Controlled release of microencapsulated docosahexaenoic acid (DHA) by spray–drying processing

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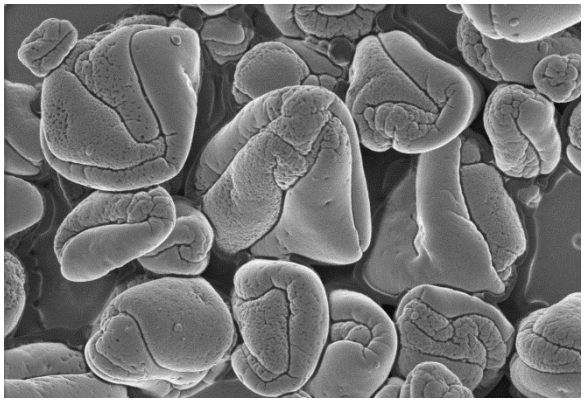
Graphical abstract



Highlights

- First study to consider encapsulation of DHA in complementary food
- Microencapsulation of fish oil using a pH dependant release polymer
- Oxidation protection of encapsulated fish oil
- Direct spray drying compared to solid lipid nanoparticle formulation of DHA

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Abstract

The omega-3-fatty acid, docosahexaenoic acid (DHA) 22:6 *n*-3, is an important food component for the visual and brain development of infants. In this study two approaches have been explored for the encapsulation of DHA in the pH dependant polymer hydroxyl-propyl-methyl-cellulose-acetate-succinate (HPMCAS). In the first approach Direct Spray Drying (DSD) was implemented for the microencapsulation of DHA/HPMCAS organic solutions, whilst in the second approach solid lipid nanoparticle (SLN) dispersions of DHA, were first produced by high-pressure homogenization, prior to being spray dried in HPMCAS aqueous solutions. The DSD approach resulted in significantly higher quantities of DHA being encapsulated, at 2.09 g/100 g compared to 0.60 g/100 g in the spray-dried SLNs. The DHA stability increased with the direct spray-drying approach. Release studies of DHA in the direct sprayed dried samples revealed a lag time for 2 hours in acidic media followed by rapid release in phosphate buffer (pH 6.8).

Keywords

Nutraceuticals; Fish oil microencapsulation; Hydroxy propyl methyl cellulose acetate succinate (HPMCAS); Docosahexaenoic acid (DHA); Spray-drying; Solid Lipid Nanoparticle (SLN)

1. Introduction

The interest in marine oils originally stemmed from epidemiological studies of Greenland Eskimos, where cardiovascular disease risk was lower compared to a Danish population, despite high fat intake, this was thought to be due to their diets being rich in *n*-3 polyunsaturated

fatty acids from fish and seal meat (Cui et al., 2006). Docosahexaenoic acid (DHA) 22:6 *n*-3 is the most abundant *n*-3 fatty acid in the central nervous system and is specifically concentrated within membrane lipids of the grey matter within the brain and the visual elements of the retina. Some intervention and epidemiological studies have associated low plasma and blood cell lipid DHA concentrations with increased risk of poor visual and neural development in infants (Innis, 2008).

In the western diet the consumption of *n*-3 LCPUFA (Long Chain Polyunsaturated Fatty Acid), eicosapentaenoic acid (EPA) 20:5 *n*-3 and DHA, is below recommended levels. Furthermore conversion in the body from the precursor α-linoleic acid (18:3 *n*-3) does not appear to overcome this deficiency (Vongsvivut et al., 2012). A preliminary study to evaluate the total daily intake of essential fatty acids of an infant's diet based on the consumption of fortified infant formula and commercial 'ready-to-feed' complementary infant foods in the UK indicates that infants are not meeting the requirements for LCPUFA, in particular DHA (Loughrill et al., 2016). This may have implications for brain and visual development, as well as inflammatory and immune functions. Furthermore due to there being limited food sources rich in DHA, infant food products may need to be fortified to meet current legislative requirements (Innis, 2008).

Fish oil is a rich source of *n*-3 LCPUFA, especially DHA and EPA, and is currently the main commercial source for nutritional supplements and food fortification of these LCPUFA (Vongsvivut et al., 2012). However LCPUFA are highly susceptible to oxidative deterioration during food processing and storage, which limits their use in food products due to off-flavour and odour production. In addition, the primary oxidation products, hydroperoxides, are considered to be toxic (Kagami et al., 2003). Microencapsulation can be used as a method to protect the LCPUFA against oxidation so as to enable their successful incorporation into food products (Vongsvivut et al., 2012).

Encapsulation is a rapidly developing area of technology with great potential in the food industry, where spray drying is a common technique used to encapsulate oils (Jafari *et al*., 2008). Furthermore microencapsulation of materials that are susceptible to oxidation have been shown to significantly hinder oxidation (Kagami et al., 2003). In particular for fish oils encapsulation can also offer improved handling properties of the oil, allow easy storage and mask the taste or odour of the core to offer improved palatability of the product (Botrel et al., 2014).

Fish oils have been encapsulated using a range of technologies, including fluidized bed coating, spray dried emulsions and single and multi-core complex coacervation (Vongsvivut et al., 2012). Spray drying is the most commonly used technology in the food industry due to low cost and ready availability of equipment (Aghbashlo et al., 2012) and has therefore been used in this study for the microencapsulation of fish oil.

Solid Lipid Nanoparticles (SLN) have attracted increased attention as a carrier system for both pharmaceutical drugs and nutraceuticals, due to their controlled drug delivery and bioavailability enhancement properties. High-pressure homogenisation, is a robust technique for nanoparticle preparation (Potta et al., 2010; 2011) and it has proven beneficial in comparison to solvent evaporation methods as they do not require the use of organic solvents (Patil et al., 2014). Therefore, high pressure homogenisation will be employed for the production of SLN in this study.

Many of the spray dried fish oil formulations on the market and in the literature use water soluble polymers as wall materials, which are not compatible with aqueous based food products, as the fish oil would be released from the microparticles within the food product prior to consumption. Hydroxy propyl methyl cellulose acetate succinate (HPMCAS) is a water insoluble polymer, which is available in three commercially available grades (L, M and H

grades) which differ in their acetyl and succinoyl content and vary in their pH solubility. It was originally developed as an enteric polymer for aqueous dispersion coating. The enteric coating prevents dissolution of the core in acidic pH **(**Sarode et al., 2014). In addition to the polymer being water insoluble to prevent fish oil release in aqueous based food products, HPMCAS also offers encapsulation of the fish oil under certain pH conditions. In particular to this study, selected infant foods are typically within the pH range of 3-4; therefore the fish oil will remain encapsulated in the food product and be released within the intestine for absorption after consumption. Therefore HPMCAS has been employed as the polymer for coating in this study.

Here we present a two-fold study, aiming to encapsulate a fish oil product in HPMCAS by using two different microencapsulation approaches (Loughrill, 2015). The first approach involved DSD of the fish oil /HPMCAS organic solution, whilst the second approach included microfluidization of lipid formulations to form nano-emulsions which subsequently were spray dried in HPMCAS aqueous solutions. Both methodologies were evaluated in terms of DHA content, solid yield % and DHA encapsulation efficiency in order to determine the most appropriate method of production. Finally the spray dried material produced by the selected production methodology was taken forward for further characterisation. In addition, the stability of the encapsulated DHA has been compared to its plain fish oil counterpart and pH dependant release of DHA will be assessed using a dissolution methodology (Loughrill, 2015).

2. Materials and Methods

Fish oil (Baby's DHA) was purchased from Nordics Naturals (Watsonville, California); containing 9 kcal, 1 g of fat, 210 mg of omega 3 fatty acids, 70 mg of EPA and 97 mg of DHA per mL of fish oil. The polymer hydroxy propyl methyl cellulose acetate succinate (HPMCAS) AS-LG was supplied by Shin-Etsu Chemical Co., Ltd (Tokyo, Japan). Tristearin and

Poloxamer 188 solution (Pluronic F68) were purchased from Sigma Aldrich (Bellefonte, PA). Acetone, methanol and hexane (HPLC grade) were purchased from Fisher UK. All other general chemicals used in this study were of analytical grade.

2.1. Direct Spray Drying of DHA-HPMCAS microparticles

5.0 g of the polymer HPMCAS was added to 250 mL of acetone/methanol (5:1, v/v) whilst stirring until completely dissolved. Fish oil (1.5 g) was then progressively added to the polymer solution while stirring to make feed solutions. Scaled up DHA/HPMCAS microparticles were produced using a Buchi mini spray dryer (B-290, Switzerland). Operating parameters were: inlet temperature, 78°C; feed rate 17%; aspirator, 85%; compressed air, 40%. In order to provide enough volume for characterisation, this procedure was scaled up by the magnitude of 7.

2.2. Preparation and Spray Drying of Solid Lipid Nanoparticles (SLN)

The lipid (tristearin) phase (1 g) was fully melted at 75°C and dispersed with heated fish oil (2 mL). The aqueous surfactant solution was prepared by adding surfactant (Pluronic F68, 0.5 g) to 50 mL pre-heated deionised water (75°C). A pre-emulsion was formed by mixing the hot lipid phase with the aqueous surfactant solution and held at 75°C using an IKA® T25 digital Ultra Turrax® (Germany) at 18, 000 rpm for 5 min. The crude dispersion was processed through a Micro DeBEE (B.E.E. International Inc. Easton, MA) high pressure homogenizer at 75°C and 15,000 psi for 10 minutes. The Micro DeBEE was heated (75°C) prior to homogenisation. The emulsion was left to cool down at room temperature whilst stirring. The produced SLN were stored at 5°C prior to spray drying (Loughrill, 2015).

The polymer (HPMCAS, 2.5g) was dissolved in 200 mL of deionised water, whilst stirring and the pH was adjusted to 6.5 using sodium hydroxide, and then filtered to remove any remaining particulates. 14.5 mL of the fish oil SLN were added to the polymer solution, whilst stirring to

make feed solutions. SLN encapsulation in HPMCAS was achieved by using a Buchi mini spray dryer (B-290, Switzerland). Operating parameters were: inlet temperature, 160°C; feed rate, 7%; aspirator, 85%; compressed air, 40%.

The solid yield was calculated as the ratio of the encapsulated powder weight collected after every spray-drying experiment to the initial amount of solids in the sprayed dispersion volume (Martinez et al., 2015).

The solid yield (Y, %) was calculated as follows:

Y = Collected encapsulated powder weight (g)/ initial amount of solids (g) x 100

2.3. Fatty acid composition and efficiency of encapsulated powders

The two encapsulated powders produced by the different methodologies were analysed by ILS laboratories (Derbyshire, UK) for fatty acid composition. Briefly, after the removal of the extracting solvent, the extracted fat is saponified with methanolic sodium hydroxide, to form soaps with the liberated fatty acids. Upon acidification, the free acids undergo an acid catalysed esterification reaction to form methyl esters of the respective fatty acids. These methyl esters are finally extracted into hexane and the fatty acid profile is determined using capillary gas chromatography using a flame ionisation detector.

Encapsulation efficiency (EE) is a significant indicator to appraise the quality of the encapsulated products (Qv et al., 2011). A more common way to describe encapsulation efficiency of microencapsulated essential oils in the food industry is the so-called non-encapsulated oil or extractable oil %, which is a key parameter to evaluate the quality of the capsules, because extractable oil is prone to oxidation and limits the shelf-life by rancidity development (Jin et al., 2008; Kralovec et al., 2012).

The EE of the DHA in this study was calculated indirectly using % of surface oil as follow (Aghbashlo et al., 2012):

$$EE = \frac{DHA_{encapsulated} - DHA_{surface}}{DHA_{initially\ added}} \times 100$$

2.4. Estimation of Surface Oil

The amount of surface oil (i.e. the free or non-encapsulated oil) on the encapsulated powder was immediately measured after production by spray drying. Hexane (15 mL) was added to 1.5 g of encapsulated powder followed by shaking of the mixture for 2 minutes. The suspension was then filtered through a Whatman no.1 filter paper, the residue was rinsed three times with 20 mL hexane. The filtrate solution containing the surface oil was then transferred to a Fistreem vacuum oven (UK) until evaporation of the solvent, finally for complete evaporation the residue was dried for 1 hour in a Thermo Scientific Heraeus® oven (Germany) at 70°C. The amount of surface oil was calculated by the difference in initial and final weights of slurry container (Aghbashlo et al., 2012; Carneiro et al., 2013).

The surface oil percentage was calculated as follows:

SO/TO x 100

TO: Total oil (g), SO: Surface oil (g)

2.5. Characterisation of the encapsulated powder

Following the evaluation of the two approaches to produce fish oil encapsulated powders based on solid yield %, DHA encapsulation efficiency and DHA content, the most appropriate methodology was selected (Loughrill, 2015) and the selected encapsulated powder characterised using the following techniques.

2.5.1. Moisture content

The moisture content of the encapsulated powder was determined using two methods, oven drying and thermal gravimetric analysis.

2.5.2. Oven Drying

Two grams of the generated encapsulated powder was dried in a Thermo Scientific Heraeus® oven (Germany) at 70°C for 24 hours (Aghbashlo et al., 2012). Weight measurements were performed using an AAA, 250 L balance (Mettler Toledo, China) with precision of ± 0.0001 g.

The moisture content was calculated as follows:

$$\text{Loss of drying} = \frac{\text{Wet weight of sample (g)} - \text{weight of sample after drying (g)}}{\text{Wet weight of sample (g)}} \times 100$$

2.5.3. Thermal gravimetric analysis (TGA)

The moisture content and physicochemical properties of the polymer (HPMCAS) and encapsulated powder samples were analysed using TA Instruments' TGA Q5000 (UK). Sample weight of 2.7 ± 0.6 mg was heated from ambient temperature to 600 °C in aluminium pans at 10 °C/min, under nitrogen atmosphere at a flow rate of 25 mL/min. Results were analysed using TA universal analysis software.

2.5.4. Water activity

The water activity of the encapsulated powder was measured using an Aqua Lab Dew Point water activity meter 4TE at 25 ± 0.5 °C (USA).

2.5.6. Scanning electron microscopy (SEM)

The encapsulated powder was sputter coated with chromium using a K575X Turbo-Pumped Chromium Sputter Coater (Quorum Technologies Ltd, UK). The morphological features were

observed using a Hitachi SU 830 cold-cathode Field Emission Gun Scanning Electron Microscope (Japan) with an accelerated voltage of 1 kV and magnification of 1000-20,000 x.

2.5.7. Particle size distribution (PSD)

The particle size distribution of the dry encapsulated material was measured using a Mastersizer 2000 (Malvern instruments) and results were analysed using Mastersizer 2000 analysis software.

2.5.8. X -ray powder diffraction (XRPD)

The crystalline state of HPMCAS and encapsulated powder was evaluated using a D8 Advance X-ray Diffractometer (Bruker, Germany) using Cu Kα radiation at 40 kV and 40 mA. Samples were analysed at angles from 3 to 40^{o} in 2θ with an increment of 0.03^{o} and a counting time of 0.5 seconds per step. Data was collected using DIFFRAC plus XRD Commander version 2.6.1 software (Bruker-AXS) (ICDD, 2008).

2.5.9. Fourier transform infrared (FTIR)

Fourier-transformed infrared (FT-IR) spectra of fish oil, HPMCAS, and produced encapsulated powder were obtained on a Spectrum Two FTIR spectrophotometer (Perkin Elmer, UK) coupled with an attenuated total reflectance (ATR) accessory (Perkin Elmer, UK). The crystal was washed with iso-propanol between each sampling. The background spectrum was obtained by measuring the empty chamber. A resolution of 4 cm^{-1} was used and the ATR spectra were averaged on 10 scans. The scanning range was 450 to 4000 cm^{-1}. Results were analysed using Perkin Elmer spectrum analysis software (Loughrill, 2015).

2.5.10. Differential Scanning Calorimetry (DSC)

The thermophysical properties of the fish oil, HPMCAS and the encapsulated powder were studied using TA Instruments' Q2000 DSC (UK) equipped with a refrigerated cooling system (RCS) under nitrogen atmosphere (50 mL/min) in hermetic aluminium pans with a single pin-hole in lid. 2.1 ± 0.5 mg of HPMCAS and encapsulated powder and 6.6 ± 0.7 mg of fish oil were cooled and held isothermal for 2 minutes at -90°C and heated to 100°C for fish oil and 200°C for HPMCAS and the encapsulated powder at 10°C/min. Results were analysed using TA universal analysis software (Loughrill, 2015).

2.5.11. Stability testing

For the stability tests an accelerated storage test was conducted to determine whether the microencapsulation process offers protection of DHA over its plain fish oil counterpart. A comparison between the loss of DHA from plain fish oil and microencapsulated fish oil were assessed. The encapsulated powder and un-encapsulated fish oil were sealed in brown glass containers and stored either at room temperature or at 40°C in a Thermo Scientific Heraeus® oven (Germany), in order to accelerate oxidation, for one week. After one week all samples were analysed for DHA content (Loughrill, 2015).

2.5.12. Dissolution

Dissolution testing of the encapsulated powder was carried out in pH 1.2 and pH 6.8 buffers. The dissolution was carried out at 37°C with a stirring speed of 50 rpm, by adding 5 g of the encapsulated powder into 500 mL of acidic dissolution medium (0.1 mol/L HCl) to mimic the stomach environment for 2 hours. Following this acidic stage, a buffer stage was employed consisting of a phosphate buffer at pH 6.8, prepared by mixing 0.1 M HCl with 0.20 M tribasic sodium sulphate (3:1) for a subsequent 1.5 hours to mimic the intestinal environment. Aliquots

were taken at 30 minute intervals and centrifuged at 3000 rpm to remove any encapsulated material and the supernatant was analysed for DHA content (Loughrill, 2015).

3. Results and Discussion

3.1. Yield and fatty acid composition of the encapsulated fish oil

Two different production methodologies were employed for the production of fish oil spray dried encapsulated powders. Firstly, SLN of encapsulated fish oil were subsequently spray dried in HPMCAS and secondly solid dispersions of fish oil and polymer (HPMCAS) were directly spray dried (DSD). Following production by spray drying solid yield % was calculated and the encapsulated powders were analysed for DHA content and determination of the DHA encapsulation efficiency. The concentration of DHA in the formulations was 0.60 g/100g in the SLN and 2.09 g/100g in the DSD, which shows that the concentration of DHA is 3.5 times greater in the directly spray dried production method compared to the spray dried nano-emulsion technique. Furthermore, the DHA encapsulation efficiency was greater in the DSD method compared to the SLN; 88% (DSD) 38% (SLN). Finally the DSD process offered higher yield production, 50-60% (DSD) 40-45% (SLN) (Loughrill, 2015).

3.2 Estimation of surface oil and Encapsulation Efficiency

The fish oil encapsulation efficiency was determined indirectly by extracting the surface oil (or the un-encapsulated oil). The DSD offered much greater DHA encapsulation efficiency, 88% (DSD), 38% (SLN). The percentage of surface oil of the encapsulated powder was also determined to be 12.4%. The lower DHA encapsulation efficiency of the SLN may be due to its poor drug loading capacity. Furthermore, production of SLN involved a multi-step, batch process, whereas directly spray drying provides a continuous process offering decreased production costs, space requirements, labour and resources (Patil et al., 2014).

3.3. Characterisation of the encapsulated powder

Due to these results the encapsulated powder produced by the directly spray dried production method was taken forward for further characterisation due to the fact that less of the microencapsulated material would need to be added to the final food product for fortification.

3.3.1 Moisture content

The moisture content of the encapsulated powder was determined to be 1.11% using the oven drying method (loss of drying) and 1.39% using TGA which is under the minimum specification of 3-4% for most dried powders used in the food industry (Klinkesorn et al., 2006). The moisture content (water content) of polymer HPMCAS determined by TGA was higher at 2.19%. Moisture content is well known to have a significant effect on the lipid stability. It has been observed that low water contents are usually associated with low water activities, which might prevent lipid oxidation (Klaypradit and Huang, 2008). The water activity (a_w) (amount of water available for biological reactions) of the encapsulated powder was found to be 0.324 a_w, which again is within the ideal range of 0.2–0.4 a_w for storage stability in lipid oxidation as reported elsewhere (Rockland and Beuchat, 1987; Rükold et al., 2001).

3.3.2. Scanning electron microscopy (SEM)

Fig. 1 illustrates the SEM images of the encapsulated powder, showing no apparent cracks or pores, which are necessary to protect the core material from oxygen and undesired release of oil droplets to the surface of particles, which is an advantage, since it suggests that capsules have lower permeability to gases, increasing protection and retention of the fish oil. In addition, the encapsulated powder appears wrinkled, with concave surfaces and in a variety of sizes which is typical of powders produced by spray-drying. This type of morphology has also been observed by Kolinowski et al. (2004) and Davidov-Pardo et al. (2008) when encapsulating fish

oils. The formation of hollow particles can be explained by the formation of a vacuole inside the particles, immediately after the crust development. This crust inflates when the particle temperature exceeds the local ambient boiling point and the vapour pressure within the vacuole rises above the local ambient pressure (Nijdam and Langrish, 2006). The lack of pores on the encapsulated powder surface was related to the good encapsulation efficiency obtained in the experimental design and the low surface oil content was a consequence of a continuous wall surface (Roccia et al., 2014).

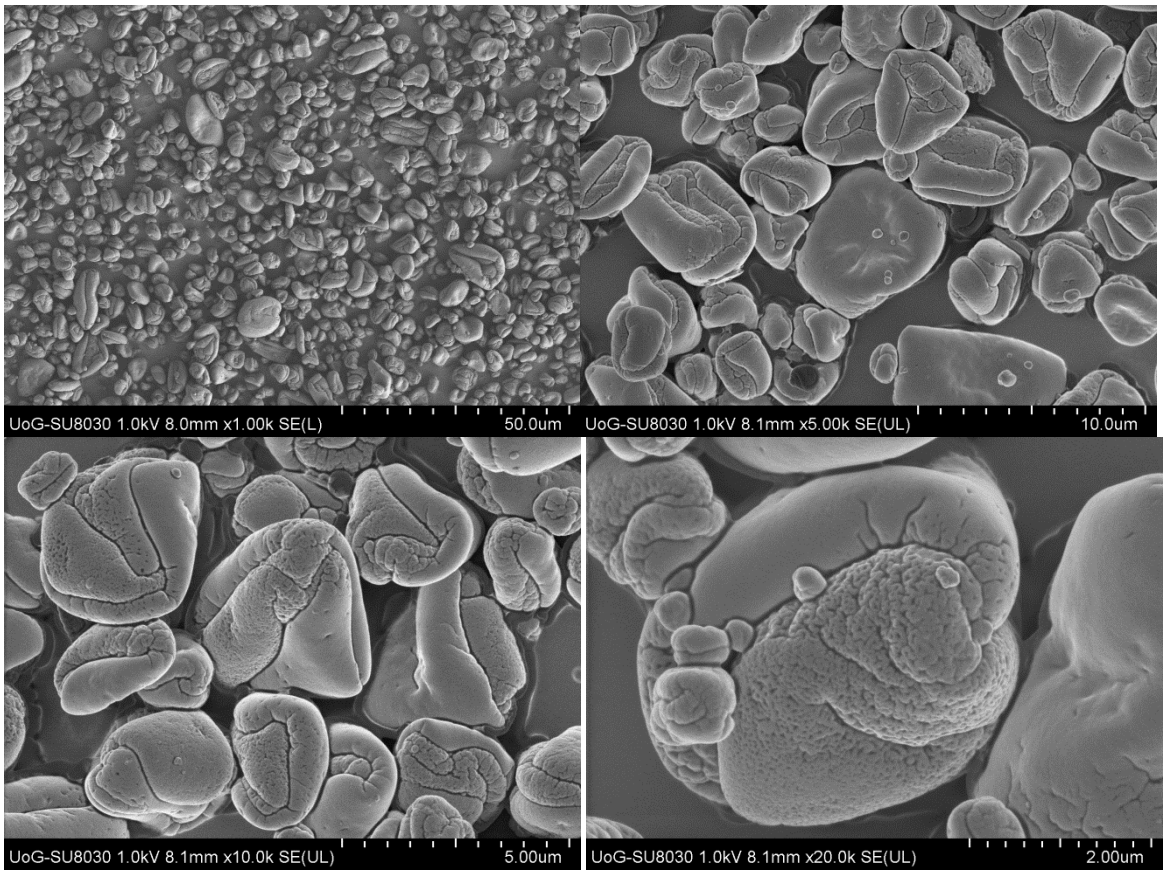

Figure 1. SEM micrographs of fish oil encapsulated powder using varying magnifications (1k – 20k x).

3.3.3. Particle size distribution (PSD)

The particle size distribution of the encapsulated powder was measured by laser diffraction analysis. The particle size varied from d(0.1) 3.366 μm to d(0.9) 18.204 μm, with a median particle size of d(0.5) 7.435 μm. The span value observed was 1.996, a relative span less than 2 is normally considered as a narrow distribution in spray drying (Gottlieb and Schwartzbach, 2004). A high span value would indicate a wide size distribution and a high polydispersity (Dubey and Parikh, 2004).

3.3.4. X ray powder diffraction (XRPD)

XRPD was applied to identify the degree of crystallinity in the polymer (HPMCAS) and encapsulated powder. In general, a crystalline material presents sharp peaks while amorphous products provide a broader peak pattern (Caparino et al., 2012). As shown in **Fig. 2**, the polymer HPMCAS and the encapsulated powder exhibited an amorphous structure with minimum organisation based on the occurrence of large diffuse peaks. It is known that amorphous solids are in general more soluble and more hygroscopic (Botrel et al., 2014). An amorphous nature dictates higher dissolution profiles as the amorphous form has a higher degree of disorder (higher free energy) (Al-Obaidi and Buckton, 2009).

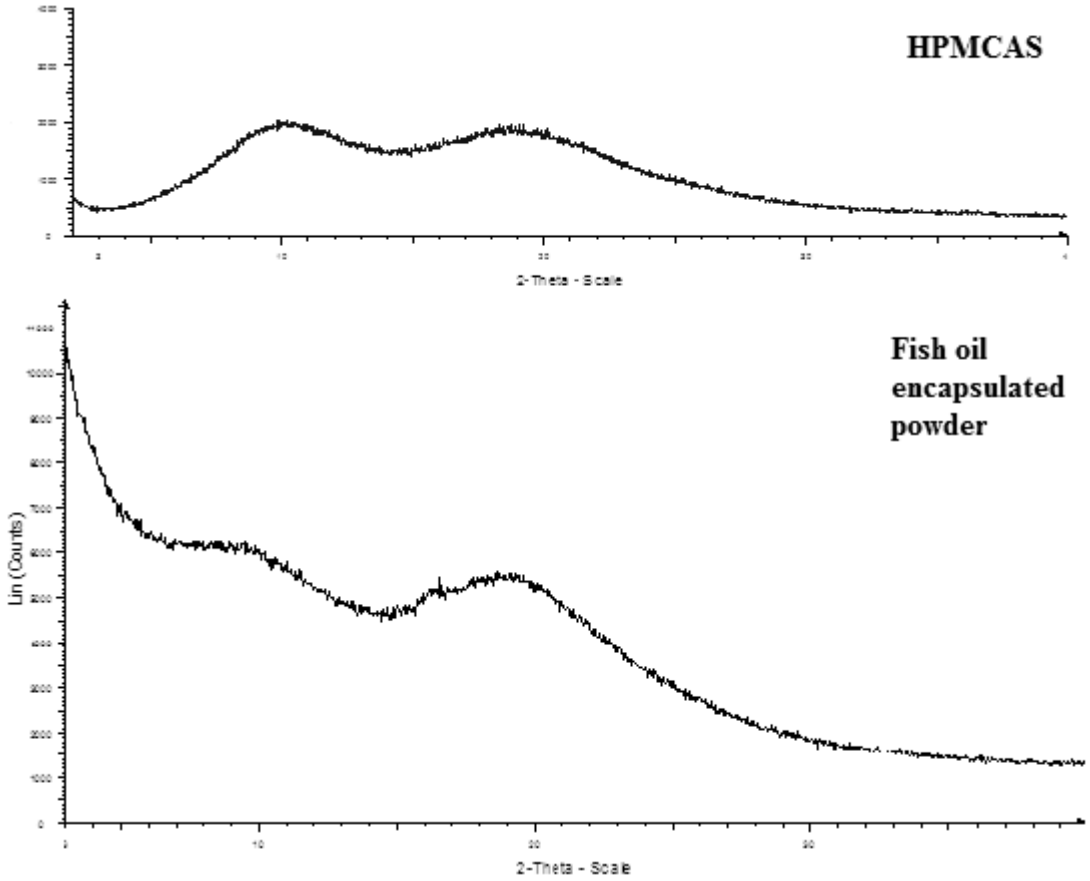

Figure 2. XRPD spectra of HPMCAS and fish oil encapsulated powder.

3.3.5. Fourier transform infrared (FTIR)

FTIR was used to explore fish oil-polymer interactions in the matrix of the encapsulated powder. IR spectra of fish oil, HPMCAS and the produced encapsulated powder are shown in **Fig. 3**.

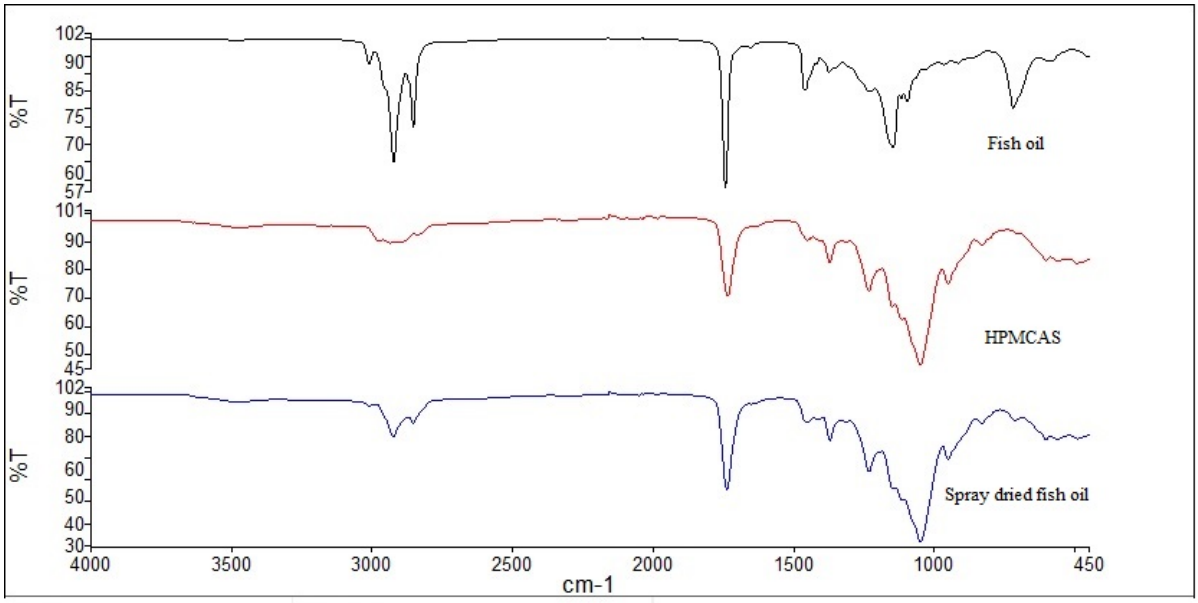

Figure 3. FTIR spectra of fish oil, HPMCAS and corresponding spray dried encapsulated powder.

Pure HPMCAS shows a strong absorption at 1738 cm^{-1} (C=O stretch), which is the same in the encapsulated powder spectrum. The absorption at ~3485 cm^{-1} shows broad O-H stretching.

A band at 3011 cm^{-1} in the fish oil is related to C-H stretch (cis-alkene HC=CH-) which specifically represents unsaturated fatty acids and triplet bands present within 3000-2800 cm^{-1} which are a feature of the C-H stretching modes of the methyl and methylene backbones of lipids. A sharp band a 1744 cm^{-1} is assignable to C=O stretches of ester functional groups from lipids and fatty acids. C-O stretching in the fish oil was observed at 1147 cm^{-1}. Additional bands relevant to lipids are those at 1458 cm^{-1}, 1147 cm^{-1} and 1097 cm^{-1} assigned to asymmetrical deformation scissor from methylene (-CH2), CH2 out-of-plane deformation modes and C-O-C symmetrical stretches respectively, these are mainly from triglycerides and cholesterol esters (Vongsvivut et al., 2012).

The FT-IR spectrum the encapsulated powder was approximately equal to the virtual mixture of initial component spectrums (fish oil and polymer HPMCAS). The nature of the peaks did not vary for the combined fish oil and polymer HPMCAS with the generated encapsulated powder, indicating the absence of any strong chemical interaction between fish oil and wall material.

3.3.6. Thermal analysis: DSC and TGA

DSC provides information about the physical behaviour of materials by detecting processes such as melting, solid-solid transition, dehydration and glass transitions, which is useful for understanding the physical nature of solids i.e. crystalline or amorphous, and how it is influenced by formulation and/or processing methods. Here DSC was employed to investigate the presence of fish oil in the encapsulated powder after spray-drying. A recent article demonstrates that HPMCAS undergoes cross-linking esterification between the pendent carboxyl and hydroxyl groups above 200°C (Li et al., 2013). For this reason the HPMCAS and the encapsulated powder were heated to below 200°C. The DSC results (**Fig. 4a**) show HPMCAS to undergo dehydration (removal of moisture) and a glass transition process at 68 ± 1°C and 133 ± 2°C respectively (**Table 1**). The fish oil undergoes two melting processes when heated between -90 and 100°C with peak temperatures at -51 ± 1°C and -15 ± 1°C. The encapsulated powder product exhibits the same processes detected in both starting materials (**Fig. 4a and Table 1**). This indicates the presence of both the fish oil and HPMCAS in their initial forms and confirms that no chemical reaction(s) or change in physical state occurs when these materials are combined and spray dried. The glass transition detected in DSC analysis supports the findings from XRPD studies in that the HPMCAS and the encapsulated powder product are amorphous. The results presented in **Table 1** show that the glass transition temperature (T_g) of the product (124 ± 1°C) is ~10°C lower than the pure HPMCAS (133 ±

2°C). The lower T_g detected for the product is due to the presence of the fish oil which plasticizes the HPMCAS.

Table 1: Mean temperatures at the peak maxima and energies associated with the processes detected in DSC analysis.

Sample	Melting process				Dehydration process		Glass transition	
	1		2					
	°C	ΔH (J/g)	°C	ΔH (J/g)	°C	ΔH (J/g)	°C	ΔC_p (J/(g.°C))
Fish oil	51±1	43±3	15±1	54±5	-	-	-	-
HPMCAS*	-	-	-	-	68±1	53±4	133±2	0.11±0.02
Product	56±1	4±1	14±1	6±1	67±1	42±2	12 ±1	0.08±0.01

*HPMCAS - hydroxyl-propyl-methyl-cellulose-acetate-succinate

4a

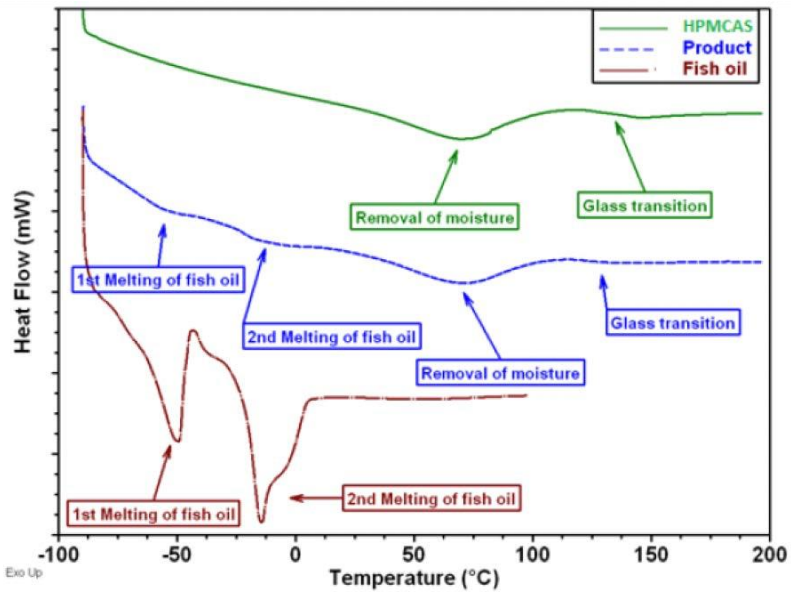

Figure 4a. Overlay of the DSC data observed for HPMCAS, fish oil and encapsulated powder product heated from -90 to 100˚C for fish oil and 200°C for HPMCAS and encapsulated powder product at 10˚C/min.

4b

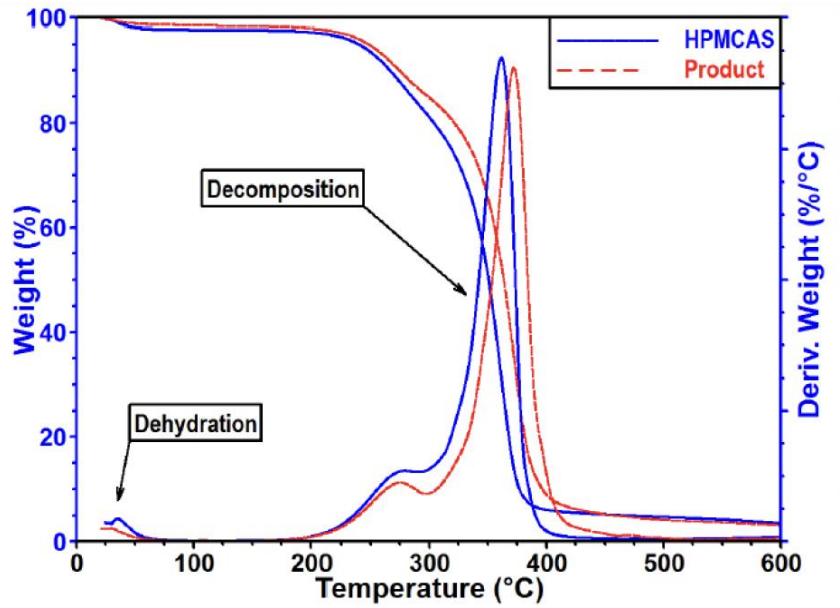

Figure 4b. TGA curve overlay of HPMCAS and encapsulated powder product heated from ambient temperature to 600°C at 10°C/min.

The TGA results for HPMCAS and the encapsulated powder product are presented in **Fig. 4b** and show that both the polymer and encapsulated powder product undergo the same three processes when heated from ambient temperature to 600°C with a total percentage weight loss in each sample of 96 ± 1 %. This demonstrates that no chemical changes of the starting materials occurred during the formulation. The 1st weight loss process (below 100°C) is a dehydration process in which 2.2 ± 0.1 % of moisture is removed for HPMCAS and 1.4 ± 0.1 % for the product, indicating lower moisture content in the encapsulated powder product. The 2nd weight loss (277 ± 2°C) is likely to be the removal of water molecules as a result of the cross-linking esterification reaction of the HPMCAS reported above 200°C (Li et al., 2013). Following this process the samples decompose (3rd weight loss process). The peak temperature

of this decomposition process is higher for the encapsulated powder product (372 ± 1°C) than the HPMCAS (362 ± 1°C). The higher decomposition temperature detected for the encapsulated powder product is possibly the result of the fish oil acting as a protective layer around the HPMCAS. As such greater amount of heat is required to reach the temperature at which the HPMCAS decomposes.

Thermal analysis results demonstrate that the starting materials did not undergo changes in their physical state and no chemical reaction(s) occurred during the formulation of the encapsulated powder. In addition, the increase in decomposition temperature observed for the encapsulated powder product suggests the fish oil forms a protective layer around the HPMCAS, indicating good encapsulation as inferred from the FTIR analysis.

3.3.7. DHA Stability testing

For the stability tests, a comparison between the loss of DHA from the microencapsulated fish oil was assessed, along with the corresponding plain fish oil over one week at 40°C. There was a 0.29 mg (1.78%) and 0.58 mg (3.56%) reduction in DHA in the microencapsulated fish oil and plain fish oil respectively. This indicates that encapsulating the fish oil in HPMCAS protects DHA against degradation. Further investigations into the oxidation, such as the primary and secondary oxidation products, of the microencapsulated product are warranted.

3.3.8. Dissolution

HPMCAS is an enteric coating polymer, meaning that it is insoluble in acidic gastric fluid but will dissolve in the small intestine, due to the pH change. The pH dependant dissolution profile of the encapsulated powders produced by both production methodologies was evaluated in order to determine the release patterns of the encapsulated DHA, and shown in **Fig. 5**. As expected there was no DHA release detected in the simulated gastric fluid and both formulations showed as lag time of 2 hours. At 90 minutes 99.5% DHA release was seen in the

directly spray dried fish oil – HPMCAS, whereas only 28.2% DHA release was observed from the spray dried SLN – HPMCAS. These results strengthen the choice of selection of the directly spray dried methodology. Furthermore the release patterns also indicate that the encapsulated powder will remain intact within the food product (at low pH) and after ingestion through the stomach and release DHA in the intestine at the site of absorption.

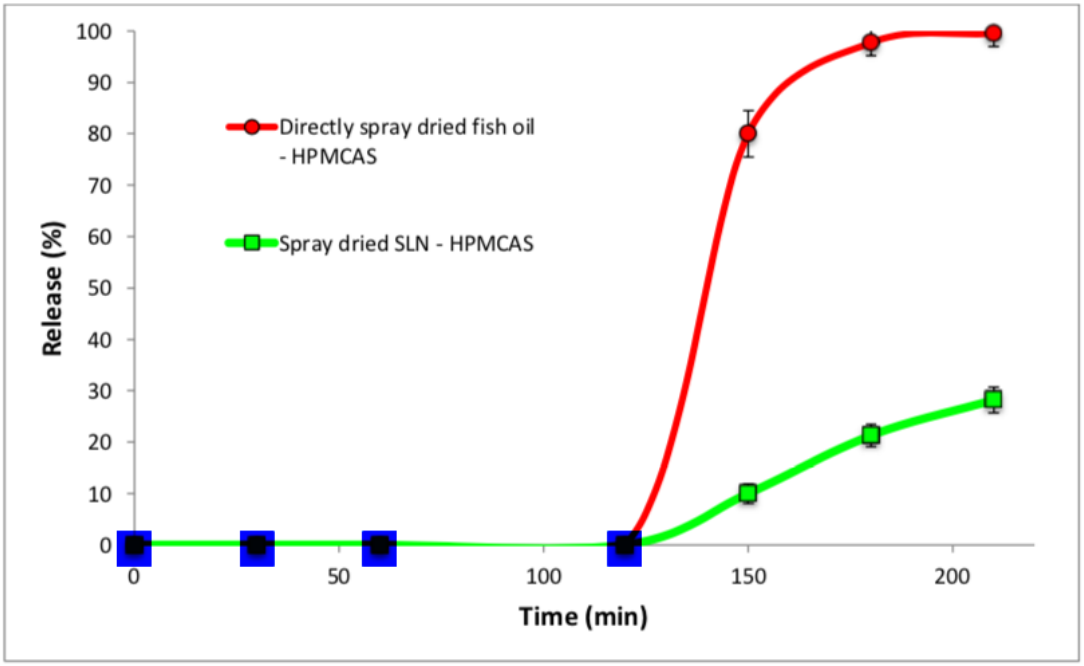

Figure 5. DHA release profile of Red – directly spray dried fish oil – HPMCAS; green – spray dried SLN – HPMCAS) in 0.1M hydrochloric acid (120 minutes) and pH 6.8 phosphate buffer.

4. Conclusion

A directly spray dried fish oil technique has proven beneficial as a production method over a spray dried nano-emulsion of fish oil in terms of DHA content, DHA encapsulation efficiency and solid yield %. The use of the encapsulated powder produced would offer protection against degradation as well as masking the odour and taste to offer palatability of the product. The encapsulated powder may therefore offer a source of DHA with potential to be incorporated into infant foods to meet recommendations (Food and Agriculture Organisation, 2010). Further work into the storage stability and the effects of the incorporation of the encapsulated powder into the food product are required.

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