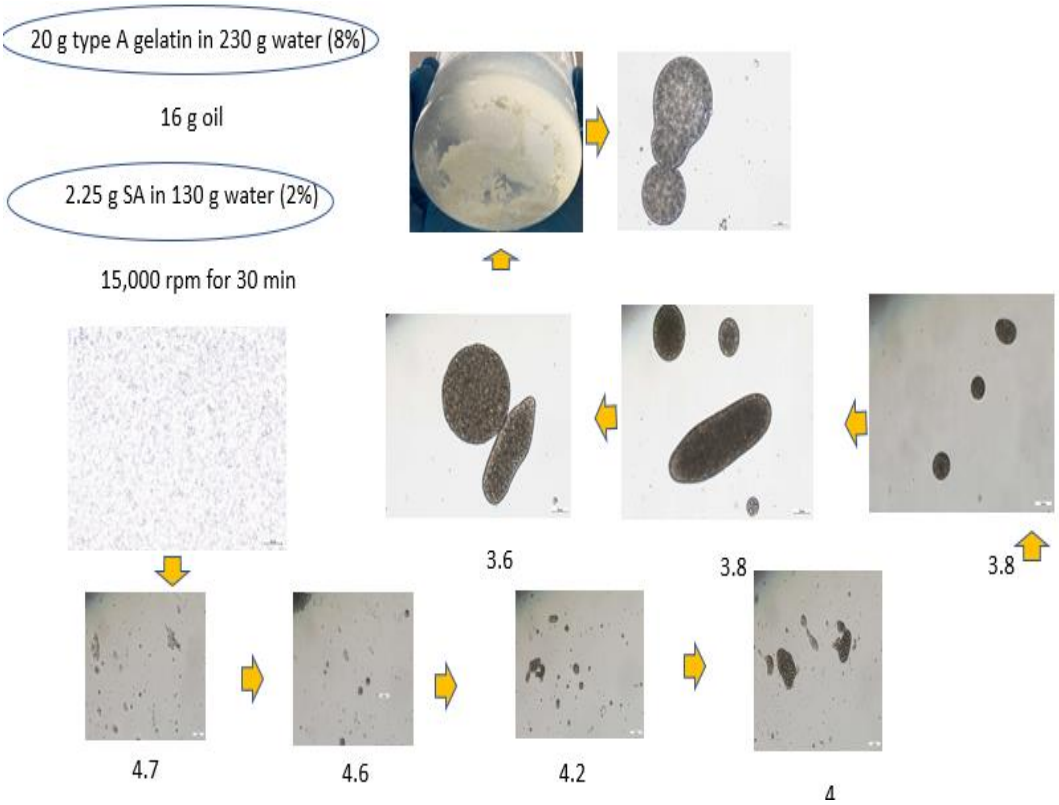

Figure 10: Microscopic images of optimization of complex coacervation for encapsulation of omega-3 oil.

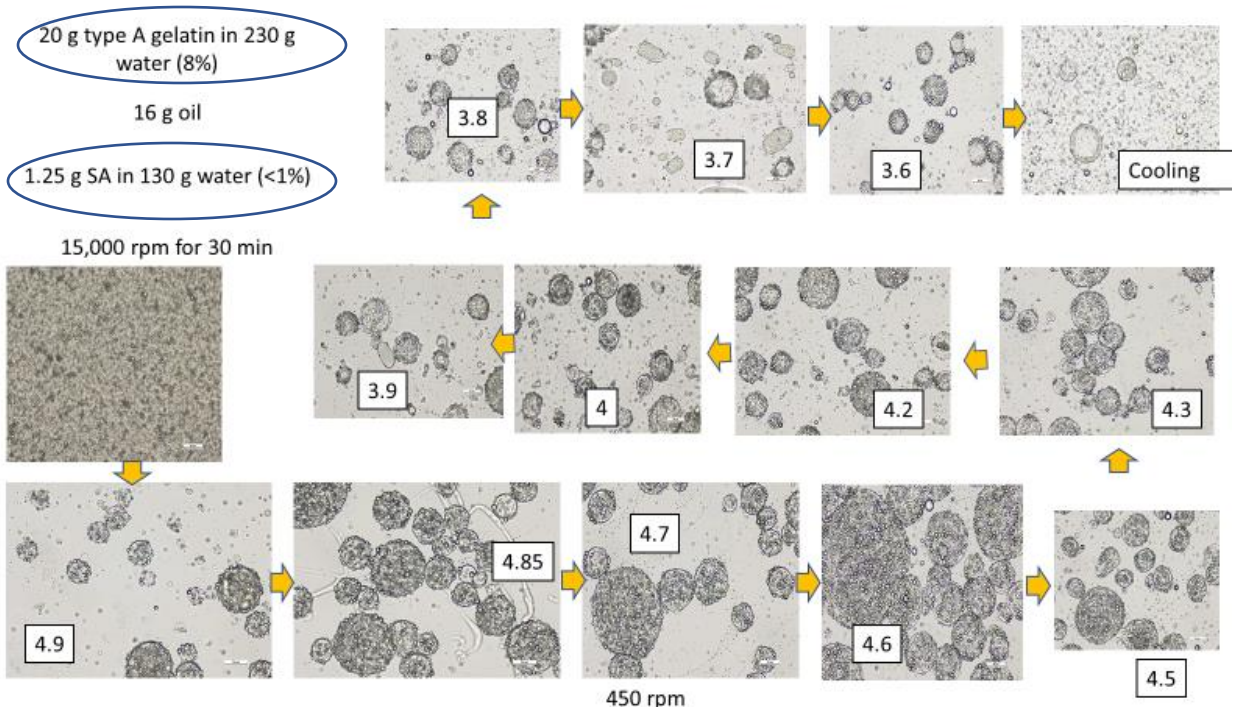

Figure 11. Microscopic images of the optimized process for complex coacervation for encapsulation of omega-3 oil

Table 7: Encapsulation parameters of coacervates of omega-3 oil

Encapsulation parameters	**Coacervates of omega-3 oil**
Total oil (%)	70±0.56
Surface oil (%)	6.1±0.06
Encapsulation efficiency (%)	91.4±0.311

3.5. Oxidative test

The peroxide values of omega-3 oil (control), freeze-dried coacervates of gelatin, alginate beads with oil/ biomass (10%v/v oil), beads of maltodextrins, gelatin and sodium alginate with oil are presented in (Fig 14). The coacervates exhibited better oxidative stability ($p<0.05$) as compared to the control (Fig 14). Whereas the least stability was found on day 5 in alginate freeze-dried beads as shown in (fig 14). The oil encapsulated with maltodextrins, gelatin and sodium alginate

exhibited good stability and lesser PV value on the 5th day (Fig 14) as compared to the other wall material i.e. alginate. This may be due to highly porous structure of the freeze-dried beads which were less stable against the oxidation (Eratte et al., 2014) The porous structures make it easier to diffuse the oxygen thorough the porous shell structure to access the encapsulated oil and weakens the oxidative stability (Siow and Ong, 2013) Therefore, during the ionic gelation when more wall material was added such as maltodextrins and gelatin, the shell strength gets strengthened and provides the better oxidative stability as compared to one wall material i.e. alginate. On a similar note biomass loaded alginate beads also show less PV value as compared to other matrices used. This may be due to the presence of natural antioxidants such as astaxanthin, polyphenols in the algal biomass (Kalidasan et al., 2015). These natural antioxidants delay lipid peroxidation by inhibiting the propagation of the oxidizing chain reaction by destroying the free radicals (Xia et al., 2017).

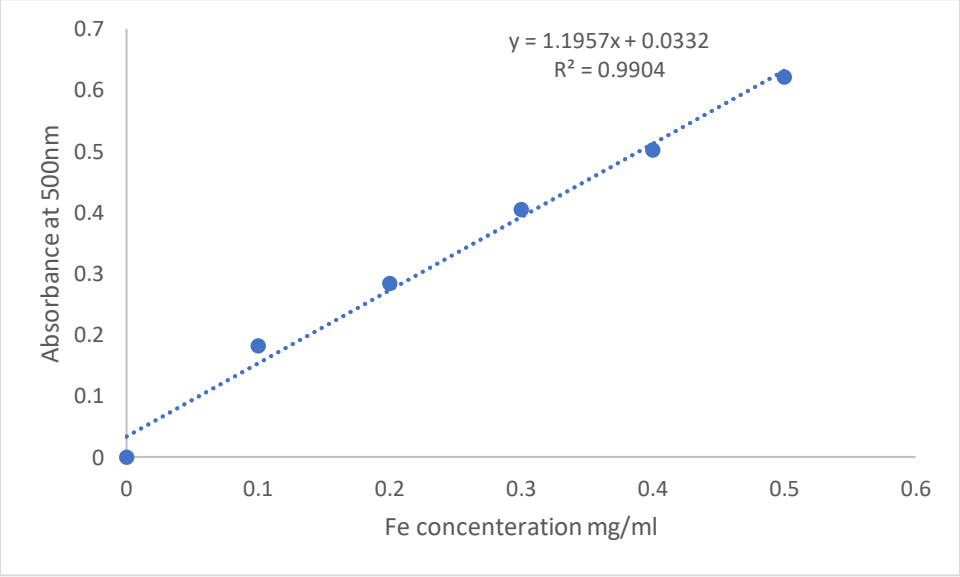

Figure 12: Standard curve of Fe^{2+} solution.

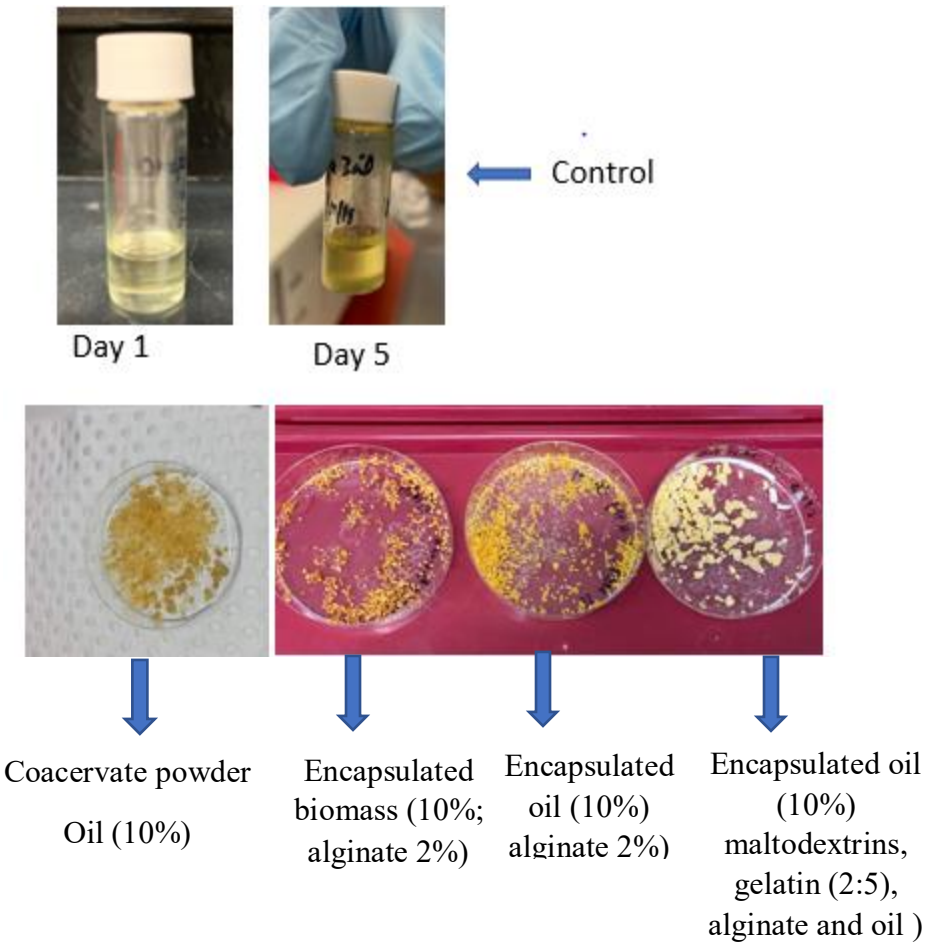

Figure 13 : Oven-drying test of the encapsulated Omega-3 oil.

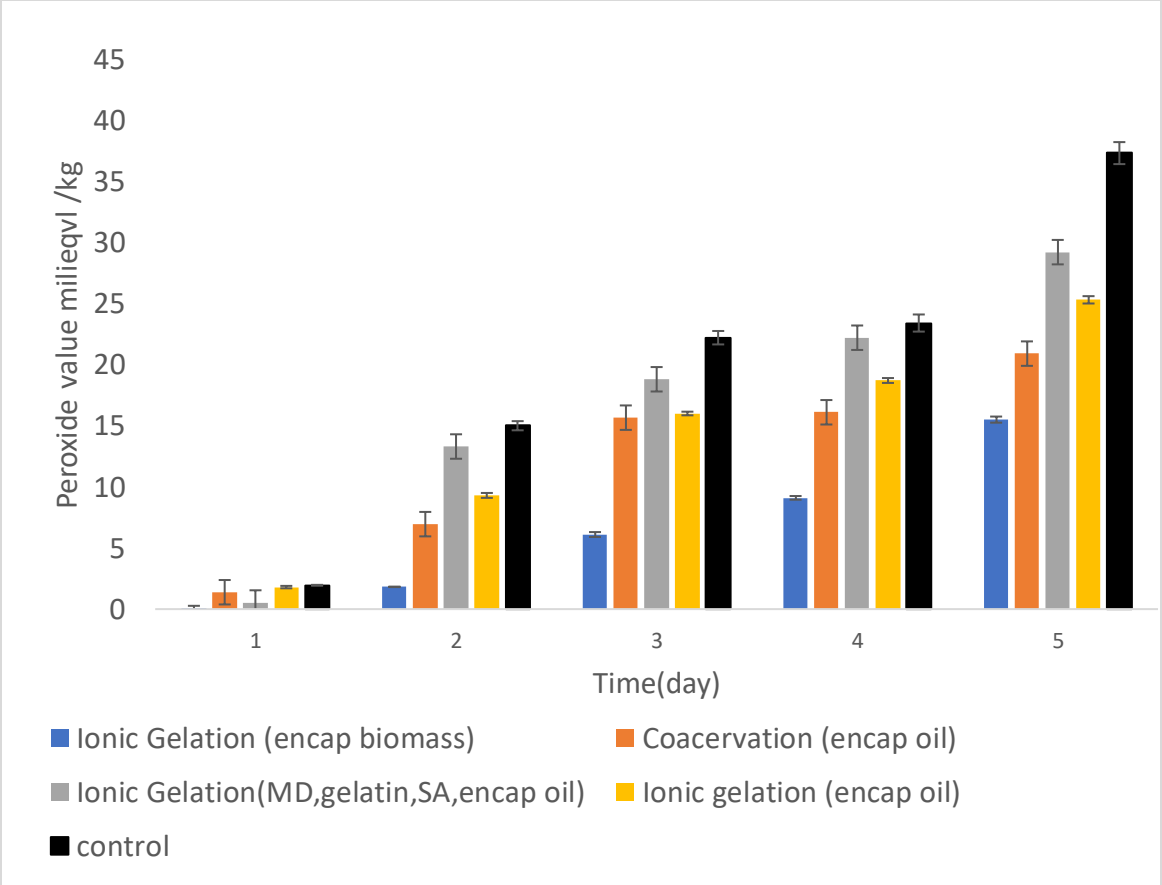

Figure 14: Changes in the peroxide values were observed in the encapsulated as well as unencapsulated (control) of omega-3 oil and biomass under the storage for 5 days at 60°C.

Conclusion

In the present research work, encapsulation of biomass and omega-3 fatty acids was achieved. Alginate bead formation with oil and biomass led to, the optimized concentrations were 2%, whereas, in the case of maltodextrins and proteins, the optimized ratios were (2:5). The storage stability was observed to be highest in maltodextrins and gelatin (as wall materials) encapsulated beads. Encapsulation efficiency of 94% was observed. In the complex coacervation, protein: polysaccharide ratios (1.4:0.8) and pH range (3.6) was optimized that exhibited 91% of encapsulation efficiency. Based on the oxidative stability test, the complex coacervation showing the highest stability as compared to the other wall materials. However, presence of natural antioxidants in the biomass, led to fewer peroxides formation as compared to the sodium alginate and maltodextrin wall materials.

References

ADARME-VEGA, T. C., LIM, D. K., TIMMINS, M., VERNEN, F., LI, Y. & SCHENK, P. M. 2012. Microalgal biofactories: a promising approach to sustainable omega-3 fatty acid production. *Microbial Cell Factories,* 11**,** 96.

BANNIKOVA, A., EVTEEV, A., PANKIN, K., EVDOKIMOV, I. & KASAPIS, S. 2018. Microencapsulation of fish oil with alginate: In-vitro evaluation and controlled release. *LWT,* 90**,** 310-315.

BARROW, C., WANG, B., ADHIKARI, B. & LIU, H. 2013. Spray drying and encapsulation of omega-3 oils. *Food enrichment with omega-3 fatty acids.* Elsevier.

BATISTA, A. P., GOUVEIA, L., BANDARRA, N. M., FRANCO, J. M. & RAYMUNDO, A. 2013. Comparison of microalgal biomass profiles as a novel functional ingredient for food products. *Algal Research,* 2**,** 164-173.

BYREDDY, A. R. 2016. Thraustochytrids as an alternative source of omega-3 fatty acids, carotenoids, and enzymes. *Lipid Technology,* 28**,** 68-70.

BYREDDY, A. R., BARROW, C. J. & PURI, M. 2016. Bead milling for lipid recovery from thraustochytrid cells and selective hydrolysis of Schizochytrium DT3 oil using lipase. *Bioresource Technology,* 200**,** 464-469.

CAN KARACA, A., LOW, N. & NICKERSON, M. 2013. Encapsulation of flaxseed oil using a benchtop spray dryer for legume protein–maltodextrin microcapsule preparation. *Journal of Agricultural and Food Chemistry,* 61**,** 5148-5155.

CARNEIRO, H. C., TONON, R. V., GROSSO, C. R. & HUBINGER, M. D. 2013. Encapsulation efficiency andoxidative stability of flaxseed oil microencapsulated by spray drying using different combinations of wall materials. *Journal of Food Engineering,* 115**,** 443-451.

CHAN, E.-S. 2011. Preparation of Ca-alginate beads containing high oil content: Influence of process variables on encapsulation efficiency and bead properties. *Carbohydrate Polymers,* 84**,** 1267-1275.

CHANG, C. & NICKERSON, M. T. 2018. Encapsulation of omega 3-6-9 fatty acids-rich oils using protein-based emulsions with spray drying. *Journal of Food Science and Technology***,** 1-12.

CHANG, C., VARANKOVICH, N. & NICKERSON, M. 2016. Microencapsulation of canola oil by lentil protein isolate-based wall materials. *Food Chemistry,* 212**,** 264-273.

CHANG, K. J. L., DUNSTAN, G. A., ABELL, G. C., CLEMENTSON, L. A., BLACKBURN, S. I., NICHOLS, P. D. & KOUTOULIS, A. 2012. Biodiscovery of new Australian thraustochytrids for the production of biodiesel and long-chain omega-3 oils. *Applied Microbiology and Biotechnology,* 93**,** 2215-2231.

CHANG, K. J. L., MANSOUR, M. P., DUNSTAN, G. A., BLACKBURN, S. I., KOUTOULIS, A. & NICHOLS, P. D. 2011. Odd-chain polyunsaturated fatty acids in thraustochytrids. *Phytochemistry,* 72**,** 1460-1465.

CHATTERJEE, S. & JUDEH, Z. M. 2015. Encapsulation of fish oil with N-stearoyl O-butylglyceryl chitosan using membrane and ultrasonic emulsification processes. *Carbohydrate polymers,* 123**,** 432-442.

CHEN, W., WANG, H., ZHANG, K., GAO, F., CHEN, S. & LI, D. 2016. Physicochemical properties and storage stability of microencapsulated DHA-rich oil with different wall materials. *Applied Biochemistry and Biotechnology,* 179**,** 1129-1142.

COUSTETS, M., JOUBERT-DURIGNEUX, V., HÉRAULT, J., SCHOEFS, B., BLANCKAERT, V., GARNIER, J.-P. & TEISSIÉ, J. 2015. Optimization of protein electroextraction from microalgae by a flow process. *Bioelectrochemistry,* 103**,** 74-81.

CUELLAR-BERMUDEZ, S. P., AGUILAR-HERNANDEZ, I., CARDENAS-CHAVEZ, D. L., ORNELAS-SOTO, N., ROMERO-OGAWA, M. A. & PARRA-SALDIVAR, R. 2015. Extraction and purification of high-value

metabolites from microalgae: essential lipids, astaxanthin and phycobiliproteins. *Microbial Biotechnology,* 8**,** 190-209.

DE JESÚS BONILLA-AHUMADA, F., KHANDUAL, S. & DEL CARMEN LUGO-CERVANTES, E. 2018. Microencapsulation of algal biomass (Tetraselmis chuii) by spray-drying using different encapsulation materials for better preservation of beta-carotene and antioxidant compounds. *Algal research,* 36**,** 229-238.

DESAI, K. G. H. & JIN PARK, H. 2005. Recent developments in microencapsulation of food ingredients. *Drying Technology,* 23**,** 1361-1394.

DRUSCH, S. 2007. Sugar beet pectin: A novel emulsifying wall component for microencapsulation of lipophilic food ingredients by spray-drying. *Food Hydrocolloids,* 21**,** 1223-1228.

ERATTE, D., WANG, B., DOWLING, K., BARROW, C. J. & ADHIKARI, B. P. 2014. Complex coacervation with whey protein isolate and gum arabic for the microencapsulation of omega-3 rich tuna oil. *Food & Function,* 5**,** 2743-2750.

FE NE MA, O., SRI, N. & PARKIN, K. 2007. Food Che mis try. 4th Edi tion. Ed. CRC Press. New York.

GHARSALLAOUI, A., ROUDAUT, G., CHAMBIN, O., VOILLEY, A. & SAUREL, R. 2007. Applications of spray-drying in microencapsulation of food ingredients: An overview. *Food Research International,* 40**,** 1107-1121.

GHASEMI, S. & ABBASI, S. 2014. Formation of natural casein micelle nanocapsule by means of pH changes and ultrasound. *Food Hydrocolloids,* 42**,** 42-47.

GHEYSEN, L., BERNAERTS, T., BRUNEEL, C., GOIRIS, K., VAN DURME, J., VAN LOEY, A., DE COOMAN, L. & FOUBERT, I. 2018. Impact of processing on n-3 LC-PUFA in model systems enriched with microalgae. *Food Chemistry,* 268**,** 441-450.

GOUIN, S. 2004. Microencapsulation: an industrial appraisal of existing technologies and trends. *Trends in Food Science & Technology,* 15**,** 330-347.

GUPTA, A., ABRAHAM, R. E., BARROW, C. J. & PURI, M. 2015. Omega-3 fatty acid production from enzyme saccharified hemp hydrolysate using a novel marine thraustochytrid strain. *Bioresource Technology,* 184**,** 373-378.

GUPTA, A., BARROW, C. J. & PURI, M. 2012. Omega-3 biotechnology: Thraustochytrids as a novel source of omega-3 oils. *Biotechnology Advances,* 30**,** 1733-1745.

GUPTA, A., SINGH, D., BARROW, C. J. & PURI, M. 2013a. Exploring potential use of Australian thraustochytrids for the bioconversion of glycerol to omega-3 and carotenoid production. *Biochemical Engineering Journal,* 78**,** 11-17.

GUPTA, A., SINGH, D., BYREDDY, A. R., THYAGARAJAN, T., SONKAR, S. P., MATHUR, A. S., TULI, D. K., BARROW, C. J. & PURI, M. 2016. Exploring omega-3 fatty acids, enzymes, and biodiesel producing thraustochytrids from Australian and Indian marine biodiversity. *Biotechnology Journal,* 11**,** 345-355.

GUPTA, A., WILKENS, S., ADCOCK, J. L., PURI, M. & BARROW, C. J. 2013b. Pollen baiting facilitates the isolation of marine thraustochytrids with potential in omega-3 and biodiesel production. *Journal Of Industrial Microbiology & Biotechnology,* 40**,** 1231-1240.

JAFARI, S. M., HE, Y. & BHANDARI, B. 2007. Role of powder particle size on the encapsulation efficiency of oils during spray drying. *Drying Technology,* 25**,** 1081-1089.

JANG, S. H., JEONG, H. J. & KWON, J. E. 2017. High contents of eicosapentaenoic acid and docosahexaenoic acid in the mixotrophic dinoflagellate Paragymnodinium shiwhaense and identification of putative omega-3 biosynthetic genes. *Algal Research,* 25**,** 525-537.

JANSEN-ALVES, C., FERNANDES, K. F., CRIZEL-CARDOZO, M. M., KRUMREICH, F. D., BORGES, C. D. & ZAMBIAZI, R. C. 2018. Microencapsulation of propolis in protein matrix using spray drying for application in food systems. *Food and Bioprocess Technology,* 11**,** 1422-1436.

JOE, M.-H., KIM, J.-Y., LIM, S., KIM, D.-H., BAI, S., PARK, H., LEE, S. G., HAN, S. J. & CHOI, J.-I. 2015. Microalgal lipid production using the hydrolysates of rice straw pretreated with gamma irradiation and alkali solution. *Biotechnology For Biofuels,* 8**,** 125.

JÓNASDÓTTIR, S. H. 2019. Fatty Acid Profiles and Production in Marine Phytoplankton. *Marine Drugs,* 17**,** 151.

KALIDASAN, K., SUNIL, K., NARENDRAN, R. & KATHIRESAN, K. 2015. Antioxidant activity of mangrove-derived marine thraustochytrids. *Mycosphere,* 6**,** 602-611.

KATZ, Y., RAJUAN, N., GOLDBERG, M., COHEN, A., HEYMAN, E. & LESHNO, M. 2009. Avoidance of early regular exposure to cow's milk protein is a major risk factor for the development of Ige mediated cow's milk allergy (cma). *Journal of Allergy and Clinical Immunology,* 123**,** S211.

KAUSHIK, P., DOWLING, K., BARROW, C. J. & ADHIKARI, B. 2015. Microencapsulation of omega-3 fatty acids: A review of microencapsulation and characterization methods. *Journal of Functional Foods,* 19**,** 868-881.

KIM, S. M., JUNG, Y.-J., KWON, O.-N., CHA, K. H., UM, B.-H., CHUNG, D. & PAN, C.-H. 2012. A potential commercial source of fucoxanthin extracted from the microalga Phaeodactylum tricornutum. *Applied Biochemistry and Biotechnology,* 166**,** 1843-1855.

KOÇ, M., GÜNGÖR, Ö., ZUNGUR, A., YALÇıN, B., SELEK, İ., ERTEKIN, F. K. & ÖTLES, S. 2015. Microencapsulation of extra virgin olive oil by spray drying: effect of wall materials composition, process conditions, and emulsification method. *Food and Bioprocess Technology,* 8**,** 301-318.

KUHN, K. R., E SILVA, F. G. D., NETTO, F. M. & DA CUNHA, R. L. 2019. Production of whey protein isolate–gellan microbeads for encapsulation and release of flaxseed bioactive compounds. *Journal of Food Engineering,* 247**,** 104-114.

LI, Z. Q., HOU, L. D., LI, Z., ZHENG, W. & LI, L. Study on shape optimization of calcium–alginate beads. Advanced Materials Research, 2013. Trans Tech Publ, 125-130.

LIN, S.-F., CHEN, Y.-C., CHEN, R.-N., CHEN, L.-C., HO, H.-O., TSUNG, Y.-H., SHEU, M.-T. & LIU, D.-Z. 2016. Improving the stability of astaxanthin by microencapsulation in calcium alginate beads. *PloS One,* 11**,** e0153685.

LIVNEY, Y. D. 2010. Milk proteins as vehicles for bioactives. *Current Opinion in Colloid & Interface Science,* 15**,** 73-83.

LOWREY, J., ARMENTA, R. E. & BROOKS, M. S. 2016. Recycling of lipid-extracted hydrolysate as nitrogen supplementation for the production of thraustochytrid biomass. *Journal of Industrial Microbiology & Biotechnology,* 43**,** 1105-1115.

MAKRI, A., BELLOU, S., BIRKOU, M., PAPATREHAS, K., DOLAPSAKIS, N. P., BOKAS, D., PAPANIKOLAOU, S. & AGGELIS, G. 2011. Lipid synthesized by micro-algae grown in laboratory-and industrial-scale bioreactors. *Engineering in Life Sciences,* 11**,** 52-58.

MENG, Y. & CLOUTIER, S. 2014. Gelatin and other proteins for microencapsulation. *Microencapsulation in the food industry.* Elsevier.

MOZURAITYTE, R., KRISTINOVA, V., STANDAL, I. B., EVENSEN, H. & RUSTAD, T. 2017. Applicability of traditional and advanced methods for oxidative quality and stability on marine phospholipids. *European Journal of Lipid Science and Technology,* 119**,** 1600103.

NG, S. K., JESSIE, L. Y. L., TAN, C. P., LONG, K. & NYAM, K. L. 2013. Effect of accelerated storage on microencapsulated kenaf seed oil. *Journal of the American Oil Chemists' Society,* 90**,** 1023-1029.

NGUYEN, H. C., SU, C.-H., YU, Y.-K. & HUONG, D. T. M. 2018. Sugarcane bagasse as a novel carbon source for heterotrophic cultivation of oleaginous microalga Schizochytrium sp. *Industrial Crops and Products,* 121**,** 99-105.

OKUDA, T., ANDO, A., SAKURADANI, E. & OGAWA, J. 2013. Isolation and Characterization of a Docosahexaenoic Acid-Phospholipids Producing Microorganism Crypthecodinium sp. D31. *Journal of the American Oil Chemists' Society,* 90**,** 1837-1844.

PARTANEN, R., RAULA, J., SEPPANEN, R., BUCHERT, J., KAUPPINEN, E. & FORSSELL, P. 2008. Effect of relative humidity on oxidation of flaxseed oil in spray dried whey protein emulsions. *Journal of Agricultural and Food Chemistry,* 56**,** 5717-5722.

PATIL, V., KÄLLQVIST, T., OLSEN, E., VOGT, G. & GISLERØD, H. R. 2007. Fatty acid composition of 12 microalgae for possible use in aquaculture feed. *Aquaculture International,* 15**,** 1-9.

PEANPARKDEE, M., IWAMOTO, S. & YAMAUCHI, R. 2016. Microencapsulation: A review of applications in the food and pharmaceutical industries. *Reviews in Agricultural Science,* 4**,** 56-65.

PEPPAS, N. A. & KHARE, A. R. 1993. Preparation, structure and diffusional behavior of hydrogels in controlled release. *Advanced Drug Delivery Reviews,* 11**,** 1-35.

PRASAD, E. 2016. Omega-3 Market to Reach $6,955 Million, Globally, by 2022

Allied Market Research.

REN, M. & OGDEN, K. 2014. Cultivation of Nannochloropsis gaditana on mixtures of nitrogen sources. *Environmental Progress & Sustainable Energy,* 33**,** 551-555.

RYCKEBOSCH, E., BRUNEEL, C., MUYLAERT, K. & FOUBERT, I. 2012. Microalgae as an alternative source of omega-3 long chain polyunsaturated fatty acids. *Lipid Technology,* 24**,** 128-130.

SAHU, A., PANCHA, I., JAIN, D., PALIWAL, C., GHOSH, T., PATIDAR, S., BHATTACHARYA, S. & MISHRA, S. 2013. Fatty acids as biomarkers of microalgae. *Phytochemistry,* 89**,** 53-58.

SCHEFFLER, S. L., WANG, X., HUANG, L., SAN-MARTIN GONZALEZ, F. & YAO, Y. 2009. Phytoglycogen octenyl succinate, an amphiphilic carbohydrate nanoparticle, and ε-polylysine to improve lipid oxidative stability of emulsions. *Journal of Agricultural and Food Chemistry,* 58**,** 660-667.

SCHRIEBER, R. & GAREIS, H. 2007. *Gelatine handbook: theory and industrial practice*, John Wiley & Sons.

SINGH, D., BARROW, C. J., PURI, M., TULI, D. K. & MATHUR, A. S. 2016. A combination of calcium and magnesium ions prevents substrate inhibition and promotes biomass and lipid production in thraustochytrids under higher glycerol concentration. *Algal Research,* 15**,** 202-209.

SINGHASUWAN, S., CHOORIT, W., SIRISANSANEEYAKUL, S., KOKKAEW, N. & CHISTI, Y. 2015. The carbon-to-nitrogen ratio affects the biomass composition and the fatty acid profile of heterotrophically grown Chlorella sp. TISTR 8990 for biodiesel production. *Journal of biotechnology,* 216**,** 169-177.

SIOW, L.-F. & ONG, C.-S. 2013. Effect of pH on garlic oil encapsulation by complex coacervation. *J. Food Process. Technol,* 4**,** 199-204.

SPRAGUE, M., DICK, J. R. & TOCHER, D. R. 2016. Impact of sustainable feeds on omega-3 long-chain fatty acid levels in farmed Atlantic salmon, 2006–2015. *Scientific Reports,* 6**,** 21892.

SUN, D., ZHANG, Z., MAO, X., WU, T., JIANG, Y., LIU, J. & CHEN, F. 2017. Light enhanced the accumulation of total fatty acids (TFA) and docosahexaenoic acid (DHA) in a newly isolated heterotrophic microalga Crypthecodinium sp. SUN. *Bioresource Technology,* 228**,** 227-234.

TALEBI, A. F., MOHTASHAMI, S. K., TABATABAEI, M., TOHIDFAR, M., BAGHERI, A., ZEINALABEDINI, M., MIRZAEI, H. H., MIRZAJANZADEH, M., SHAFAROUDI, S. M. & BAKHTIARI, S. 2013. Fatty acid profiling: a selective criterion for screening microalgae strains for biodiesel production. *Algal Research,* 2**,** 258-267.

TAMM, F., HÄRTER, C., BRODKORB, A. & DRUSCH, S. 2016a. Functional and antioxidant properties of whey protein hydrolysate/pectin complexes in emulsions and spray-dried microcapsules. *LWT,* 73**,** 524-527.

TAMM, F., HERBST, S., BRODKORB, A. & DRUSCH, S. 2016b. Functional properties of pea protein hydrolysates in emulsions and spray-dried microcapsules. *Food Hydrocolloids,* 58**,** 204-214.

TANG, C.-H. & LI, X.-R. 2013. Microencapsulation properties of soy protein isolate Influence of preheating and/or blending with lactose. *Journal of Food Engineering,* 117**,** 281-290.

TONON, R. V., GROSSO, C. R. & HUBINGER, M. D. 2011. Influence of emulsion composition and inlet air temperature on the microencapsulation of flaxseed oil by spray drying. *Food Research International,* 44**,** 282-289.

UGALDE, V., ARMENTA, R. E., KERMANSHAHI-POUR, A., SUN, Z., BERRYMAN, K. T. & BROOKS, M. S. 2018. Improvement of culture conditions for cell biomass and fatty acid production by marine thraustochytrid F24-2. *Journal of Applied Phycology,* 30**,** 329-339.

VAN WAGENEN, J., MILLER, T. W., HOBBS, S., HOOK, P., CROWE, B. & HUESEMANN, M. 2012. Effects of light and temperature on fatty acid production in Nannochloropsis salina. *Energies,* 5**,** 731-740.

VELOZA, A. J., CHU, F.-L. E. & TANG, K. W. 2006. Trophic modification of essential fatty acids by heterotrophic protists and its effects on the fatty acid composition of the copepod Acartia tonsa. *Marine Biology,* 148**,** 779-788.

WANG, B., ADHIKARI, B. & BARROW, C. J. 2014. Optimisation of the microencapsulation of tuna oil in gelatin–sodium hexametaphosphate using complex coacervation. *Food Chemistry,* 158**,** 358-365.

WU, K. G. & XIAO, Q. 2005. Microencapsulation of fish oil by simple coacervation of hydroxypropyl methylcellulose. *Chinese Journal of Chemistry,* 23**,** 1569-1572.

XIA, Q., AKANBI, T. O., WANG, B., LI, R., YANG, W. & BARROW, C. J. 2019. Investigating the Mechanism for the Enhanced Oxidation Stability of Microencapsulated Omega-3 Concentrates. *Marine Drugs,* 17**,** 143.

XIA, Q., WANG, B., AKANBI, T. O., LI, R., YANG, W., ADHIKARI, B. & BARROW, C. J. 2017. Microencapsulation of lipase produced omega-3 concentrates resulted in complex coacervates with unexpectedly high oxidative stability. *Journal of Functional Foods,* 35**,** 499-506.

XIE, Y., SEN, B. & WANG, G. 2017. Mining terpenoids production and biosynthetic pathway in thraustochytrids. *Bioresource Technology,* 244**,** 1269-1280.

YANG, H.-L., LU, C.-K., CHEN, S.-F., CHEN, Y.-M. & CHEN, Y.-M. 2010. Isolation and characterization of Taiwanese heterotrophic microalgae: screening of strains for docosahexaenoic acid (DHA) production. *Marine Biotechnology,* 12**,** 173-185.

ZHANG, B.-B., WANG, L., CHARLES, V. R., ROOKE, J. C. & SU, B.-L. 2016a. Robust and biocompatible hybrid matrix with controllable permeability for microalgae encapsulation. *ACS Applied Materials & Interfaces,* 8**,** 8939-8946.

ZHANG, K., ZHANG, H., HU, X., BAO, S. & HUANG, H. 2012. Synthesis and release studies of microalgal oil-containing microcapsules prepared by complex coacervation. *Colloids and surfaces B: Biointerfaces,* 89**,** 61-66.

ZHANG, Z., ZHANG, R., CHEN, L., TONG, Q. & MCCLEMENTS, D. J. 2015. Designing hydrogel particles for controlled or targeted release of lipophilic bioactive agents in the gastrointestinal tract. *European Polymer Journal,* 72**,** 698-716.

ZHANG, Z., ZHANG, R. & MCCLEMENTS, D. J. 2016b. Encapsulation of β-carotene in alginate-based hydrogel beads: Impact on physicochemical stability and bioaccessibility. *Food Hydrocolloids,* 61**,** 1-10.

ZHAO, X.-H. & TANG, C.-H. 2016. Spray-drying microencapsulation of CoQ10 in olive oil for enhanced water dispersion, stability, and bioaccessibility: Influence of type of emulsifiers and/or wall materials. *Food Hydrocolloids,* 61**,** 20-30.

ZHOU, P. P., LU, M. B., LI, W. & YU, L. J. 2010. Microbial production of docosahexaenoic acid by a low temperature-adaptive strain Thraustochytriidae sp. Z105: screening and optimization. *Journal of Basic Microbiology,* 50**,** 380-387.