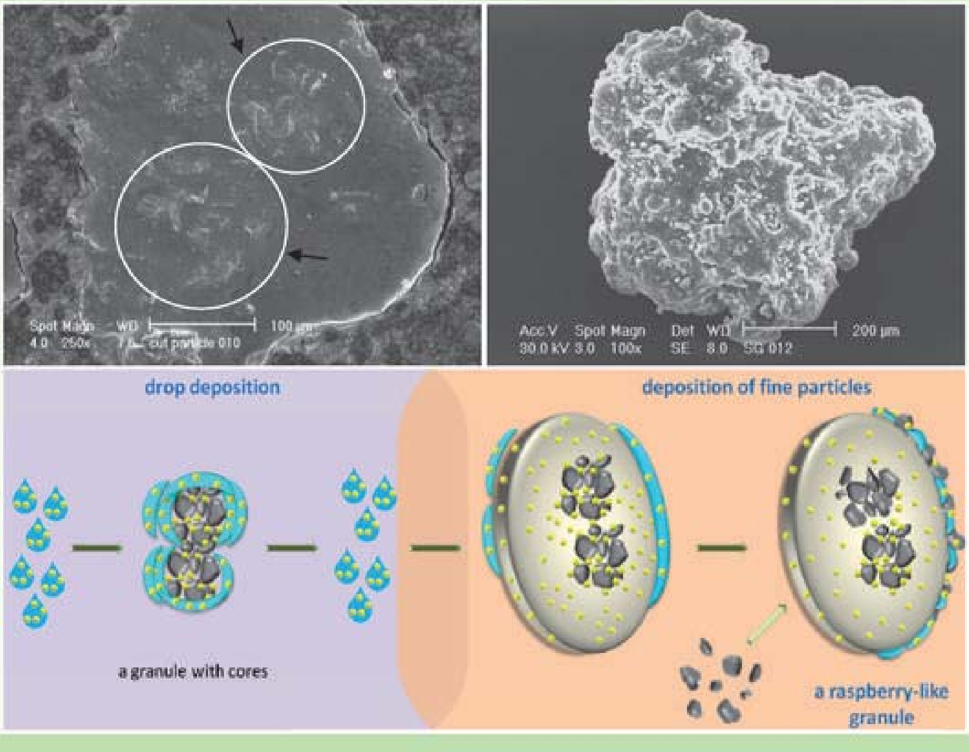
Sri Haryani Anwar

Microencapsulation of Fish Oil Using Spray Granulation, Spray Drying and Freeze Drying


Spot Magn WD 100 µm
4.0 250x 7.6 cut particle 010
Acc.V Spot Magn Det WD 200 µm
30.0 kV 3.0 100x SE 8.0 SG 012
drop deposition
deposition of fine particles
a granule with cores
a raspberry-like granule

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Bonn, December 2010

Sri Haryani Anwar

To my husband and children

Abstract

The stability of microencapsulated fish oil prepared using various drying methods is investigated. In the preliminary study, two production processes, i.e., spray granulation (SG) and SG followed by film coating (SG-FC) are examined and compared. First, three types of fish oil (10/50, 33/22, and 18/12) based on the ratios of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are used in the SG process. Each type of fish oil was emulsified with soybean soluble polysaccharide (SSPS) and maltodextrin to produce 25% oil powders. Second, a 15% film coating of hydroxypropyl betacyclodextrin (HPBCD) is applied to the SG granules. The powder stability against oxidation is examined by measurement of peroxide values (PV) and GC-headspace propanal after 6-week's storage at room temperature (± 21 ºC) and at 3 - 4 ºC. The results show that the coated powders have lower stability than uncoated powders and this indicates that the film coating by HPBCD ineffectively protected the fish oil as the coating process might have induced further oxidation.

In the main research, emulsions of 33/22 fish oil are prepared with four combinations of matrices and microcapsules are produced by SG, spray drying (SD), and freeze drying (FD). The objective is to identify the most critical factors which determine powder stability and to further examine the superiority of the SG process compared to other drying processes. Oxidation parameters and analytical methods remain the same as in the preliminary study, but the storage time is extended to 8 weeks.

The best matrices are a combination of 10% (w/w) SSPS and 65% (w/w) OSA-starch. Microencapsulation of 620 mg/g fish oil with these coating materials then dried by SG is able to produce fish oil powder having a very low propanal content and with a shelf life of five weeks at ± 21 ºC. The ability of SSPS to form thick membranes at the oil/water interface and the role of both matrices to stabilize emulsion by steric repulsion are critical to prevent early formation of peroxides. The results of the present research indicate that instead of layering a single concentrated core, microcapsule formation by the SG process is actually started by agglomeration of seed particles. The seed particles are then covered by the growth of droplet deposition and the granule surface is coated by fine particles. This assumption is supported by scanning electron microscope (SEM) examinations which verify the raspberry-like microstructure of the final granules. Therefore, it can be assumed that the SG process produces "multiple encapsulations" granules and provides maximum protection to the oil droplets.

Comparison of the SG, SG-FC, SD, and FD processes confirms that combination of matrices, drying temperature, microcapsule morphology, and processing time are among the most critical factors governing the stability. Exposure to high drying temperature or heat is proved to be a limiting factor for drying unstable emulsion. If a process does not apply high drying temperature, the particle morphology becomes a determining factor for product stability.

The main contribution of this study is to provide in-depth evaluation of four different drying processes with comprehensive information on the drying mechanisms in relation to how they affect the stability of microcapsules. The amount of polyunsaturated fatty acids (PUFAs), fish oil quality, type of matrix, and their physicochemical characteristics are also discussed in this study.

Kurzreferat

Die Stabilität von mikroverkapseltem Fischöl, hergestellt durch verschiedene Trocknungsverfahren, wird untersucht. In Voruntersuchungen werden beispielhaft die Prozesse Sprühtrocknung (SG) und Sprühtrocknung mit einer anschließenden Filmbeschichtung (SG-FC) untersucht und miteinander verglichen. Zunächst werden Fischöle mit unterschiedlichen Verhältnissen con Eicosapentaensäure (EPA) und Docosahexaensäure (DHA) (10/50, 33/22 und 18/12) im SG-Prozess verwendet. Alle Fischöle werden mit löslichen Sojabohnenpolysacchariden (SSPS) und Maltodextrin emulgiert, um 25%ige Ölpulver zu produzieren. Anschließend wird eine Filmbeschichtung dieser Ölpulver mit 15% Hydroxypropyl-Betacyclodextrin (HPBCD) durchgeführt. Die Stabilität der Pulver gegen Oxidation wird mithilfe der Peroxid-Zahl (PV) und des Propanal-Gehaltes (Headspace-GC) nach 6-wöchiger Lagerung bei ± 21 ºC und bei 3 - 4 ºC untersucht. Die Ergebnisse zeigen, dass die beschichteten Pulver eine geringere Stabilität als die unbeschichteten aufweisen, was auf einen ineffizienten Schutz des Fischöls durch die Filmbeschichtung mit HPBCD und möglicherweise auf eine durch den Beschichtungsprozess induzierte zusätzliche Oxidation hindeutet.

In den Hauptuntersuchungen werden Fischöl-Emulsionen (Typ 33/22) mit vier Matrix-Kombinationen hergestellt und Mikrokapseln durch SG, Sprühtrocknung (SD) und Gefriertrocknung (FD) produziert. Das Ziel ist, die für die Pulverstabilität entscheidenden Einflussfaktoren zu identifizieren und die Vor- und Nachteile des SG-Prozesses gegenüber anderen Trocknungsprozessen zu ermitteln. Die Parameter und die analytischern Methoden werden wie in den Voruntersuchungen verwendet, allerdings wird die Lagerzeit auf 8 Wochen erweitert.

Die beste Verkapselungsmatrix ist eine Kombination aus 10% (w/w) SSPS und 65% (w/w) OSA-Stärke. Durch die Mikroverkapselung und anschließende SG von 620 mg/g Fischöl mit diesen Kapselmaterialien wird Fischölpulver mit einem sehr geringen Propanal-Gehalt und einer Lagerstabilität von 5 Wochen bei ± 21 ºC erzeugt. Die Fähigkeit von SSPS, Filme an der Öl/Wasser-Grenzfläche zu bilden und die emulsionsstabilisierende Wirkung beider Matrices sind entscheidend, um die frühe Bildung von Peroxiden zu verhindern. Die Ergebnisse der vorliegenden Arbeit deuten darauf hin, dass die Mikrokapselbildung im SG-Prozess zunächst durch eine Agglomeration der Keimpartikeln (Nuclei) erfolgt. Anschließend wachsen die Agglomerate durch Tröpfchenablagerung und die Kapseloberfläche wird durch feine Partikeln umschlossen. Diese Annahme wird durch Rasterelektronenmikroskop-Aufnahmen (SEM) unterstützt, die die himbeerartige Struktur der Endpartikeln bestätigen. Daher kann angenommen werden, dass die SG „mehrfach verkapselte" Partikeln erzeugt und so einen maximalen Schutz der Öltröpfchen bewirkt.

Der Vergleich von SG, SG-FC, SD und FD bestätigt, dass die Kombination der Kapselmatrices, die Trocknungstemperatur, die Kapselmorphologie und die Verarbeitungszeit die kritischen Einflussfaktoren auf die Pulverstabilität sind. Es zeigt sich, dass eine hohe Prozesstemperatur ein limitierender Faktor bei der Trocknung von instabilen Emulsionen ist.

Diese Arbeit liefert eine ausführliche Bewertung der genannten Trocknungsprozesse und umfassende Informationen darüber, wie die Trocknungsmechanismen die Kapselstabilität beeinflussen. Die Menge an mehrfach ungesättigten Fettsäuren (PUFAs), Fischölqualität, Matrixart und ihre physiko-chemischen Eigenschaften werden ebenfalls in dieser Arbeit diskutiert.

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I Introduction

Microencapsulation is defined as “the technology of packaging solid, liquid, and gaseous materials in matrices or small capsules that release their contents at controlled rates over prolonged periods of time” [28;242;280]. The substance to be encapsulated is called “core”, while the microencapsulating agent surrounding the core is defined as “wall”. The core is also known as “active agent”, and the term “wall” is also referred to “matrix, coating material, or shell”. Microcapsules often have a diameter between 3 and 800 microns and contain 10 to 90 wt % core. The shell is designed to prevent diffusion of material from a microcapsule or into a microcapsule [282], to protect the core from deterioration, and to release it under the desired conditions [313]. Microencapsulation can suppress the volatility of flavor, odor and reactivity of food ingredients [118].

The first commercial application of microencapsulation technology began in the late 1930s and 1940s with the development of “carbonless paper” by the National Cash Register. Gelatine was used to coat a colourless dye precursor by a process known as coacervation. Using the same wall (gelatine) drugs were microencapsulated as early as 1931 [42]. Microcapsules are also used in applications of pharmaceuticals, pesticides and scented strips [251]. Nowadays, the encapsulation technology has been applied broadly in the food industry to microencapsulate sensitive food ingredients such as flavors, spices, vitamins, carotenoids, and omega-3 oils. The technology is aimed to protect sensitive ingredients from chemical degradation by blocking the direct influence of oxygen, pressure, heat, pH, heavy metals and other influences that may cause or accelerate degradation [247]. In the case of vitamins, the protection is essential to maintain vitamin levels, while flavor encapsulation is important to avoid unwanted off-taste. Microencapsulation of pigments such as β-carotene is necessary to achieve special physical effects such as high colour strength and special colour hue [247].

As is the case with vitamins, flavours, and pigments, sensitive oils such as fish oil have become increasingly important in the food industry, particularly because the nutritional values. The nutritional benefits of fish oil are generally attributed to their long-chain omega-3 polyunsaturated fatty acids (PUFAs) [137], including both Docosahexaenoic Acid (22:6n-3) or DHA and Eicosapentaenoic Acid (20:5n-3) or EPA [129].

PUFAs have been claimed to have a broad range of beneficial effects including lowering cholesterol, decreasing the risk of arrhythmia, lowering the blood pressure, preventing diabetes in pregnancy, and beneficial effects on joints (relief of arthritis) [185]. Both omega-3 and omega-6 PUFA are precursors of hormone-like compounds, which are involved in many important biological processes in human body [287]. In addition, DHA and EPA play an important role in early infant nutrition particularly for the development of vital human organs such as the neural tube [137]. The imbalance of these PUFAs is believed to cause a variety of diseases [143].

In functional food development, incorporation of PUFAs into food products is dominated by omega-3 fatty acids (α-linolenic acid (ALA) C18:3n-3, eicosapentaenoic acid (EPA) C20:5n-3, docosahexaenoic acid (DHA) C22:6n-3) and omega-6 fatty acids (γ-linolenic acid (GLA) C18:3n-6 and arachidonic acid (AA) C20:4n-6) [6]. Soybean, canola, flaxseed, hemp, and perilla oils are the major sources of ALA, while GLA is mostly found in evening primrose, blackcurrant and borage oils [287]. Oils from the marine algae *Cryptecondium conchii* are mainly rich in DHA only, while fish oil contains both EPA & DHA [287].

Although the nutritional values of fish oil are recognized, adequate daily intake is difficult to achieve. Fish consumption is relatively low in many countries, especially consumption of oily fish with high levels of omega-3 PUFAs [137]. Because of their sensitivity to oxidation, fish oils need to be stabilized to protect them from oxidation. In food application, fish oil interaction with other food ingredients needs to be prevented [6]. Attempts to prevent fish oil oxidation to allow omega-3 fatty acids to fulfill their functions are not trouble-free.

Fish oils in their natural state have a taste and smell that make them less attractive to consumers [185]. Processing technology for masking the smell and taste in food systems faces great challenges. Therefore, to address the problems concerning the susceptibility of fish oil to oxidation and its unpleasant smell, microencapsulation, where the oil is packaged within carrier materials, may be used in place of bulk oils.

Intensive research in the field of fish oil microencapsulation has been done in food industries, food research centres as well as in university laboratories (Table 1.1). A number of coating materials including sodium and calcium caseinate [32;129;142], soy protein [32], whey protein [32;129], gelatine [127], maltodextrin with a wide range of DE [104;129;147;290], sucrose and lactose [127], starches, modified starches [55;274], gum acacia [127], modified cellulose (MC and HPMC) [147], as well as highly branched cyclodextrin (HBCD) [129] have been reported to protect fish oils against oxidation.

Despite types of coating material, Table 1.1 also informed the importance of encapsulation process to products' stability. Thies (2004) classified the encapsulation process as chemical (A) or mechanical (B) processes. A chemical process may rely only on the physical phenomena, while in a mechanical process a chemical reaction may actually be involved. Some typical processes used for producing microcapsules for food application are: (A) complex coacervation, polymer-polymer incompatibility and submerged nozzle processes, and (B) spray drying, spray chilling, fluidised bed coaters, liquid extraction, melt extrusion, suspended nozzles, and spinning or rotating discs [282]. Similarly, Shahidi and Han (1993) divided the technology for forming microcapsules into three groups: physical methods, chemical methods and physicochemical methods [255]. The selection of a method is dependent on economic benefits, sensitivity of core, size of microcapsules, physicochemical properties of both core and wall, application for food ingredients, and release mechanisms.

Literature review indicates that even the best combination of biopolymers for microencapsulating fish oil used with different drying techniques can produce both stable and unstable products. It is necessary to determine which combinations are the best for microencapsulating the right amount of PUFAs in fish oil. To date, there are data gaps in the field of fish oil microencapsulation that need to be filled. No data was found on microencapsulation of fish oil by spray granulation (SG) and application of secondary coating after SG by the fluid bed film coating process (SG-FC). Most importantly, there are no studies where various production methods are compared. The present study therefore is conducted to evaluate and compare fish oil microencapsulation by spray granulation (SG), spray granulation followed by fluid bed film coating (SG-FC), spray drying (SD), and freeze drying (FD). Previous research usually investigated microencapsulation by a commonly used method such as spray drying or only by freeze drying. Unsatisfied outcomes obtained by a certain method might be different if produced by other processes. Each drying method offers advantages and disadvantages and so does each coating material.

Selection of the best coating materials and microencapsulation process are crucial steps in food microencapsulation. By carefully examining these two factors, this research is designed to answer the questions associated with the stability of fish oil microcapsules against oxidation in relation to how they are produced.

TABLE 1.1. Summary of research on fish oil microencapsulation

Year	Author(s)	Shell/wall materials	Encapsulation process	Antioxidant	Type of oil (brand)	Parameter analysed	Author(s) conclusions
1999	Heinzelmann and Franke [97]	● maltodextrin ● lactose ● sodium caseinate	● freeze drying using a lab-scale freeze dryer with slow, medium and fast freezing rate	with and without antioxidant	sand eel oil	● formulation parameters (composition of matrices & antioxidant) ● homogenization condition ● freezing rate	● antioxidant give a better shelf life ● slow and fast freezing show the best oxidative stability (OS) ● replace lactose by maltodextrin ↑ OS ● ↓initial FD temp had no + effects on the shelf life
2001	Keogh et al. [142]	● sodium caseinate (80% protein) ● calcium caseinate (80% protein) ● skim milk powder (38% protein, 50% lactose)	● previously homogenized ● spray drying: ▪ 2 fluid nozzles ▪ inlet temp. 177 +/- 2°C ▪ outlet temp. 75 +/- 2°C	ALT 1, mixture of: ● ascorbic acid (8.6%) ● lecithin (5.2%) ● α-tocopherol (86.2%)	sand eel (Ammodytes species)	● free fat content ● surface fat content ● air content (vacuola) of powder particles ● powder's shelf life ● fat globule & powder diameters	● possible to ↓ (VV) vacuola volume using 2-fluid nozzle ● shelf life ↑ → pressure homgenizat. ● off-flavor detc, shelf life: 31 wks at 4°C ● total solid levels: 30.0 - 34.5% (emulsion) ● if VV can be 0, then shel-life can be 36 wks at 4°C

Year	Author(s)	Shell/wall materials	Encapsulation process	Antioxidant	Type of oil (brand)	Parameter analysed	Author(s) conclusions
2003	Cho et al. [32]	• soluble wheat protein (SWP) • whey protein isolate (WPI) • na-caseinate • isolate soy protein (ISP) • protein cross linking by MTGase powder from s. moboraense	• double emulsification & enzymatic gelation • combination of enzymatic & heat gelation • double emulsification & heat gelation	• no antioxidant • but used emulsifier (span 80)	Marinol D-40, wormerveer, The Netherlands	• physicochemical properties: (rheology, electrophoretic, particle-size distribution) • microstructure • controlled release • core retention • oxidative stability	• core retention ↓ than by spray drying • ISP → highest emulsion stability & gelling capability. • double emulsification → protect fish oil • storage time for oxidative stability observed was only 14 days at 50°C PV:5-9
2003	Kagami et al. [129]	• sodium caseinate (SC) • whey protein isolate • maltodextrin of (DE 2-5, DE 4, 11-12, 18) • HBCD (highly branched cyclic dextrin)	• spray drying: ▪ centrifugal atomizer at 30000 rpm ▪ inlet temp. 150°C ▪ outlet temp. 90-100°C ▪ inner chamber diameter = 800 mm	• tocopherol 6000 ppm • ascorbyl palmitate 500 ppm	refined fish oil: • 17.3% DHA • 5.5% EPA	• surface oil • oxidative stability (OS) • microstructure • particle size & density • drying speed of dextrin solution	• HCBD → ↑ OS • SC + HCBD prevent lipid oxidation • ↑ DE → OS ↑ except HCBD • void of DE18/SC smallest and HBCD/SC, intermediate • at 46% oil load → OS was lowest but at 83% decrease • HBCD highly recommended • oxidation mechanism remains unclear

Year	Author(s)	Shell/wall materials	Encapsulation process	Antioxidant	Type of oil (brand)	Parameter analysed	Author(s) conclusions
2003	Hogan et al. [104]	● NaCas (protein: 85%) ● carbohydrates (DE 5.5, 14, 18.5, 28 & 38)	● spray drying ▪ 1.5 mm nozzle atomizer ▪ lab-scale	sodium azide (0.01%) as anti microbial agent	fish oil (menhaden or herring oil)	● effects of combining NaCas & carbohydrates, core/wall ratio & NaCas/DE28 ratio ● particle size distribution & ME ● lipid oxidation in emulsion & powder → PV & AV	● for DE 18.5-38, storage time → little effect for PV but ↑ for low DE ● droplet size emulsion → no relation to oxidative stability ● DE≥14 → ME ↑ ● α-tocopherol effective in retarding lipid oxidation ● NaCas (1.27-11.39% w/w) ● carbohydrates (28.93-38.65%) ● emulsion (30% w/w) total solids Unexpected results: ● low temp storage → lipid oxidation ↑ ● core/wall ratio & NaCas/DE28 had no effect on ME ● fish oil powders had strong fish-like odor
	Objective: Examine influence of DE,core/wall ratio, addition of water or oil soluble antioxidant on the microcapsule properties & stability						

Year	Author(s)	Shell/wall materials	Encapsulation process	Antioxidant	Type of oil (brand)	Parameter analysed	Author(s) conclusions
2005	Turchiuli et al. [290]	• acacia gum (CIRX 40693) • maltrodextrin glucidex (DE12)	• spray drying ▪ niro minor dryer (lab) ▪ rotating disc atomizer ▪ inlet temp. 200 & 220°C ▪ outlet temp. 100 -130°C • fluid bed agglomeration ▪ use water spray, temp. (45+/-2°C)	40-60 mg/100g α-tocopherol	vegetable oil (ISIO4, France) composed of: • 35% sun-flower oil (SO) • 25% SO enriched in oleic acid • 7% rapeseed oil • 3% grapes pip oil	• size of oil droplet in emulsion • particle size distribution • particle diameter • powder's total oil content, lipid oxidation • non-encapsulated oil	• powder water content ↑ → emulsion flow rate ↑ • regular spherical shape only by dry matter ≤ 40% • 87% yield at end of agglomeration process • low surface oil • behaviour of veg. oil → similar to α-tocopherol
2004	Kolanowski et al. [147]	• hydroxypropyl cellulose (HPMC) • methylcellulose (MC) • maltodextrin → as filling agent	• spray drying ▪ pilot scale ▪ atomizer nozzle (500µm) ▪ drying chamber 1.5 m • inlet temp. 160 +/- 2°C • outlet temp. 60 +/- 2°C	Soy lecithin phosphatydyl choline → as emulsifier	fish oil (Marineol omega-3)	• encapsulation efficiency • oxidative stability • capsule morphology	• MC & HPMC more soluble in cold water • powder poorly soluble at 20 & 30°C, not soluble at 60°C • fat deterioration detected during storage • MC better then HPMC • oil retention 98.5% (400g/kg) • 500 g/kg→ more structural failures

Year	Author(s)	Shell/wall materials	Encapsulation process	Antioxidant	Type of oil (brand)	Parameter analysed	Author(s) conclusions
2004	Baik et al. [10]	• corn syrup • lecithin • sodium caseinate	• spray drying ▪ inlet temp. 210°C ▪ outlet temp. 95°C	• α-tocopherol • ascorbyl palmitate	refined menhaden oil (Omega Pure^{TM})	• Effect of processing on the OS • Effect of various antioxidants and RH on OS	• encapsulated fat with antioxidant 10 x more stable than surface fat • α-tocopherol showed a greater antioxidative effect than ascorbyl palmitate • addition of >200 ppm α-tocopherol in a 10-30% RH range prolonged the OS
2005	Tan et al. [274]	• modified food starch → capsul$^{®}$	• spray drying ▪ a pilot-scala spray dryer (Niro-Denmark) ▪ rotary atomizer ▪ inlet temp. 150°C ▪ outlet temp. 80°C ▪ atomizer speed 20000 rpm	n.a	marine oil ROPUFA$^{®}$	• emulsion stability • microsphere morphology • microsphere size • yield determination oil content of ME	• high oil:wall ratio → amount of starch insufficient to encapsulate oil • oil remains on the surface • high oil load → ↓ ME • oil load ↑ → droplet size in emulsion • droplets coalesced but not separated • oil load affected size, roundness, yield & ME
	Objectives: To study the effect of high oil loading on emulsion stability & its relationship to product particle size, roundness, yield & microencapsulation efficiency (ME)						

Year	Author(s)	Shell/wall materials	Encapsulation process	Antioxidant	Type of oil (brand)	Parameter analysed	Author(s) conclusions
2005	Jónsdóttir et al. [127]	● protein: caseinate, gelatin ● carbohidrat: lactose, maltodextrin, sucrose, acacia ● gelatin of cod skin	● spray drying ▪ Büchi B-191 mini spray dryer ▪ inlet temp. 170°C ▪ outlet temp. 100-110°C	● span 80 (0.4%) ● lechitin (6.5%)	fish oil: (18% EPA & 12% DHA)	● surface oil ● morphology ● oxidative stability ● sensory analysis ● SPME ● identification & quantification of volume compounds	● no differences in capsule size among samples ● great amount of free fat on lactose matrix but least rancid odor ● fish-gelatin-based capsules → ↓ OS compared to carbohydrates based capsules ● samples with tocopherol → lowest rancidity after 20 wks ● SPME → sensitive enough to detect oxidative changes during storage when using PDMS/DVB fiber
	Objectives: Compare the stability of m.e fish oil coated with caseinate & carbohydrates mixtures with gelatin-based coating materials. Used SPME → lipid oxidation						
2006	Velasco et al. [299]	● sodium caseinate ● D-lactose	● freeze drying ● emulsions were frozen at -50°C for 24 h and freeze dried for 48 h in a Heto FD3 freeze drier	mixture of antioxidant contained in fish oil	● refined sunflower oil ● fish oil (sand eel oil with & without antioxidant	● characterization of oil and DMO ● oxidation pattern in bulk oil and DMO	● free oil fraction had oxidative pattern of bulk oil, having induction period (IP) where peroxides accumulated before oxidation accelerated

Year	Author(s)	Shell/wall materials	Encapsulation process	Antioxidant	Type of oil (brand)	Parameter analysed	Author(s) conclusions
2006	Drusch & Schwarz [55]	• 2 types of derivatised starch > (n-OSA starch) low viscosity (L), 132 mPas at 30% solids at 25°C medium viscosity (M), 340 mPas at 30% solids at 25°C	• spray drying ▪ lab-scale (1-7 kg/h) ▪ rotating disk atomizer ▪ rotation 18000 rpm ▪ moderate: inlet temp. 160°C, outlet temp. 60°C ▪ severe: inlet temp. 210°C outlet temp. 90°C	n.a addition of glucose syrup, (DE 38)	fish oil: 12.3% DHA 18.0% EPA	• viscosity & stability emulsion • morphology of microcapsules • lipid oxidation	• high oil load (50%) + L n-OSA → large oil droplets → stability ↓ • M n-OSA capable to emulsify 50% oil load • at higher drying temp → ballooning occurred and particle size ↑ • repeat passing of emulsion to homogenizer → more efficient to reduce oil droplet size than ↑ homogenization pressure • moderate spray drying → advantages → ballooning & lipid oxidation were limited
	Objectives: To evaluate the suitability of two different type of n-OSA (n-octenylsuccinate derivatised starch)						

Year	Author(s)	Shell/wall materials	Encapsulation process	Antioxidant	Type of oil (brand)	Parameter analysed	Author(s) conclusions
2006	Augustin et al. [7]	● Na caseinate ● WPI, SPIe ● skim milk powder ● glucose, dried glucose syrup ● oligosaccharide ● mix of protein-carbohydrate was heated to form maillard reaction product	● spray drying using a Drytec lab-spray dryer with a twin fluid nozzle at 2.0 bar atomizing pressure ● emulsion was heated to 60°C ● Inlet temp. 180°C ● Outlet temp. 80°C	n.a.	tuna oil and primrose oil (Clover corp)	● effect of time-temp. treatment on the extent of maillard reaction ● effect of different protein carbohydrate formulation to powder properties	● increasing extent of maillard reaction will increase powder stability ● morphology and physical barrier properties of powder contributed to powder stability
2006	Drusch et al. [56]	● n-OSA starch ● glucose syrup	● spray drying ■ lab-scale (1-7 kg/h) ■ rotating disk atomizer ■ rotation 18000 rpm ■ moderate: inlet temp. 170°C, outlet temp. 70°C	n.a	refined cold-pressed fish oil with 33% n-3 fatty acids (Henry Lamotte GmbH), EPA 18% and DHA 12.3%	● oxidation of bulk fish oil ● microcapsule morphology ● lipid oxidation during microencapsulation ● OS at different temp. and RH	● lipid oxidation depends to a certain extent on storage temp. & to a greater extent on RH ● hydroperoxide reached 88 & 146 mmol/kg in samples stored at 11 and 33% RH, respectively. ● hydroperoxide ↑ with ↑storage temp. was observed at 48.59% RH

Year	Author(s)	Shell/wall materials	Encapsulation process	Antioxidant	Type of oil (brand)	Parameter analysed	Author(s) conclusions
2006	Drusch et al. [57]	● glucose syrup ● trehalose	● spray drying ■ rotating disk atomizer ■ rotation 30000 rpm ■ moderate: inlet temp. 170°C, outlet temp 70°C homogenization at 200/50 bar	antioxidant, free fatty acids, pigments, mono and diglycerides were removed by column chromatography	refined cold-pressed fish oil with 33% n-3 fatty acids (Henry Lamotte GmbH), EPA 18% and DHA 12.3%.	● physicochemical properties of powder: particle size, oil droplet size, true density and BET surface	● at low RH, oxidation ↓ in trehalose samples →in amorphous state, trehalose better than glucose syrup ● at 54% RH, oxidation↑ upon trehalose crystallization ● trehalose suitable for low humidity storage
2007	Drusch,S. [51]	● sugar beet pectin ● glucose syrup	● spray drying ■ lab-scale (7 kg/h) ■ rotating disk atomizer ■ homogenization at 200/50 bar and 500/100	n.a.	refined cold-pressed fish oil with 33% *n*-3 fatty acids (Henry Lamotte GmbH), EPA : DHA 18 : 12.3	● composition & homogenization condition ● median of oil droplet size ● morphology ● particle size ● extractable fat	● oil droplet emulsion influenced by oil, pectin, & dm content ● 1 & 2% pectin → enough for preparing stable emulsion up to 3 days ● non-extractable fat is higher in 50% oil load than in 20% ● OS of microcapsule was not examined ● % of non-extractable fat & ME were not mentioned

Year	Author(s)	Shell/wall materials	Encapsulation process	Antioxidant	Type of oil (brand)	Parameter analysed	Author(s) conclusions
2006	Rusli et al [248]	● whey protein isolate (WPI) ● soy protein isolate (SPI) ● dried glucose syrup (DGS)	● spray drying ▪ lab-scale (7 kg/h) ▪ rotating disk atomizer ▪ homogenization at 200/50 bar and 500/100	n.a	● tuna oil (HiDHA 25N Food) ● palm stearin and their mixture	● effect of oil types ● homogenization pressure (35+10 or 18+8 MPa) ● total solids (TS) of emulsions (26.1 or 33.3%) ● emulsion size ● emulsion viscosity ● ME ● oxidative stability	● oxidative stability decreased when oil load was increased from 20-60% ● at higher oil load, increase in oil/protein ratio results in thinner encapsulating film around the oil droplets ● properties of film surrounding the droplet size affect ME ● ME not affected by decreasing TS of emulsion from 33 to 26% ● powder with heated WPI-DGS mixture was superior to powder containing SPI

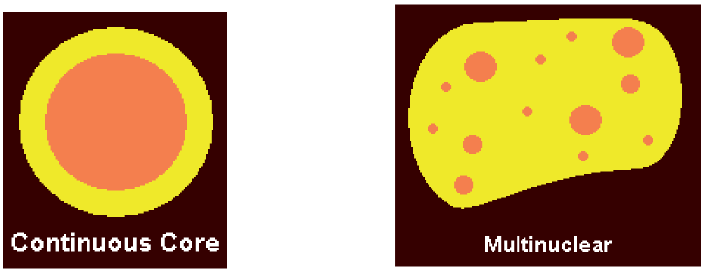
II Theoretical background

2.1 Microencapsulation

Microencapsulation is a technology for enveloping sensitive ingredients as a ‘core’ material within a polymer matrix or ‘wall’ [104]. The aim of the microencapsulation process is to convert a liquid into solids, thus improving product stability and handling, and also masking unpleasant flavours [55]. A growing demand for functional and sophisticated food products has led to an increasing application of microencapsulated food ingredients.

2.1.1 Type of microcapsules

The two types of microcapsules in the food industry are continuous core/shell microcapsules (Type A) and multinuclear microcapsules (Type B; Fig. 2.1). The processing technique used influences the structure of the microcapsules produced [282]. In type A, the core material is concentrated in a centralised interior volume element in the capsule and the protective shell materials surround the central core. This structure is typically produced by a process called complex coacervation, fluidised bed and droplet co-extrusion. The positive feature of this type is that there is no shell mat located in the interior of the capsules. The shell thickness is maximised for a constant loading and size. A high core loading of 90% of the total capsule weight can be achieved. The mechanism of release is usually when the shell is ruptured at any point [281].



Type A Type B

Fig. 2.1 Structure of microcapsules in the food industry; adapted from [282].

The Type B microcapsule has a core that is dispersed throughout the shell material. Therefore, the protective barrier is distributed throughout the interior and is not present at the outer surface of the capsule. The shell can be very thin; the core loading varies from 20-30% of the total capsule weight and rarely has a shell loading above 75%. These microcapsules are typically produced by spray drying, pressure extrusion, and droplet desolvation.

2.2 Fats and oils

The terms fats and oils are often used interchangeably. The selection of a term is usually based on the physical state of the material at ambient temperature. Fats are solid at ambient temperatures and oils liquid. Fats and oils occur naturally in a wide range of sources. All animals produce fat, while seeds and fruits contain oils, and marine sources provide oils. All edible fats and oils are water-insoluble substances that consist of glyceryl esters of fatty acids or triglycerides [212].

2.3 Fish oil as the source of omega-3 polyunsaturated fatty acids (PUFAs)

2.3.1 Types and sources of fatty acids

Fatty acids are important nutrients, which cannot be synthesized by the human body but have to be obtained through nutrition uptake. Fatty acids with double bonds beyond the ninth carbon from the carboxyl end of the compound are classified as essential for human health [212]. This includes polyunsaturated fatty acids (PUFAs) such as linoleic (C-18:2) and linolenic (C-18:3). These fatty acids are also known as ***omega 6 (linoleic)*** and ***omega-3 (linolenic)*** [212]. Vegetable oils are the main source of omega-6 PUFAs such as safflower oil (~78%), sunflower oil (~68%), corn oil (~60%) and soybean oil (~54%). Canola oil and soybean oil contain relatively high linolenic (8.8%) or omega-3 PUFAs (7.6%). However, the main sources of omega-3 PUFAs are fatty fish species such as herring, mackerel, sardine and salmon [142]. Marine oils, including fish oil, are the complex mixture of fatty acids with varying lengths and degrees of unsaturation [257].

2.3.2 Structure of polyunsaturated fatty acids

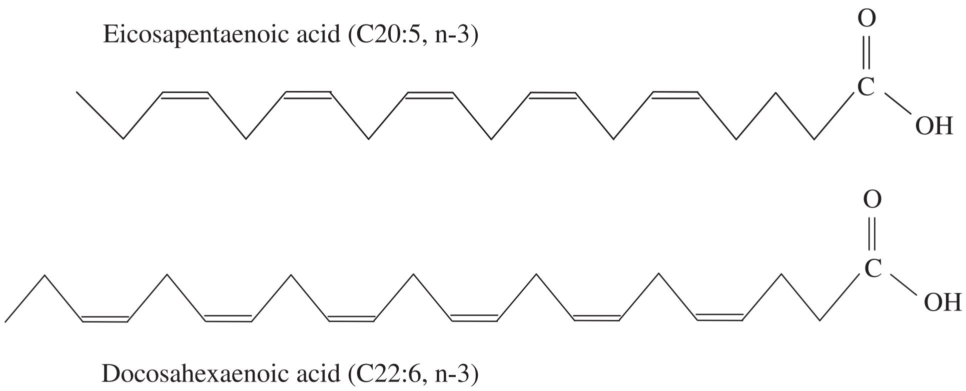
Polyunsaturated fatty acids (PUFAs) are composed of 18 or more carbon atoms and a terminal carboxylate group having two or more double carbon bonds. Classification of these fatty acids is determined by the position of the first double bond, as counted from the methyl terminus. The one with its first double bond at position 3 as counted from the methyl terminus is called omega-3 PUFA, while the one located at position 6 is omega 6. The symbol omega (ω) and its synonym *n* is often used to classify PUFAs [263]. Alpha-linolenic acids (18:3 Δ9, 12, 15), eicosapentaenoic acid (EPA, 20:5 Δ5, 8, 11, 14, 17), and docosahexanoic acid (DHA, 22:6 Δ4,7,10,13,16,19) are the most studied PUFAs within this group (Table 2.1; Fig. 2.2).

Table 2.1. ω-3 polyunsaturated fatty acids, adapted from [263]

Common name	**Systematic name (with all double bonds in *cis*-configuration)**	**Short name**
α-Linolenic acid	Δ9, Δ12, Δ15-Octadecatrienoic acid	ω-3 18:3
	Δ6, Δ9, Δ12, Δ15-Octadecatetraenoic acid	ω-3 18:4
	Δ8, Δ11, Δ14, Δ17-Eicosatetraenoic acid	ω-3 20:4
Eicosapentaenoic acid	Δ5, Δ8, Δ11, Δ14, Δ17-Eicosapentaenoic acid	ω-3 20:5
	Δ7, Δ10, Δ13, Δ16, Δ19-Docosapentaenoic acid	ω-3 22:5
Docosahexaenoic acid	Δ4, Δ7, Δ10, Δ13, Δ16, Δ19-Docosahexaenoic acid	ω-3 22:6
	Δ5, Δ8, Δ11, Δ14, Δ17, Δ20-Tetracosahexaenoic acid	ω-3 24:6

2.3.3 Nutritional benefits of omega-3 PUFAs

A high intake of PUFA is associated with a low incidence of coronary heart disease (CHD) and reduced risk of cancer [300]. Docosahexaenoic acid (DHA) is an essential component of the cell membranes of human tissues and accounts for over 60% of the total fatty acids in the rod outer segment in the retina [79]. It is also regarded essential for the proper visual and neurological development of infants because of its role as a structural lipid component. Furthermore, DHA reduces the risk factors of diseases such as cardiovascular, hypertension, arthritis, arteriosclerosis and thrombosis.



Eicosapentaenoic acid (C20:5, n-3)

Docosahexaenoic acid (C22:6, n-3)

Fig. 2.2 Schematic representation of EPA and DHA

The Western diet typically contains high levels of total fat. However, the intake of omega-3 PUFA is too low. In fact, the most commonly consumed PUFAs originating from plant oils are dominated by omega-6. Increasing omega-3 consumption and reducing that of omega-6 is needed in order to balance the intake of PUFAs and give optimal benefit for human health [263].

2.4 Lipid oxidation

Lipid oxidation is a cycle of chemical interactions between active oxygen with unsaturated fatty acids in lipids. Its mechanism depends on the type of active ingredients present, the physical properties of the food, and their physicochemical environment [184;208]. Lipid oxidation has become a serious concern in the food industry particularly because of the quality deterioration caused by the oxidation products. Oxidation affects food texture, color, nutrition, flavor (by development of rancidity), and limits the enrichment of important food ingredients into food products [27;71;208].

2.4.1 Autoxidation of PUFAs

The main lipid components of food are triacylglycerols (triglycerides), which are commonly referred to as fats and oils and phosphoglycerides (phospholipids).

Triglycerides consist of fatty acids which esterified to glycerol, contribute to flavor, odor, color, and texture of foods. Fatty acids include saturated, monounsaturated, and polyunsaturated fatty acids. The PUFAs are characterized by having two or more *cis* double bonds separated by a single methylene group or a 1,4-diene structure [71].

The oxidative stability of triglycerides, which are rich in long-chain *n*-3 PUFAs, remains an open and important question [72]. Some factors affecting the stability of fish and algae oils with a high content of PUFAs are the physical and colloidal states of the lipids, antioxidants, and the presence and activity of transition metals. These oils are initially much more stable to oxidation in the bulk systems than in the corresponding oil-in-water emulsions [72]. The ease to oxidation of oil-in-water emulsions is due to the high surface area that increases lipid interactions with aqueous pro-oxidants [27].

The oxidation of unsaturated fatty acids is commonly known as autoxidation. Autoxidation is the direct reaction of molecular oxygen with organic compounds. It involves a chain process occurring autocatalytically through free radical intermediates. This process occurs spontaneously under mild conditions and is initiated by light, heat, trace metals, peroxides or hydroperoxides, and has an induction period.

2.4.2 Pro-oxidants and antioxidants

Pro-oxidants are compounds that are commonly found in most of food systems and function as initiators, facilitators, and accelerators of lipid oxidation. Pro-oxidants react directly with unsaturated fatty acids to develop lipid hydroperoxides (such as singlet oxygen and lipoxygenases) and to form free radicals (transition metals and light) [27]. The presence of pro-oxidants at high temperature can cause rapid decomposition of hydroperoxides after their formation. Their detection is difficult or reported as no accumulation during use and storage, particularly the oxidation of frying oil.

Addition of antioxidants into food systems and the way they retard lipid oxidation can vary and depend on the type of antioxidants and the physical properties of food. Huang et al. [112] reported that some antioxidants impede lipid oxidation under certain circumstances but in other conditions they can promote the oxidation. Reische et al.

[235] classified antioxidants based on their mechanisms of action as primary and secondary antioxidants.

Primary antioxidants, also known as the chain-breaking antioxidants or as free radical scavenging antioxidants, can suppress lipid oxidation by hindering the initiation, propagation or scission reaction. They interfere with the reaction by accepting free radicals and transforming them into stable free radicals so that they will no longer be able to promote initiation and propagation reactions. Tocopherols are natural chain-breaking antioxidants, while butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), porpyl gallate (PG) and tertiary butylhydroquinone (TBHQ) are synthetic ones [235].

Secondary antioxidants prevent oxidation in many ways and can act as chelators, oxygen scavengers and reducing agents or as singlet oxygen quenchers. The chelator type can bind, inactivate, and reduce the activity of pro-oxidant metals. Some common examples of chelators are citric acids and ethylenediaminetetraacetic acid (EDTA). Some chelators can be ineffective when used alone [235] and may increase the oxidation by increasing metal solubility or changing the metal redox potential [172]. EDTA will work effectively when the ratio of its concentration to the pro-oxidant metals such as iron is equal or more than 1 [172].

Tocopherols, carotenoids, amino acids, peptides, proteins, urate, and ascorbate have the ability to inactivate singlet oxygen formed by the enzymatic reactions in biological systems or in the presence of light, photosensitizers and triplet oxygen [43]. This research applied fish oils to which antioxidants of mixed tocopherols had been added by the manufacturers. Therefore, the further review of literature will only focus on tocopherols, including their antioxidant activity, mechanisms of reaction to food lipids, and effectiveness.

2.4.3 Effect of tocopherol on autoxidation of fish oil

In nature, there are four tocopherol homologs, based on the degree of methylation of the chromanol ring: α, β, γ, and δ tocopherols. The concentration of tocopherols varies in vegetable oils and can be removed unintentionally during oil processing. The mixture of tocopherols is added to fats and oils to improve the oxidative stability [131;235]. The addition is essential for PUFAs protection [53], and their antioxidative activities are mainly by biochemical and biophysical mechanisms [130].

The inhibition of oxidation by tocopherols has been described by Frankel (1996) and follows the reactions according to equation 1-5. It is started when PUFAs (LH) form alkyl radicals ($L^\bullet$) in the presence of initiator (X). The alkyl radicals decompose hydroperoxides in the existence of trace metals to produce alkoxy radicals ($LO^\bullet$), which can react very rapidly with oxygen to form peroxy radicals ($LOO^\bullet$). If these radicals further react with other lipids, then hydroperoxides (LOOH) will be developed [70].

Tocopherol (A), which is known as a chain-breaking antioxidant, competes with polyunsaturated fatty acids (LH) for the chain-carrying peroxy radicals. As reported by McClements and Decker (2000), chain-breaking antioxidants have a higher affinity for peroxyl radicals than for lipids. This property enables this type of antioxidant to scavenge free radicals that already pre-formed or to stop their formation during initiation and propagation steps. Tocopherol (TOH) inhibits free radicals oxidation in two ways: (1) by donating a hydrogen to peroxy radicals ($LOO^\bullet$) to stop chain propagation (eq.4) resulting in a α-tocopheryl semiquinone radical ($A^\bullet$) and (2) by reacting with alkoxy radicals ($LO^\bullet$) to prevent the further decomposition of hydroperoxide and limiting the formation of aldehydes [184]. The α-tocopheryl semiquinone$^\bullet$ can further donate another hydrogen to $LOO^\bullet$ and form unstable methyltocopherylquinone or react with another α-tocopheryl semiquinone radical to form an α-tocopheryl dimer. The α-tocopheryl dimer is the regeneration form of TOH and continues to show antioxidant activity [235].

$LH + X^\bullet \longrightarrow L^\bullet + XH$ (1)
$L^\bullet + 02 \longrightarrow LOO^\bullet$ (2)
$LOO^\bullet + LH \longrightarrow LOOH + L$ (3)
$LOO^\bullet + AH \longrightarrow LOOH + A^\bullet$ (α-tocopheryl semiquinone$^\bullet$) (4)
$LO^\bullet + AH \longrightarrow LOH + A^\bullet$ (5)
α-tocopheryl semiquinone$^\bullet$ + $LOO^\bullet$ $\longrightarrow$ LOOH + methyltocopherylquinone$^\bullet$ (6)
α-tocopheryl semiquinone$^\bullet$ + α-tocopheryl semiquinone$^\bullet$ $\longrightarrow$ α-tocopheryl dimer (7)

Though α-tocopherol is well known as a natural antioxidant, the activity of TOH homolog in fats and oils is reported in the order of δ-tocopherol > γ-tocopherol > β-tocopherol > α-tocopherol [152;199]. However, Kulas et al. (2003) and Frankel (2005) reported that the antioxidant activity of tocopherol homolog is not consistent in all food systems and is dependent on many factors, including the physical nature of the food, the characteristics of lipid substrates, antioxidant concentrations, experimental conditions, and method used to evaluate oxidation [71;153].

Alpha-tocopherol is the only tocopherol homolog that is present naturally in fish oil, and its level can be reduced by at least half to below 100 ppm by deodorization and refining processes. Kulas and Ackman (2001) studied the effect of tocopherol concentrations up to 2000 ppm on the formation and decomposition of hydroperoxides in purified fish oil triacylglycerols (TAG). They reported that at low level of addition (100 ppm), the relative ability of tocopherols to retard hydroperoxides formation in fish oil was decreased in the order of α-tocopherol > γ-tocopherol > δ-tocopherol. However, at a higher initial concentration (1000 ppm), a reverse order was found. Alpha-tocopherol and γ-tocopherol have concentration limits for their maximal antioxidant activity, which are 100 ppm and 500 ppm, respectively. Above these values, an inversion activity was observed. Meanwhile, δ-TOH showed increasing antioxidant activity with an additional level of up to 1500 – 2000 ppm. The pro-oxidant activity was not detected for all tocopherols [152].

From the same study, contrary to the effect of hydroperoxide formation, the formation of volatile secondary oxidation products decreased with increasing tocopherols concentration. The authors also underline that the formation of propanal (a product of β-scission of an alkoxyl radical) is inhibited by α-tocopherol by hydrogen donation to form a stable product. At low concentrations in fish oil, α-tocopherol reveals strong antioxidant activity by donating a hydrogen atom to inhibit formation of hydroperoxides, while γ- tocopherol and δ- tocopherol need a higher concentration [152].

In previous research, Hamilton et al. (1998) reported different results. They found that at a 2.0% concentration, α-TOH had a pro-oxidant effect in fish oil. The main reason for the contradictory finding was the process of fish oil purification prior to addition of the antioxidant [91]. In this work, the refining process left significant levels of iron (0.18 ppm Fe) and copper (< 0.01 ppm Cu) in the refined oil, while in the study by Kulas and Ackman (2001), the purification by column chromatography resulted in fish oil free of natural tocopherol. Therefore, the substrate containing natural TOHs and subsequently receiving an addition of several thousand ppm yielded extremely high concentrations thus exhibited the pro-oxidant effect. Strong antioxidant activity by α-tocopherols retard oxidation even if the TOHs are only present in very small amounts in triacylglycerol (TAG) [154].

The ternary blends of 2% δ-tocopherol, 0.1% ascorbyl palmitate, and 0.5% lecithin showed superior protection of refined fish oil with no significant peroxidation during a 6-month storage period [91]. This finding is in agreement with recent work by Drusch et al. (2008). They observed that formulation of antioxidants rich in γ- and δ-tocopherols but low in α-tocopherol, in combination with ascorbyl palmitate, carnosic acid from rosemary extract, citric acid and lecithin, would be efficient in stabilizing oils rich in PUFAs [53].

2.4.4 Lipid oxidation in oil-in-water emulsions

An emulsion is dispersion of two immiscible liquids, in which one is dispersed in the other (usually oil and water, with droplet size in a range of 0.1 to 100 µm) and thermodynamically unstable due to different densities of oil and water [183]. This property needs positive free energy to increase the surface area between water and oil phase. In order to increase the emulsion stability for a period of time, emulsifiers are usually added prior to homogenization. Emulsifiers are surface-active molecules that absorb to the surface of oil droplets immediately after homogenization and have the ability to form a protective membrane to prevent droplet aggregation. Many food lipids are in the form of this type of emulsion, such as milk, dressings, mayonnaise, beverages, sauces, etc., while margarine and butter are two examples of a water-in-oil emulsion [48;183].

Theoretically, an oil-in-water emulsion consists of three regions: the interior of the oil droplets, the aqueous or continuous phase, and the interfacial layer. The interfacial layer is a few nanometer-thick layer surrounding each droplet in the emulsion, and this narrow region consists of a mixture of emulsifier molecules, oil, water and any other surface-active compounds attracted toward this region. For relatively small droplets with thick interfacial layers, the volume occupied by the interface is quite significant. Thus, it is highly possible that low concentrations of molecules (few µM to mM) such as antioxidants, pro-oxidants, and hydroperoxides are localized within the interfacial region. Molecule position in the emulsion depends on their polarity and surface activity. Polar molecules are usually located in the aqueous phase, non-polar ones in the oil phase, and amphiphillic molecules at the interface [49].

Type and concentration of molecules present in the interface determine its characteristic and most importantly have a great impact on the rate of lipid oxidation in the emulsion [49]. Another factor that must be taken into account is the lipid molecules orientation at the interfacial layer, whether parallel or perpendicular to the interface. This orientation will affect molecule accessibility to the water-soluble antioxidants or pro-oxidants. If oxidation occurs, oxygen can increase the polarity of fatty acids, and this will change the emulsion properties as well as the susceptibility of fatty acid to oxidation [101].

A very good review has been addressed by McClements and Decker (2000). The authors highlight the importance of molecular environments providing details and causes of lipid oxidation in oil-in-water emulsions. They describe the factors involved in determining the rate of oxidation as follows:

1. Chemical structure of lipids

   The number and location of double bond in a lipid molecule determine its susceptibility to oxidation. The rate of fatty acid oxidation increases as the degree of unsaturation increases [208]. The closer the double bonds to the methyl end of a fatty acid, the greater its stability with respect to oxidation [194].

2. Oxygen concentration

   In food oils, oxygen is reported three times more soluble than in water, thus oxygen can act as a fuel to accelerate lipid oxidation unless an action is taken to exclude oxygen [136].

3. Antioxidants

   The effectiveness of chain-breaking antioxidants is determined by their chemical properties and physical location within a system. Lipophilic antioxidants are more effective in oil-in-water emulsions than the hydropihilic ones. Similarly, non-polar antioxidants work better in emulsions than in bulk oil compared to the polar antioxidants. Tocopherols are examples of lipophilic and non-polar antioxidants while Trolox and ascorbic acid are in the group of hydrophilic and polar antioxidants. The effectiveness of tocopherols in emulsion increases as their polarity decreases or their surface activity increases. This is due to their position will be at the oil-water interface when oxidation occurs [113]. EDTA and phytate are transition metal chelators that retard oxidation by binding and removing iron from the droplet surface [174;187].

4. Interfacial characteristics

   The rate of lipid oxidation in emulsion is highest if the droplets are negatively charged (because they can attract the positively charged iron ions electrostatically to the droplet surface), and fairly similar for uncharged and positively charged droplets [173;174]. Oxidation rate is significantly lower when the lipid droplets are cationic at a pH below the isoelectric point (pI) of the emulsifying proteins [51;108]. The oxidation is lowest in the system where the droplets are stabilized by surfactants with longest polar head groups because they can act as a thick physical barrier to separate the lipid from pro-oxidants [264]. Protein also has the ability to built thick and viscoelastic membranes surrounding the oil droplets, thus partly inhibiting oxidation [51].

5. Droplet characteristics

   Contradictive findings exist with regard to the role of droplet size in the emulsion. If a system has excess of reactants, decreasing droplet size means doubling their concentration, thus the oxidation rate increases. In contrast, if there is only a small amount of hydroperoxides on the surface of the oil droplets, decreasing the droplet size means that there is no effect of free radicals on oxidation [239].

6. Interaction with aqueous phase components

   Casein has the ability to form an interfacial layer of up to 10 nm around oil droplets, which is very thick compared to the 1-2 nm for whey protein [49]. A thick interfacial membrane may explain why an emulsion stabilized by casein is more stable to oxidation than an emulsion stabilized by whey protein [109]. Proteins were also reported as effective transition metal chelators that reduce iron and hydroperoxide interaction [33;90;108-111]. However, recent research shows that the protein function as an iron chelator might be changed during food processing if the protein chain is unfolded, and its denaturation may alter the metal-binding properties [220]. Polysaccharides may have a dual function in emulsion as thickening agents and as antioxidants by chelation of metal ion and by hydrogen donation [82].

Previous studies regarding oil-in-water emulsion emphasized that the common cause of oxidative instability in this system is the interaction between transition metal originated in the aqueous phase and the hydroperoxides located on the surface of the oil droplets. Iron is chemically reactive and has become one of the main pro-oxidants in foods [33]. As an oxidation accelerator, iron can promote lipid oxidation by coming into close proximity to the lipid substrate [184]. Transition metals or pro-oxidants have the ability to decompose hydroperoxide (ROOH) into peroxyl ($ROO^{\bullet}$) and alkoxyl ($RO^{\bullet}$) radicals, which are highly reactive (eq.(1) – eq. (6)). If within the oil droplets and at the oil-water interface these radicals react with unsaturated fatty acids (LH), the lipid radical ($L^{\bullet}$ and $LOO^{\bullet}$) will be formed. Further reactions of lipid radicals with other unsaturated fatty acids will regenerate the oxidation chain reaction [187;188]. Moreover, formation of alkoxyl radical leads to the reaction of β-scission, which results in the development of rancidity indicators such as ketones, aldehydes, alcohols and hydrocarbon.

$$\begin{array}{llll}
\mathrm{Fe3^{+} + ROOH} & \longrightarrow & \mathrm{Fe2^{+} + ROO^{\bullet} + H^{+}} & (1) \\
\mathrm{Fe2^{+} + ROOH} & \longrightarrow & \mathrm{Fe3^{+} + RO^{\bullet} + OH^{-}} & (2) \\
\mathrm{ROO^{\bullet} + LH} & \longrightarrow & \mathrm{ROOH + L} & (3) \\
\mathrm{RO^{\bullet} + LH} & \longrightarrow & \mathrm{ROH + L} & (4) \\
\mathrm{L^{\bullet} + O2} & \longrightarrow & \mathrm{LOO^{\bullet}} & (5) \\
\mathrm{LOO^{\bullet} + LH} & \longrightarrow & \mathrm{LOOH + L^{\bullet}} & (6)
\end{array}$$

McClements and Decker (2000) also underlined the importance of the physical location of reactive molecules within the emulsion. Hydroperoxides and free radicals are usually present on the surface of droplets and are surface active, whereas the transition metals and enzymes, as lipid oxidation accelerators, originate from the aqueous phase of the emulsion. If the free radicals are formed on a droplet surface, they can easily react with unsaturated fatty acids within the same droplet surface and oxidation occurs. Therefore, the rate of oxidation depends on the speed of free radicals, hydroperoxides or unsaturated fatty acids when diffusing within a droplet from one region to another [184].

A recent study reveals that iron is a cause of oxidation. Choi et al. (2009) isolated iron from fish oil emulsion and observed the rate of oxidation. Iron was encapsulated within the internal aqueous phase of the water-in-oil-in-water (W/O/W) emulsion. The first aqueous phase system was prepared by dispersing 15 wt% whey protein isolate and 0.1 wt% iron into a 20 mM phosphate buffer solution at pH 7.0. The oil phase was prepared by mixing 8 wt% polyglycerol polyricinoleate (PGPR) into corn oil. Then the first aqueous phase was mixed with the oil phase to form a W/O emulsion and subsequently dispersed into 80 wt% of an aqueous surfactant solution (0.5 wt% Tween 20, 20 mM phosphate buffer, 0.02 wt% sodium azide) at pH 7.0 using a membrane homogenizer. The final W/O/W emulsion was finally combined with a pre-mix 16 wt% fish oil with 84 wt% aqueous surfactant solution. The results show that the TBARS formed decreased compared to fish oil emulsion only and depended on the concentration and location of iron, i.e., no added iron < iron in external < aqueous phase < iron in internal aqueous phase [36].

2.5 Encapsulation methods

Thies (2004) classified the encapsulation techniques as Type A and Type B (Table 2.2). In the former process, microcapsules are produced entirely in a liquid-filled stirred tank or tubular reactor. In the latter process, microcapsules are formed by spraying droplets of coating materials on a core material being encapsulated, where the liquid droplets are solidified by spraying them into a gas phase. This process also allows the gelling droplets to be sprayed into a liquid bath, or a polymerization reaction can be carried out at solid/gas or liquid/gas interfaces of dispersed particles or droplets [282].

The selection of a method depends on economic reasons, sensitivity of the core, size of microcapsule desired, physical and chemical properties of both core and coating, application for the food ingredient and the release mechanism. Microencapsulation processes involve both physical and chemical techniques [118]. There is no single encapsulation process that is able to produce a full range of capsules needed by the users [282].

Table 2.2 Classification of encapsulation processes [282]

Type A (chemical) process	**Type B (mechanical) process**
Complex coacervation	Spray drying
Polymer/polymer incompatibility	Spray chilling
Interfacial polymerization in liquid media	Fluidised bed
	Electrostatic deposition
In-situ polymerization	Centrifugal extrusion
In-liquid drying	Spinning disk or rotational suspension separation
Thermal and ionic gelation in liquid media	Polymerization at liquid/gas or solid/gas interface
Desolvation in liquid media	Pressure extrusion or spraying into solvent extraction batch
	Hot-melt extrusion

2.5.1 Spray drying

Spray drying is the most commonly used encapsulation method in the food industry [256]. It is an old technique, and the first spray dryer was constructed in 1878 and has been used since 1930s to encapsulate flavour using gum acacia. This method uses available equipment, has high production capacities (up to 4,000 kg/h), low process cost (20% of that of freeze drying and 30% of that of vacuum drying), applies a wide choices of carrier solids, and has low effective process temperatures [232].

Table 2.3 Spray drying vs fluid bed drying, adapted from [11;295].

Main difference	***Spray drying***	***Fluid bed drying***
Feed characteristics	Fluid	solids
Residence time	5-100 sec	1-300 min
Particle size	10-500 µm	10-3000 µm

The critical performance criteria are very important when one decides to use spray drying. It is related to the physical properties of spray-dried food ingredients: particle size and shape, absolute and bulk densities, flowability, dispersibility, moisture content, appearance, active load and structural strength. Neglecting one of these criteria will result in an ingredient that will not perform properly in the final application [232].

The spray drying process involves: 1) formation of an emulsion or suspension of coating and core material, 2) atomization of the emulsion into a drying chamber containing circulating hot dry air, and 3) evaporation of moisture from the emulsion droplets when in contact with the hot air (Fig. 2.3) [118]. In the food industry, the core is generally a water-immiscible flavour, vitamin, animal fat, fish oil or plant oil. The core is emulsified in an aqueous solution of shell material until 1-3 micron oil droplets are obtained. It is crucial that the emulsion formed must be stable, not break before or during spray drying and have a low viscosity and thus can be pumped into the chamber [282].

The carrier is usually hydrated (can be by heating) until it reaches a chosen solids level. The solids level is typically maximised, and there is no need to add water. The upper limit of infeed solids is the viscosity at which the infeed cannot be adequately atomised, or if the material is not to be dried in the chamber. High infeed solids will produce large particles that may not dry, and thus impinge on the dryer wall, stick to the wall and ultimately burn on [232].

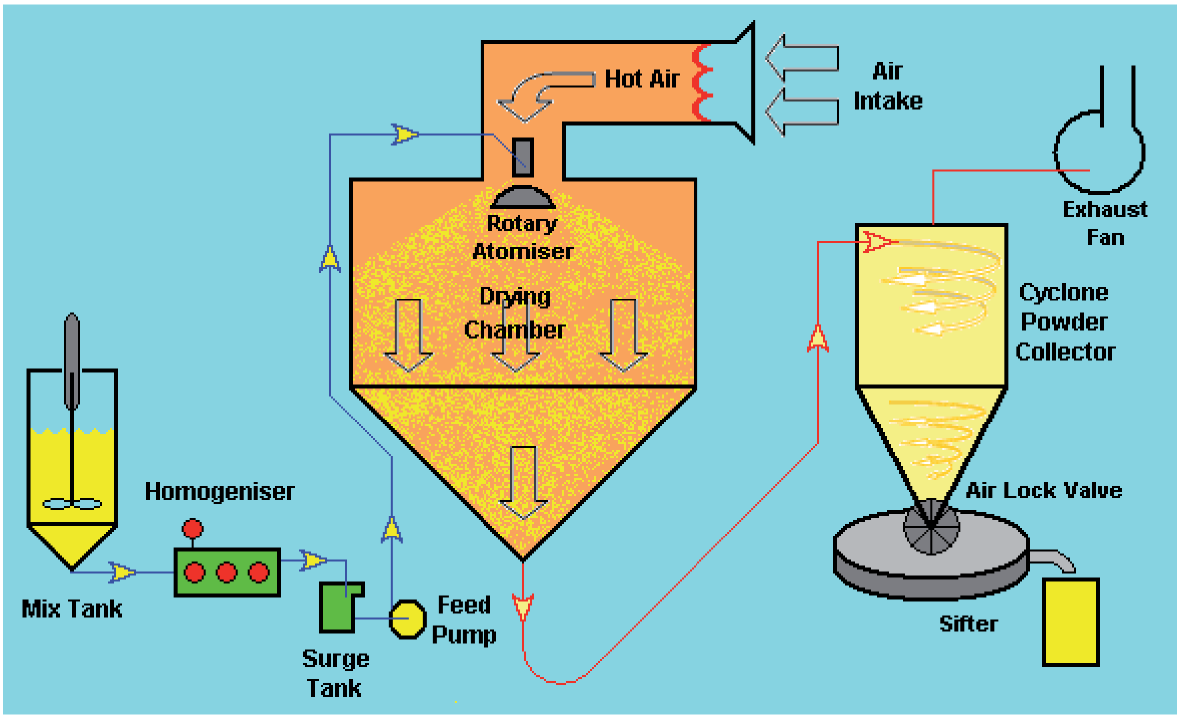

Hot Air
Air Intake
Exhaust Fan
Rotary Atomiser
Drying Chamber
Cyclone Powder Collector
Homogeniser
Air Lock Valve
Mix Tank
Feed Pump
Sifter
Surge Tank

Fig. 2.3 Conventional spray dryer

2.5.1.1 Atomization

The goal of atomization is to create a maximum heat-transferring surface between the drying air and the liquid to optimize heat and mass transfers. Atomization can be carried out by pressure or centrifugal energy through an atomizer. The higher the provided energy, the finer are the formed droplets. Increasing the feed rate for the same amount of energy used will increase the size of particles. This is also the case when viscosity and surface tension of the initial liquid are high [77].

2.5.1.2 Drying of droplets

Three consecutive steps for droplet drying are described by Gharsallaoui et al. (2007) based on the theory of drying [77]:

(1). Heat transfer causes the increase of droplet temperature up to a constant value. This value is defined as the air drying humid thermometer temperature.

(2). Evaporation of water from droplets occurs at constant temperature and water vapor partial pressure. The water diffusion rate from the droplet core to its surface is considered constant due to the rapid migration of water and is equal to the surface evaporation rate.

(3). A dry crust is formed at the droplet surface when the droplet water content reaches a critical value and the drying rate rapidly decreases. Drying is theoretically accomplished when the particle temperature is equal to the drying air.

According to the glass transition theory, if the evaporation process is rapid, the particle surface is glassy and will not stick to the wall or bottom part of the dryer. But for a certain type of matrix with low T_g (such as low molecular weight sugars), the surface of particles remain thermoplastic and causes stickiness (Fig. 2.4). The particle is in a completely glassy form when it is fully dried [20].

For any drying method, there is an optimum core concentration that can be encapsulated efficiently. The oil concentration (load) is represented by the term ‘core-to-wall’ ratio. Higher oil loads result in lower encapsulation efficiency [103;243;274] and higher surface oil content of the powder [104;141].

To prepare a stable emulsion, the exact calculation of dissolved solid content in the feed is crucial. High solid content in the prepared emulsion significantly increases the core retention by: (1) decreasing the time needed to form a semi-permeable membrane at the surface of the drying particle, (2) increasing emulsion viscosity which prevents the circulation movement inside the droplets and leads to rapid skin formation [231].

A point that must be underlined is that there is an optimum infeed solid content for the drying of food flavors and oils [234]. Adding more coating materials into a certain level of solid content exceeds its solubility and creates undissolved walls. This can also create a problem by increasing the viscosity. High viscosity leads to slow formation of discrete droplets during atomization and difficulties regarding droplet formation [231].

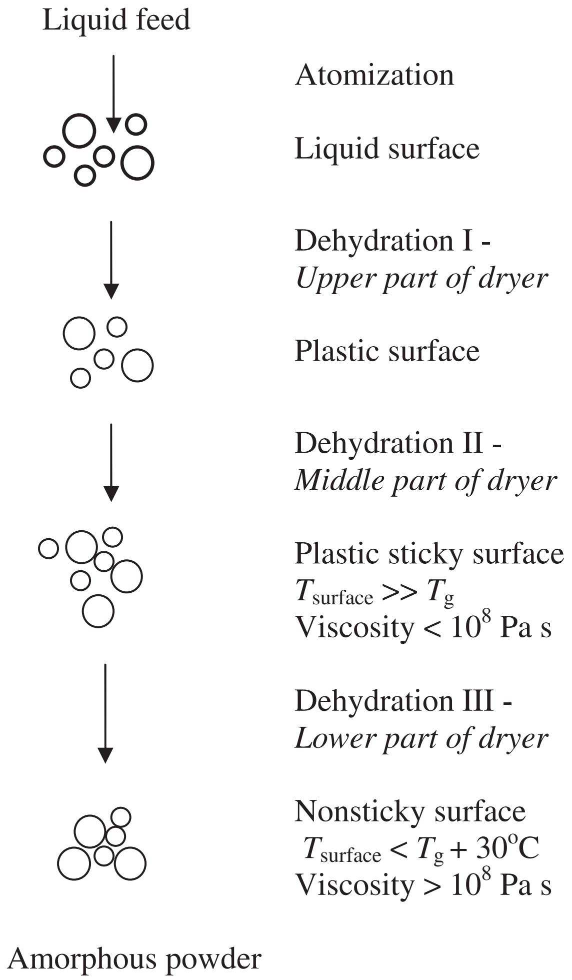

Liquid feed
Atomization
Liquid surface
Dehydration I -
Upper part of dryer
Plastic surface
Dehydration II -
Middle part of dryer
Plastic sticky surface
$T_{\text{surface}} >> T_g$
Viscosity < 10^8 Pa s
Dehydration III -
Lower part of dryer
Nonsticky surface
$T_{\text{surface}} < T_g + 30^{\circ}\text{C}$
Viscosity > 10^8 Pa s
Amorphous powder

Fig. 2.4 Particle surface behavior; T_{surface} is surface temperature of a droplet [20]

Emulsification methods in combination with the emulsion size are two important parameters in the production of a stable emulsion, and thereby to determine powder characteristics when subsequently dried by spray drying [122]. By using ultrasound homogenizer instead of Ultra-Turrax, the quality of emulsion can be improved, particularly when the wall material has low emulsifying properties and a weak viscosity such as maltodextrin. A proper emulsification method results in higher retention and better core loading [196]. Higher retention of butyric acid was also reported when using ultrasonic emulsification instead of Ultra-Turrax.

A successful spray drying process produces powders with good physicochemical properties. This can be achieved by forming a stable emulsion (by optimizing the factors mentioned above) and applying the right spray drying parameters. The latter includes the proper choice of inlet and outlet temperature, infeed temperature, atomization type, drying air flow rate and humidity, as well as the powder particle size [122].

The particle size of microcapsules is mainly determined by the physical properties of the emulsion (viscosity and solids concentration) and also by the type of atomization [65;123]. Nozzle atomization produces bigger particles than centrifugal wheel atomization, which can also be produced if the inlet air temperature is high and the difference between inlet and exit air temperature is low.

There are many critical reviews of fats and oils encapsulation [61;215;233;313]. Factors that influence the ability to encapsulate fat or oil include the fat/oil load, coating materials, and homogenization. The higher the oil load the poorer the encapsulation efficiency. Type of coating material also has a strong effect on encapsulation.

2.5.2 Freeze drying

Freeze drying or lyophilization is an attractive drying method for extending food shelf life [168] and was first developed to prevent the flavor and aroma losses that occur when a conventional drying method is used [41]. It is the best method for water removal using the lowest drying temperature than any other drying method to obtain highest quality of final products [100;230]. Freeze drying maintains the original structure of food, enables rapid complete rehydration, retains maximum volatiles and flavor retention [164].

Despite its advantages, freeze drying is known as an expensive drying method, particularly because of high operating and maintenance costs as well as long drying time under continuous vacuum, which increases energy consumption [230]. The cost of freeze drying is twice than that of vacuum belt drying and almost five times than that of spray drying. However, the high cost of freeze drying can be compensated if the products are in high demand. Some important examples of products in this category are freeze-dried instant coffee, and active enzyme preparations for cheese cultures, etc. [100]

Freeze drying requires very low pressure (vacuum) to obtain acceptable drying rates [164]. Figure 2.5 represents the phase diagram of water (pressure versus temperature) where the curve lines show the passage from solid to vapor (sublimation), or from liquid to vapor (evaporation) or from solid to liquid (fusion). Vacuum drying lies below the atmospheric pressure line (101.330 kPa), and freeze drying takes place by sublimation at a temperature lower than 0.01 °C and a vapor pressure below 0.612 kPa. If a product at a pressure and temperature corresponding to ambient conditions (point A) is to be dried by freeze drying, it will follow the line from A to D. It must be frozen first by decreasing its temperature, then the water vapor pressure should be lowered below the triple point pressure, and some heat should be supplied to help the ice to convert into vapor by sublimation.

2.5.2.1 Freezing

Freezing is removal of heat that lowers the temperature of foods below 0 °C. At this temperature, the ice crystals begin to form and the solutes present in intra- and extra-cellular fluids become concentrated in the remaining liquid water [66]. The freezing temperature is a function of solutes in solution. The freezing temperature of food is lower than that of pure water [189]. Freeze drying can be carried out at 4.58 mmHg pressure and 0 °C if the product contains water. Most foods can be freeze dried at a temperature below -10 °C and at a pressure below 2 mmHg [164].

Freezing starts by lowering the temperature below the point at which nucleation occurs. Nucleation is the formation of crystal embryos that functioned as the nucleus for crystal growth. The rate of crystal growth is controlled by the rates of mass and heat transfer. The movement of water molecules to the crystal boundaries is promoted by mass transfer and the transport of solute molecules away from these boundaries [132].

Two types of ice formation during freezing depend on how the freezing is taking place. Rapid freezing shortens the time of transition from nucleation temperature to the temperature close to the glass transition temperature in which the creation of a highly viscous environment is important. In contrast, at slow freezing, few nucleation sites are developed and formation of large ice crystals occurs [132]. During freezing, the network of ice crystals is formed, and this is particularly important for the primary and secondary drying steps.

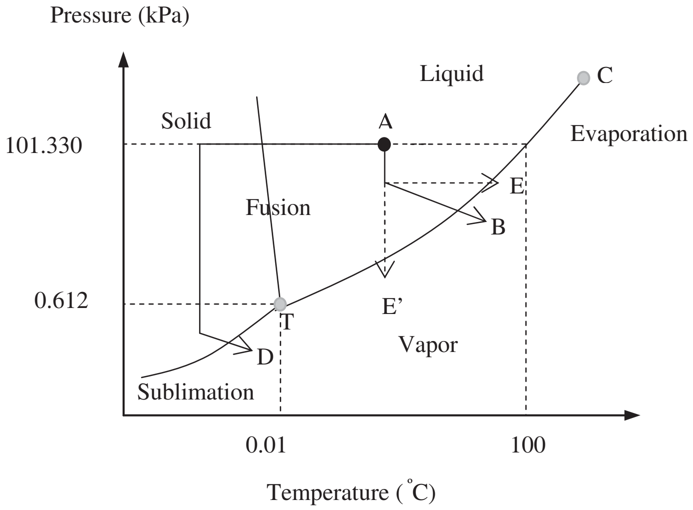


Fig. 2.5 Phase diagram of water. T is triple point of water at 0.01 °C and 0.612 kPa where liquid, vapor and solid coexist. C is critical point of water at 374 °C and 22 060 kPa; adapted from [230]

In the freezing step, the solvent (usually water) is separated from the solute to form ice, and as the freezing progresses, the solute becomes highly concentrated and creates a highly viscous amorphous matrix. The crystallization of solutes can be prevented by the rapid development of this type of matrix [132]. Freezing prior to freeze drying of food should be able to freeze the material to its maximally freeze-concentrated condition ($T_g^{'}$ is T_g at maximum freeze concentration), where maximum ice crystals have formed and the remaining unfrozen material has the lowest water content ($W_g^{'}$) [100]. Each solute has its specific glass transition temperature (T_g), and the mixture of solutes in food systems have a glass transition temperature depending on the solute mixture [132].

2.5.2.2 Primary drying

During primary drying phase, the sublimation front is formed. This front is boundary between the frozen and dried product. The heat must be transferred into the product to this front to accelerate sublimation, and the water vapor must be removed by mass transfer through the dried product [100]. The water vapor produced in the sublimation interface is removed through the outer porous layers of the product [189]. These layers are formed as a result of ice sublimation and increase as the drying proceeds [230].

The latent heat of sublimation is 2.84 MJ/kg or 1,220 Btu/lb and must be added carefully without increasing the product temperature above its melting point [100]. The drying rate at this stage is faster due to the availability of a large amount of unbound water in a frozen state.

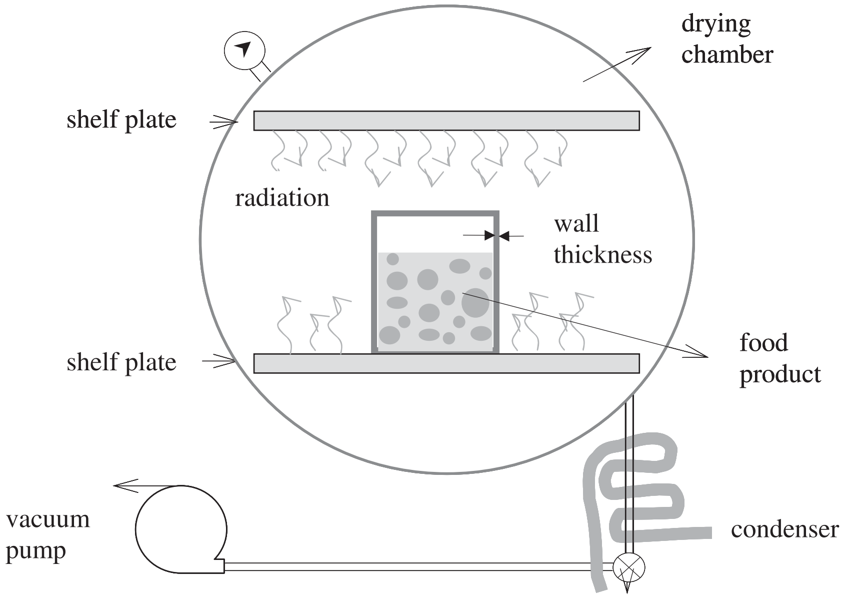


Fig. 2.6. Dehydration of food product by freeze drying; adapted from [230]

The freeze drying process (Fig. 2.6) is started when the product is placed in a vacuum chamber on a shelf plate. This bottom shelf plate supplies energy by conduction whereas the top shelf plate supplies energy by radiation. The conduction heat transfer from the bottom plate to the product container takes place through a contact points, and conduction is predominant within the product [230].

2.5.2.3 Secondary drying

The secondary drying begins when all the ice is sublimed out of the frozen product. For the drying process, heat is added at a slower rate considering that the moisture loss only takes place through diffusion of water molecules out of the freeze-dried matrix [100]. At this stage, the bound water, of which the main part is in an unfrozen state, must be dried. However, because the state is mostly unfrozen, the drying rate is very slow [295].

2.5.2.4 Structural changes of freeze-dried food

Changes in food structure occur during secondary drying. The important occurrence is called “collapse structure”. Collapse is defined as “a time-, temperature-, and moisture-dependent viscous flow that results in a loss of structure and a reduction in sample volume” [289]. This phenomenon takes place when the pockets where ice crystals that have sublimed disappear [100], and the highly porous solid matrix of the freeze-dried material can no longer support its structure against gravity [170]. The collapse structure is the result of a higher temperature in the sublimation front than the critical temperature. In the first stage, the collapse is localized in the pores in certain areas and progresses to the entire structure [19].

The collapse temperature (T_c) in freeze drying is known as the stickiness temperature (T_{sticky}) (Table 2.4). Therefore, the collapse of drying material in freeze drying is similar to the stickiness during spray drying [19]. The T_c is equivalent to $T_g^{'}$, which is the glass-transition temperature of highly concentrated unfrozen liquid in the product being dried [284;285].

Table 2.4 Glass-transition temperature ($T_g^{'}$) and collapse temperature (T_c) of selected aqueous solutions [19].

Solution (w/w)	$T_g^{'}$ (°C)	T_c (°C)
Sucrose, 10%	-32 (-32.9)	-32 (-32)
Maltodextrin DE 5-8, 10%	-9 (-9.5)	-7 (-10)
Maltodextrin DE 20, 25%	n.a.	232.22[a]
Sucrose 5% + maltodextrin (DE 5-8), 5%	-24	-19 (-20)
Orange juice, 23%	n.a.	-24[b]
Trehalose	-42[c]	n.a.

[a]cited from [289]; [b]cited from [15]; [c]cited from [266]

If collapse occurs during freeze-drying (when the temperature of sublimation is above the collapse temperature), the ice crystals seem to dissolve rather than to sublime. Food that collapsed loses its shape by becoming a highly viscous liquid, and there is loss of flavor and low aroma retention. When the ice crystals fail to sublime, the capillaries are eliminated. Severe collapse completely closes the capillaries [170;289].

To obtain an optimal process, freeze drying should follow a path (Fig. 2.7). Freezing should produce a maximum amount of ice, and the primary drying temperature should remain below the collapse temperature. The temperature and moisture content should be maintained such that the temperature is just below the glass transition temperature. Collapse may occur if the temperature exceeds the glass transition temperature

In the freeze drying of liquids, the collapse temperature depends on the viscosity of the unfrozen portion of the solution. According to the amorphous viscosity theory, the matrix is considered as a concentrated amorphous aqueous solution. If the temperature can be maintained below the collapse temperature, the matrix is sufficiently viscous and behaves like a solid or acts as a structurally rigid solid. The viscosity is correlated to moisture content and temperature [15]. Maintaining freeze drying temperatures below or close to the maximally freeze-concentrated glass-transition temperature ($T_g^{'}$) results in a material in the glassy state, and hence the shrinkage or collapse is negligible. This type of process produces a very porous product [225]. However, collapse occurs if the temperature in the frozen zone increases above the collapse temperature. Then the viscosity of concentrated amorphous solution decreases due to the dilution with water from the ice melting [15]. An increase in solute molecular weight or addition of macromolecules increases the collapse temperature of the freeze-dried solution. This means that the product will tolerate higher temperatures at a given moisture content without loss of structural qualities [289].

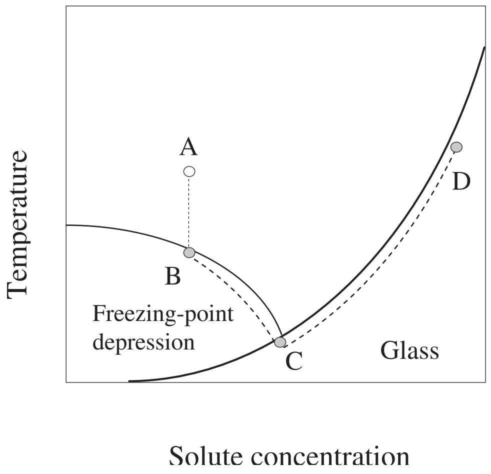


Fig. 2.7 Processing path of optimal freeze drying; adapted from [100] A is product to be freeze-dried, A-B is freezing process , C is sublimation of ice, C-D is final drying .

The glass-transition temperature is usually reported not as a single value but as a midpoint of the glass transition temperature (Table 2.5) and depends on the material [238]. The heterogeneity and multi-component nature of powder (in encapsulated flavors, for instance) such as types of matrix and the amounts of liquid flavor, play an important role in the observed T_g value and collapse characteristics [26].

Table 2.5 Glass-transition temperature (T_g) of freeze-dried sugar/gelatin matrices [134]

Matrix	T_g (^{o}C)	
	Sugar:gelatin (9:1)	Sugar:gelatin (8:2)
Lactose:gelatin	106	108
Maltose:gelatin	90	92
Sucrose:gelatin	67	70
Trehalose	112[a]	
Trehalose/cornstarch	136[a]	

[a] cited from [266]

2.5.2.5 Entrapment of oil droplets during freeze drying

A food matrix can only retain oil droplets and or flavors during drying if it is in the glassy state [156]. In freeze drying, sublimation results from the replacement of the ice layer by air, and therefore the droplets remain entrapped in the matrix (Fig. 2.8). This occurs within the solid layers, which are below T_g, and the entrapment of oil is retained as long as the storage conditions of material are kept below T_g. If the temperature is raised and T_g is exceeded, loss of flavor cannot be prevented [132].

The complete loss of flavor occurs rapidly if crystallization of the matrix takes place, and the matrix molecules assemble in crystal form and thereby exclude the oil and flavor. After elimination of its porosity, the matrix becomes dense, and flavor loss develops slowly by diffusion through the dense matrix.

Kaushik and Roos (2008) examined the entrapment of lipid in the glassy matrices formed by freeze drying. They observed that the T_g of sugar/gelatin was well above room temperature (more than 67°C). At this temperature, the encapsulated lipid is well protected in the glassy state of the matrices (i.e., sugar and gelatin mixture) because the matrices exhibit very high viscosity, and the mobility of the compound is extremely low.

Therefore, the amorphous glasses retain the dispersed compound in the emulsion during and after freeze drying [135].

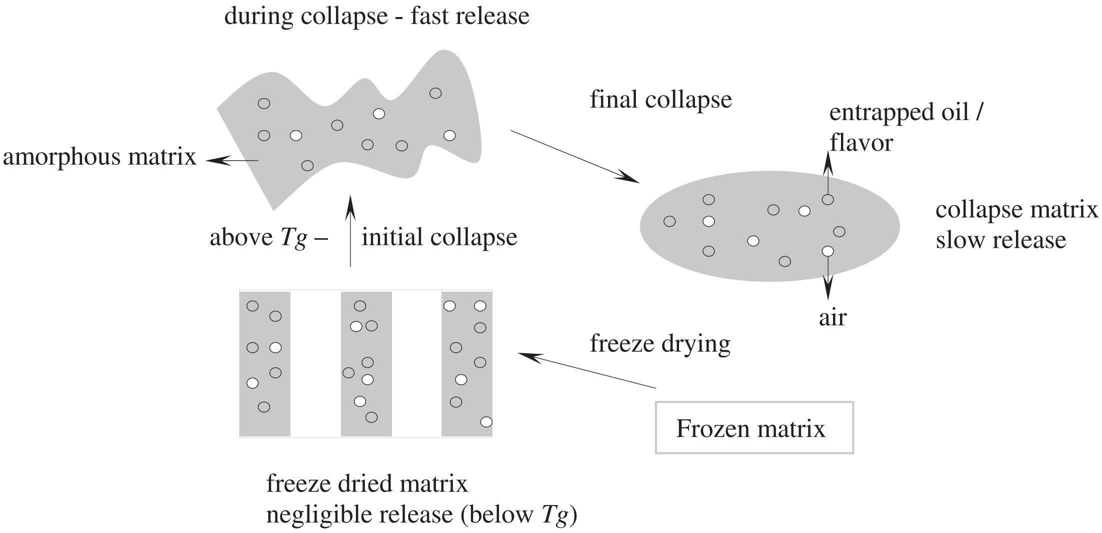

during collapse - fast release
amorphous matrix
above Tg –
initial collapse
final collapse
entrapped oil / flavor
collapse matrix slow release
air
freeze drying
Frozen matrix
freeze dried matrix negligible release (below Tg)

Fig. 2.8 Mechanism of collapse of freeze dried matrix above glass-transition temperature (T_g) and oil/flavour release; adapted from [132]

In a series of studies, Heinzelmann et al. (1999; 2000a,b) explored fish oil microencapsulation by freeze drying. The first investigation reported that the addition of a mixture of antioxidants (ascorbic acid, lecithin, and tocopherol) improved the product shelf life. The powder showed a better oxidative stability than the pellets, thus indicating that the grinding process did not decrease the stability. The high shear force inside the extruder caused mechanical stress, which damaged the milk proteins and thus reduced the protection of fish oil [97;98]. The second investigation examined several process parameters in the oxidative stability of fish oil powder during storage at 25 ^{o}C. The variables were microencapsulation matrices (sodium caseinate, lactose and maltodextrin), homogenization pressures (1 pass at 10 MPa and 3 passes at 40 MPa), and freezing rates (slow, medium and fast). In the slow freezing process, 50 ml emulsion was frozen in a petri dish at -20 °C for 10 h. The medium freezing process involved pumping the emulsion into a self-conveying scrape cooler (freezing extruder) where the twin screws were covered by a jacket cooled with a refrigerant (-40 °C). In the fast freezing process, the emulsion was dropped into stirred liquid nitrogen using a burette [99].

Although the authors found that low homogenization pressure resulted in larger oil droplet size, lower total oil content, higher free oil content and lowest ME, they concluded a negative correlation between oil globule size or microencapsulation efficiency with the storage stability. The ME increment was inversely related to the freezing rate. The highest ME corresponded to slow freezing rate, followed by medium and fast freezing. The overall results indicate that high ME did not necessarily correlate with high storage stability. Microcapsules prepared from fast freezing exhibited the longest shelf life (12 weeks) [99].

Velasco et al. (2006) found that samples prepared from oil from which its natural antioxidants had been removed had a faster oxidation rate in their free oil whereas in oils containing natural or added antioxidants, the encapsulated oil fraction was instable [299]. The surface or free oil is readily extracted with organic solvents without matrix disruptions. The encapsulated oil is embedded inside the matrix, therefore it is necessary to break up the solid structure of the matrix in order to extract the oil [176]. In a recent study, the authors investigated the influence of relative humidity on oxidation of free and encapsulated oil fractions in freeze-dried microencapsulated oils. For samples without antioxidants, the oxidation was faster in the free oil than in the encapsulated oil, as the oxygen diffusion through the matrix became a determining factor in the encapsulated oil fraction [298]. However, for samples with antioxidants, the diffusion of oxygen through the solid matrix was not a limiting factor [296]. In such system, the oxidation rate was faster in the encapsulated oil than in the free oil because the oxygen supply was higher than the oxidation rate of the encapsulated oil fraction [298].

Free oil consists of discontinuous oil deposits and oxidized as lipids in the continuous phase. The encapsulated fraction has individual oil droplets, which can be oxidized at different rates. Under conditions of no oxygen restriction, the overall surface-to-volume ratio of free oil would be lower than that of the encapsulated oil, thus the oxidation rate in the latter fraction is faster than in free oil [76;299].

2.5.3 Spray granulation

The spray granulation process uses the basic principle of fluidised bed equipment in which the gas passes through the bed material and, at a certain velocity, the bed starts to fluidise. Similar to this principle, a novel apparatus was developed, i.e. a ‘spouted bed’. It was originally developed to fluidise larger particles with a high-velocity spout of gas that penetrates to the bed and fluidise the particles upward [119].

2.5.3.1 The spouted bed

The spouted bed was originally designed to dry coarse particles. In its development, it was used in many applications, including drying of suspensions, emulsions and paste-like materials into inert bed particles, granulation, particle and tablet coating, reaction granulation, gas cleaning, solid blending, cooling of fertilizers, and many others [178;179].

The spouted bed has three distinct regions: the spout, the annulus and the fountain. The apparatus consists of a cylindrical column with a conical base. An orifice is fitted to the conical base through which the spouting fluid is injected. The ascending flow particles inside the chamber are developed by the high fluid flow rate which finally forms a fountain. These fountain particles are directed toward the outer part of the spout and fall into the annular region [179;237]. The spout is the central channel in the system, and in the spout region the particles move in the same direction as the gas flow. Due to the high velocity of the gas, particles move as in a pneumatic conveyor. The peripheral region is the dense region known as the annulus, where the particles move counter- current to the gas. The term fountain is used to describe the mushroom form above the annulus, and in this region the particles move in a decelerated regime subsequently falling into the annulus.

The main characteristics of the spouted bed are a high rate of solids circulation, intense mixing of particles, intimate fluid-particle contact and high rates of mass and heat transfer. Despite the variable cross-section area of the apparatus, which is a function of the apparatus height, the continuous circulating motion in contrast to the random particle motion in a fluidized bed are the main differences between the conventional fluidized bed and spouted bed [87]. Its ability to produce good mixing of solid particles is due to the intensive heat and mass transfer between the gas and the solid phase [86;88].

The spouted bed technology has been modified to overcome the limitation related to the existence of a maximum bed height for steady spouting and problems related with upscaling. For this purpose, the conical spouted bed, jet spouted bed with high porosity, spouted bed with draft tube, two-dimensional spouted bed, and slot spouted bed were invented [237].

Many articles related to spouted bed drying report the application of this process in food processing, i.e., drying of seeds and grains, and drying of pastes and suspensions with inert particles. The principle of drying suspension on inert particles is feeding the paste or suspension over the bed of inert particles, which are already moving in a steady spouting regime. As the process proceeds, the inert particles are coated with a thin film, and the film fractures due to its fragile form and the inter-particle collision inside the bed. Finally, the inert particles are released and the powder is then collected by a cyclone or other separation equipment. The process of deposition, drying and removal of film from inert particles takes place only if the movement of the spout is not disturbed by particle agglomeration [178;179;237]. Drying on inert particles can be performed in variety of fluid beds such as classical fluid bed, spouted bed, spout-fluid bed, jet spouted bed, vibrated fluid bed, cyclone dryer, swirling stream dryers, impinging stream dryer or pneumatic dryer [150].

To date, the application of drying with inert particles for food pastes has been studied intensively. Some important examples are drying of vegetable starch [16], concentrated skim milk and non-homogenized whole milk [177], tropical fruit pulps [186], mango pulp [40], etc. According to literature reviews, the spouted bed technology is developing rapidly, not only with respect to the enormous modification of the apparatus but also regarding its application in various fields, including the pharmaceutical and feed. For this research, spray granulation of fish oil emulsion was performed using the Glatt-ProCell Spouted Bed apparatus equipped with an adjustable gas inlet.

2.5.3.2 Glatt-ProCell spouted bed

A typical ProCell spouted bed has a rectangular shape with an extended process chamber. The bottom-end part of the chamber has inclined side walls, called the inside contour, which are supported by two cylinders of the gas throttle shaft positioned on top of a base plate (Fig. 2.9). The two cylinders are separated by a centre profile and form two parallel gaps which functioned to divert the high-velocity fluidized gas entering the chamber. The cylinders are adjustable by rotating them so that the free cross-section area of the gas inlet can be varied. This condition allows control of the gas inlet velocity and its distribution to prevent clogging and a dead zone. The adjustment can be made without interrupting the process [86-88;119].

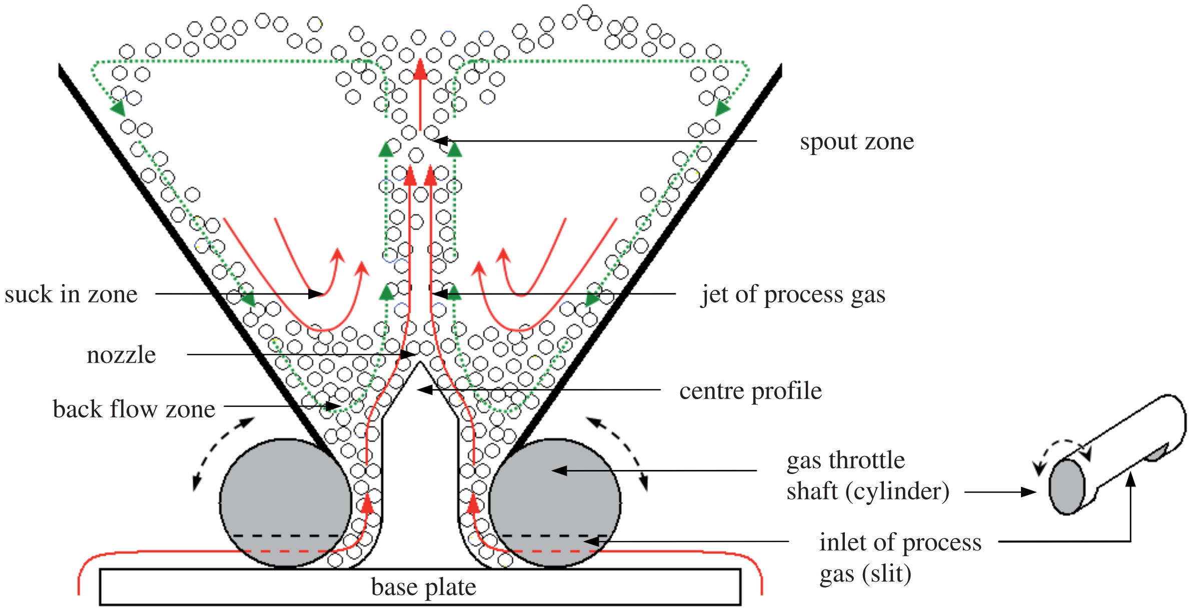

spout zone
suck in zone
jet of process gas
nozzle
centre profile
back flow zone
gas throttle
shaft (cylinder)
inlet of process
gas (slit)
base plate

Fig. 2.9 Glatt-ProCell spouted bed; adapted from Gryczka et al., 2008 [88], reprinted with permission

When the process has started, the fluidizing gas enters the equipment from the two slits horizontally and is diverted upwards by the centre profile. After passing this profile, the two flows are united and form a jet gas and pass the apparatus vertically from the bottom part to the top. The jet gas flow fluidizes seed particles (which have been inserted before the process started) and bring up the particles. At this time, the liquid (in the form of emulsion, suspension or high-viscosity liquid) can be sprayed in by a nozzle located above the centre profile in the central region. The spraying direction is usually from the bottom to the top (as in the Würster fluid bed), but top spray is also possible. In the upper process chamber, the particles are separated to the sides and transported back to the lower area toward the gas entry zone due to the slope of the inner profile. At this point, the particles fluidized by the core jet into the spout zone and re-circulated [88].

The regions inside the ProCell spouted bed can be divided into jet zone and back-flow zone. The jet zone is the area above the middle profile where the fluidization gas flows with a high velocity and streams up the particles. The back-flow zone consists of the two zones adjacent to the jet zone.

Berquin (1961; 1966) stated the principles of granulation process in the spouted bed. The solution or emulsion is atomized into the bed, which consists of product granule nuclei and is spouted by the heated gas. The products (rounded granules) of uniform structure are obtained by a mechanism of layer-by-layer growth [17;18;178]. The high velocity of the inlet gas prevents particle agglomeration during layer deposition. The latter effect is permissible in a conventional fluid bed [178;179].

Gryczka et al. (2009) underlined the advantages of the Glatt-ProCell spouted bed over the conventional fluidized beds. The apparatus is designed to fluidize various forms of particles, including fine, large, and irregular particles, and can be adapted to fluidize particles with a wide range of size distribution. As ProCell characterizes by a low drying temperature and possibility to reduce residence time in the drying chamber, therefore it is suitable for drying heat-sensitive ingredients (Fig. 2.10).

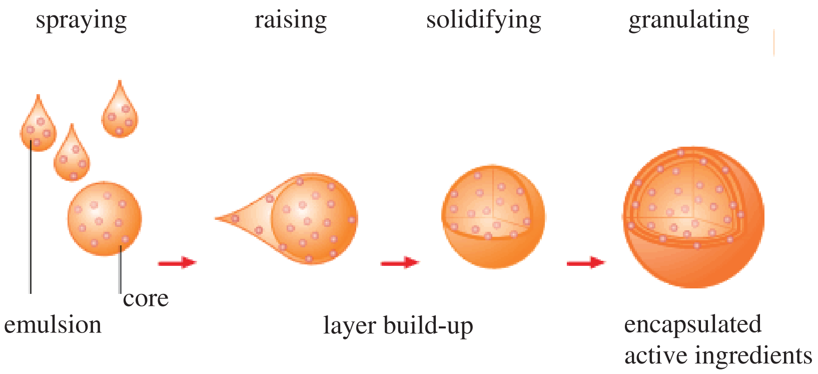


Fig. 2.10. Principle of spray granulation using Glatt-ProCell spouted bed [116] (cited from IPC Process Centre's website, 2010: reprinted with permission)

2.5.4 Fluid-bed film coating

The term film coating refers to the encapsulation technologies that utilise a spray process to deliver film material to a core particle. The technique is based on the use of fluidising air to provide a uniform circulation of particles past an atomizing nozzle. As the atomized coating materials contact the particles, fluidising air evaporates solvent or solidifies coating solids on the particles as part of a developing film. This process (Fig. 2.11) is continued until the desired film thickness is achieved [74].

The fundamentals of fluid bed coating and granulation theoretically had been described by Loeffler [162], and its mechanism explained by Uhlemann [293]. The experimental works related to the coating of single particle has been explored in detail by the group research at the University of Karlsruhe, Germany [158;159;213]. Recently, very good reviews of the state-of-the art of air-suspension particle coating in the food industry have been published by Werner at al. [303;304].

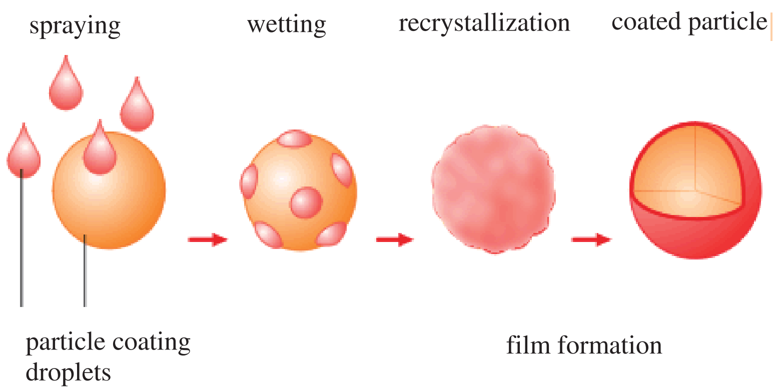


Fig. 2.11. Principle of fluid bed coating by Wurster Process (cited from IPC Process Centre website, 2010: reprinted with permission) [115].

Two terms have been identified in this field: fluidized bed spray granulation and fluidized bed film coating. The difference is the size of the particles to be coated. In the first process, a solid containing liquid is transformed into granules by atomizing it into seed particles that have the same composition as the dissolved component. The liquid can be a solution, suspension or melt and dried continuously in one step in which the solvent is evaporated thus solid is deposited on the surface of seed particles. Particle growth can take place by two ways: agglomeration or surface layering. The latter is also known as the ‘onion skin’ layered structure [158;159;213;318;319]. The main objective of the film coating process is to form individual particles in which each of the particle is well distributed and uniformly coated [291]. An existing core has a larger particle size compared to the seed particle in the granulation process. Although the particle size differs from that of the starting particles, the principle steps in granulation and coating process are identical.

In each case, the particle growth is determined by the successful collision between a droplet of the liquid and the seed or core particle. Loeffler (1988) divided particle deposition into two steps: (1) droplet movement to the particle surface, and (2) droplet adhesion on the surface of the particle. The ability of a droplet to come into contact with a particle will govern whether a droplet captures or bounces, and this is controlled by the impingement-efficiency (ε). After the initial collision, droplet deposition is determined by the adhesion-probability (h). Thus, the collection efficiency (φ) can be calculated as the impingement-efficiency and adhesion-probability [162]:

$$\varphi = \varepsilon \, . \, h$$

The fundamentals of granulation have been described in section 2.5.3.1 (spouted bed), therefore in the following only the principles of the fluidized film coating process will be discussed. The basic principle of film coating is atomization of the liquid spray through a nozzle into a bed of fluidized particles. The coating medium is the solute consisted in the spray, which is usually dissolved in a solvent. The solvent is the vehicle for transporting the coating material, evaporated by the fluidizing gas and resulting in solute layers on the particle surface [304]. Selection of a suitable coating material depends on the core characteristics [45] and the process technology to be used [144].

The coating quality can be measured by the microscopic level and as a function of coating mass uniformity and coating morphology. The coating mass uniformity is related to the amount of coating material received by each of the particles [291] and can vary depending on (1) the number of the particles passing through the spray zone, and (2) the amount of material deposited per particle per pass through the spray zone [30]. The second case might happen more significant due to the sheltering effect when the particles are further away from the source of the spray [31].

The coating morphology refers to variation of the physical properties of coating in each particle that receives the same amount of coating material. One important factor to determine coating morphology is the type of coating material. Hydrolysed gelatine produced fractures on the coating surface [47]. Maltodextrin (DE18) formed better coating quality without cracks and holes. The imperfections (fractures and cracks) can be caused by the shrinkage during drying as the result of stress fractures in the coating, and this coating rigidity can be reduced by the addition of plasticizers [47]. Non-continuous film was also formed by carboxymethylcellulose (CMC) due to its higher hygroscopicity, thus the coating was of poor quality [45].

Drying temperatures also play a key role in the coating morphology. Particles coated with lactose at lower air temperature (70 ^{o}C) had a crystallised surface morphology, whereas those dried at 120 °C produced an amorphous surface structure. The crystal structure exhibits better protection because of the lower diffusion rate to the crystalline matrix and its lower hygroscopicity compared to that of the amorphous matrix [158].

Particle coating is a complex process [145] and consists of three major operations: fluidization, atomization and drying [169]. Three techniques exist, namely bottom-spray (Würster), top-spray, and tangential-spray processes. The main difference in these methods is the position of the nozzle. The top-spray and tangential-spray processes are configured more appropriately for agglomeration or granulation application [74]. Since this research uses the Würster coating to encapsulate fish oil granule, only this process will be focussed on in the following.

The principles of Würster process are 1) the spray pattern is concurrent with the air feed, 2) the particles to be coated are accelerated inside the Würster tube and fed through the spray cone concurrently, 3) the particles move further apart as they pass through the atomized spray from the nozzle and into an expansion region of the apparatus, 4) the fluidised air evaporates coating materials onto particles prior to particle-particle contact, and 5) the coating process is uniform, even, and without agglomeration (Figure 2.12).

Werner et al. (2008) identified the life-cycle of a single coating droplet and a particle in the Würster process as the 10 micro-level processes. They are (1) particle motion, (2) atomization, (3) droplet-particle collision, (4) droplet impact and adherence, (5) droplet impact and spreading, (6) infiltration, (7) drying, (8) film formation, (9) layering, and (10) inter-particle agglomeration [198].

2.5.4.1 Particle motion

The Würster process (Fig. 2.12) starts with particle motion in the centre of the bed. The particles are entrained upwards by the fluidising gas to the upbed region and reach the expansion zone. At this point, the gas velocity decreases causing the particles to fall back to the annular zone where they are finally transported back pneumatically to the upbed region. The whole cycle is then repeated [303]. An important observation by Kleinbach and Riede (1995) [144] was that increasing gas velocity resulted in an increase in coating quality. This was because the higher velocity of air lowered the residence time of particles in the spray zone, thereby causing a decrease in particle exposure time in the high humidity area, thus preventing particle dissolution

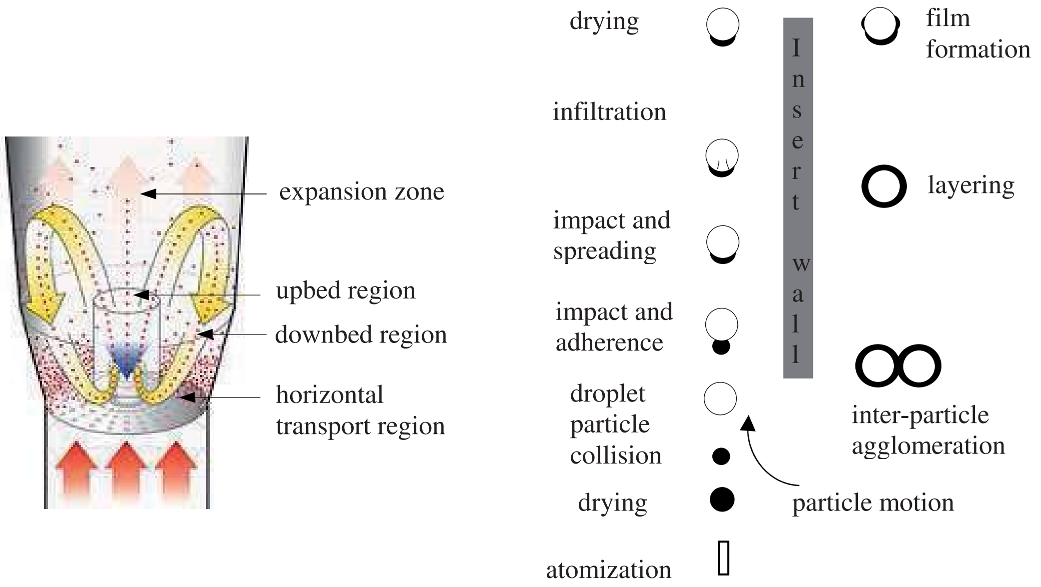

expansion zone
upbed region
downbed region
horizontal transport region
drying
infiltration
impact and spreading
impact and adherence
droplet particle collision
drying
atomization
Insert wall
film formation
layering
inter-particle agglomeration
particle motion

Fig. 2.12 Würster coating process [80] and steps in particle coating [303].

○ = particle; ● = atomized droplet.

2.5.4.2 Atomization

In order to obtain a uniform coating, the atomization system must produce a fine spray so that the droplets are small enough before colliding into the surface of the particles. Small droplets are essential for wetting, improved spreading, minimized particle agglomeration and resulting homogeneous final coating. The most common type of atomizer for particle coating is the dual-fluid nozzle, which applies high pressure gas [89;291]. The size of droplets relative to the particles to be coated is also an important variable. Lower occurrence of agglomeration can be achieved by higher particle/droplet size ratio [169]. Guignon et al. (2002) underlined that for coating purposes, the minimum particle/droplet size ratio should be at least 10, i.e., the droplet size should be around 5-10 µm in order to provide good coating for 100 µm particles [89].

2.5.4.3 Droplet-particle collision

Löffler (1988) exemplified the droplet-particle collision as dust deposition on the collector surface, which takes places by droplet transport to the particle surface and the adhesion on the surface [162]. Guignon et al. (2002) reported that droplet transport to the surface occurs by interception, inertia and diffusion. In particle coating, the collision by inertia is dominant where the particle surface and the droplet from the nozzle are in one path (Fig. 2.13) [89].

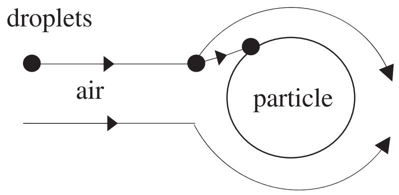


Fig. 2.13 Inertia: droplest diverge from nozzle through stream of air [89]

2.5.4.4 Droplet impact and adherence

The adherence of a droplet on the surface of a particle is started by the action of droplet to wet the surface, to the greatest extent spread the surface transversely without splashing and without move back (recoil). A number of factors control the adhesion efficiency, including droplet and particle momentum at collision, angle of incidence of both, liquid spray and inter-facial properties, and particle surface structure [89]. The adhesion will fail if, before colliding with the particle, the droplets have dried [46]. Another important point is the drying time of the wetted surface to a non-sticky state and the surface tackiness during drying. Particles that are moisturized by droplets and become wet should not come into contact with other particles, otherwise agglomeration might take place.

2.5.4.5 Droplet impact and spreading

The ability of droplets to spread across the particle surface is very important for determining the success of the coating process after impingement and droplet adhesion [279]. According to Werner et al. (2007), there are 4 possibilities of droplet impact on a flat dry surface (Fig. 2.14):

(1) Droplet rebound occurs due to high droplet velocity and causes drying of droplets (2) Droplets do not spread after adherence or there is limited spreading. A thick-pancake spreading might take place, and this may result in longer processing time; a greater quantity of coating material is needed to cover the whole surface area.

(3) Splashing may arise if the droplet impact velocity is too high during impingement.

(4) Droplet ejection can take place if the droplet recoil velocity is too high.

All these phenomena (rebound, limited spreading, splashing and droplet recoil) may result in a decrease in the protection of the particle surface by the coating layers because of the existing of voids in the coating deposit [303].

2.5.4.6 Infiltration and drying

When a droplet spreads on the porous particle surface, infiltration may take place. This can be considered as a loss if the infiltration time is the same or more rapid than the spreading time [303]. The spreading and infiltration time is similar to the inkjet droplet impact on a powder bed [105], but for food coating, it specifically depends on the properties of the coating solution, the interaction with particles, the pore size distribution and porosity. The rate of infiltration will decrease if the surface tension is lowered. Lowering the extent of droplet recoil results in a reduction of droplet splashing and in greater spreading [214].

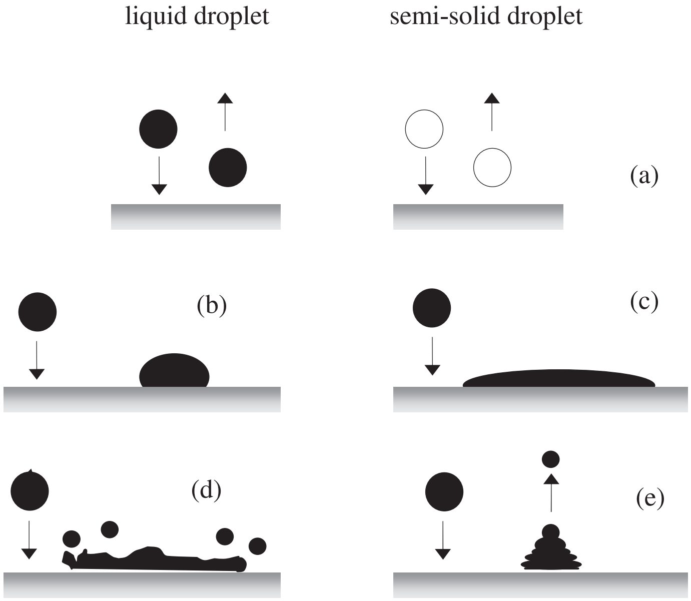


Figure 2.14 Impact of a droplet collision on a dry-flat surface: (a) rebound of liquid and solid droplet; (b) formation of thick pancake; (c) think pancake; (d) splashing during spreading; (e) droplet recoil (ejection), adapted from [303].

Drying of coating layers in a fluid bed is described as the transport of water to the surface of drying particles by diffusion [64] expected to occur in the upbed region. The drying time can be controlled by adjusting two factors, i.e., drying air condition and particle circulation time. The first factor includes drying air temperature, velocity, and outlet relative humidity, while the latter can be controlled by manipulating the air velocity, Würster height and partition gap.

Many researchers in this field are concerned about droplet over-drying before adherence, as it decreases the coating level. To achieve a successful coating process, surface stickiness is expected to enhance the droplet-particle adherence. This is possible considering that droplet drying takes place in an instant immediately after the droplets are formed [5]. The appearance of cracks and fissures are affected by the rate of drying and mechanical stress development during the formation of film coating [304].

2.5.4.7 Film formation and layering

If the solvent in a polymer solution is evaporated and the solubility limits are exceeded, the formation of films can be formed by the chain entanglement [149]. A film can be developed if the polymer has significant solubility in the solvent. The formation of a continuous and coherent film is crucial in entire particle surface so that the coating layer can function as a diffusion barrier. Thin and coherent coating can be achieved by high droplet moisture content, unimpeded spreading and coalescence of droplets upon adherence to the particle surface, and low drying rate. On the other hand, ‘a raspberry-type’ coating morphology can be observed if droplets experience an over-drying condition resulting in less protection to the particle being coated.

Droplet and particle coalescence might be fail if the surface tension and solution viscosity are too high. Imperfections, film wrinkles, cracks and fissures can be minimized by the addition of a plasticizer of polymer blending to increase plasticity. Therefore, a continuous cycle of droplet impingement, coalescence, adherence, spreading and drying is essential in order to build coherent layers over time in a film coating process [31].

2.6 Coating materials (encapsulation matrix)

The initial step in encapsulating food ingredients is the selection of a suitable coating material. The coating substances can be selected from various natural or synthetic polymers (Fig. 2.15). The properties of ideal matrices are good rheological properties, ability to disperse or emulsify the active material, being non-reactive with the material to be encapsulated, and ability to seal and hold the active material during processing [255].

The kind of material appropriate for the encapsulation of foods or food ingredients is very much dependent on the kind of material to be encapsulated. These materials have to show excellent film-forming and barrier properties to give sufficient protection. There are two groups of shell materials: hydrophilic (water-soluble) and hydrophobic (oil-soluble) [247].

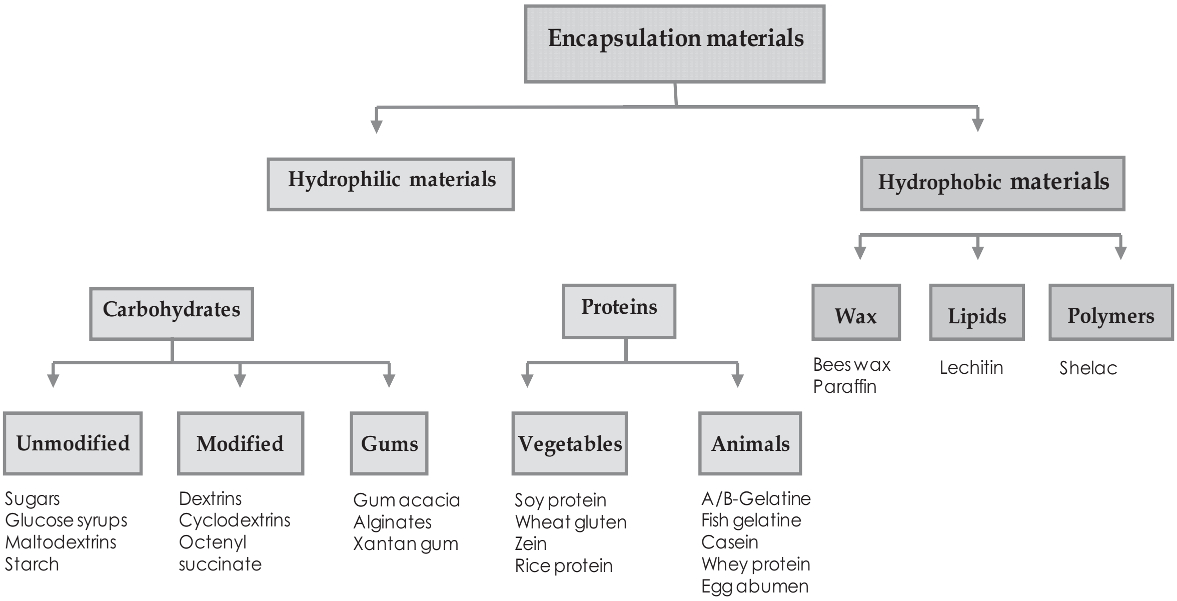

Encapsulation materials
Hydrophilic materials
Hydrophobic materials
Carbohydrates
Proteins
Wax
Lipids
Polymers
Bees wax
Paraffin
Lechitin
Shelac
Unmodified
Modified
Gums
Vegetables
Animals
Sugars
Glucose syrups
Maltodextrins
Starch
Dextrins
Cyclodextrins
Octenyl
succinate
Gum acacia
Alginates
Xantan gum
Soy protein
Wheat gluten
Zein
Rice protein
A/B-Gelatine
Fish gelatine
Casein
Whey protein
Egg abumen

Fig. 2.15 Choices of shell materials for microencapsulation; adapted from [247]

For spray drying purposes, one can divide the shell materials into four classes: hydrolysed starches, emulsifying starches, gums and proteins (gelatine, casein, soya protein and whey protein). Hydrolysed starches are very soluble, inexpensive and exhibit low viscosity in solution. The higher dextrose equivalent (DE) hydrolysates will add substantial protection against oxidation. However, this group has low emulsifying capacity and marginal retention of volatiles. The emulsifying starches provide excellent emulsification properties but poor protection of the active core against oxidation. Gum, e.g., gum acacia, is a very good emulsifier, is bland in flavour but may or may not offer protection against oxidative deterioration depending upon the gum. Proteins have excellent functionality in terms of emulsification abilities even though relatively high cost. Because of the high emulsification properties, proteins are important for encapsulation of high loads of lipophilic materials such as fish oils, butter and shortenings [232]

2.6.1 β-Cyclodextrin

Cyclodextrins (CDs) are seminatural products of cyclic oligosaccharides produced from amylase fraction of starch by the action of cyclodextrin glycosyltransferase (CGTase), an enzyme produced by several organisms such as *Bacillus macerans*. The three major cyclodextrins are built up from α-1,4-linked D-glucopyranose units. The α-Cyclodextrin (α-CD) consists of six glucopyranose units, β-Cyclodextrin (β-CD) of seven such units, and γ-Cyclodextrin (γ-CD) of eight units [249;269].

The molecular configuration of a CD is a toroid, formed by 6-8 glucose residues with the interior of hydrophobic and the exterior of hydrophilic. The monomers are connected to each other in a doublenut-shaped ring, giving cyclodextrin a hollow cavity [255]. All the primary hydroxyl groups are in the narrower base, while the secondary hydroxyl groups are located on the wider base of the toroid. Due to the absence of the hydroxyl group inside the circle of the toroid, this area is reported to have a non-polar property. In a hydrated condition, the cavity of α-, β-, and γ-CDs can accommodate 6, 11, and 17 water molecules, respectively. These water molecules can be easily replaced by guest molecules, and the complexes exist either in a solution or in a crystalline state [217].

Though the cavity is non-polar, CDs can include other molecules within their structural cavity ranging from polar compounds (alcohols, acids, amines and small inorganic anions) to apolar compounds such as aliphatic and aromatic hydrocarbons. While the interior is welcoming for the guest molecules, the exterior helps CDs to be solved in water [93]. Cyclodextrins are well known for forming inclusion complexes with many flavours, colours and vitamins. These inclusions will reduce loss by volatility or attack by oxygen and light [312]. The inclusion complexes are stable and can change the physical and chemical properties of guest molecules [255].

β-Cyclodextrin is an enzymatically modified starch consisting of seven ring-structured glucopyranoses [224]. β-Cyclodextrin is reported by many authors to be a good shell material for flavour microencapsulation. Molecular encapsulation of flavour with β-cyclodextrin was reported to improve the retention of pre-added flavour during extrusion [317]. For encapsulation properties, β-cyclodextrin forms a stable capsular structure, though it has generally lower water solubility compared to microencapsulated samples [255].

The diameter of the β-cyclodextrin cavity is larger on one side, particularly where the secondary hydroxyl groups are situated, than on the side where the primary hydroxyls are located (Fig. 2.16) [269]. The mechanism of inclusion complexes between CDs and guest molecules can be explained by the 'host-guest' substitution theory, and the resulting inclusion is referred to as the molecular encapsulation [34].

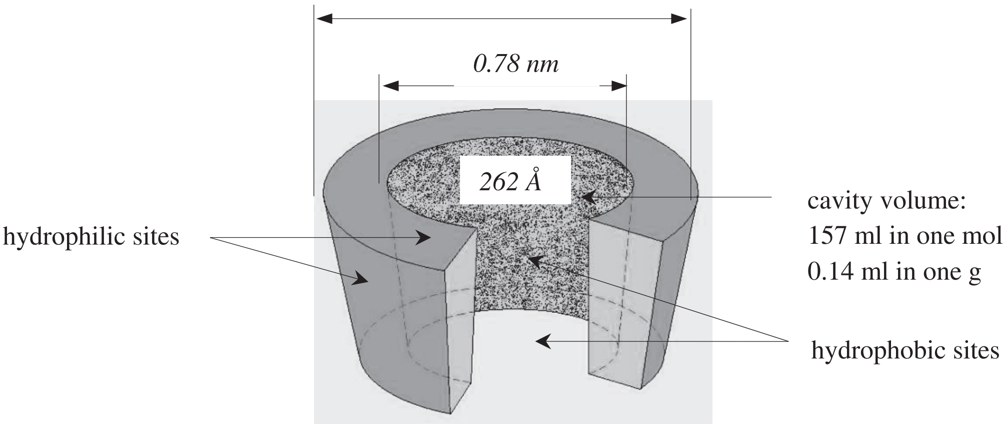


Fig. 2.16 Molecular structure of β-cyclodextrin

In aqueous solution, the cyclodextrin cavity is slightly apolar and occupied by water molecules. The existence of water molecules inside the cavity is energetically unfavorable because of polar-polar interaction. This creates a condition where the water molecules can be readily substituted by appropriate 'guest molecules' that are less polar than water. The simplest and most frequent case of complex formation is the host:guest ratio of 1:1, but 2:1, 1:2, 2:2 or more complicated associations are not impossible [262;269].

Shimada et al. (1992) found that the interfacial tension of vegetable oils-water interface decreased with the increase in cyclodextrin concentration. The number of CD molecules required to entrap a fatty acid residue of soybean oil at the interface was approximately 2.4 for both α- and β- cyclodextrin. For coconut oil, 1.8 of α-CD and 1.7 of β-CD molecules were needed to include fatty acid residues into their cavity.

Choi et al. (2010) recently investigated complexes obtained by fish oil and β-CDs self-assembly and aggregation mechanisms. The molecular inclusion of β-CD is apparently not possible to entrap fish oil as the 'guest' because of the complexity of fish oil components. Instead, the encapsulation of fish oil by β-CDs occurred by mean of the self-assembly mechanism (Fig. 2.17). The authors conclude that the high oil load (10:20) in the β-CD:fish oil ratio gave the best results in terms of encapsulation efficiency and oil load. At this ratio, 97% of the fish oil was retained in the freeze-dried powder for 3 days of storage where the release rate was slowest in de-ionized water [34].

2.6.2 Hydroxypropyl-β-Cyclodextrin (HPBCD)

The rather low solubility of native β-CD in water (18 mg/ml at 25°C) and the evidence of nephrotoxicity have driven scientists to find a CD host with better solubility [163]. Water-soluble CDs derivatives can be divided into two groups: (1) chemically modified CD derivatives and (2) naturally (enzyme) modified CD derivatives. Reaction of parent β-CD with chemical will substitute the hydroxyl groups and form various products. The degree of substitution (DS) is defined as the number of hydroxyls that are substituted in a glucose unit, with the possibility of numbers 1, 2, or 3. The average DS means the average number of substituted hydroxyls in a glucose unit, and thus the value can be any number between 0 to 3 [271].

Hydroxypropyl-β-Cyclodextrin (HPBCD) is a hydroxyalkyl derivative of cyclodextrins with improved solubility in water [292]. It is prepared in an alkaline aqueous solution by reacting propylene oxide with β-CD [218]. The reaction results in substitution of CD hydroxyl by 2-hydroxypropyl groups [219]. The disruption of the stable hydrogen bond system around the cyclodextrin rims by insertion of a substituent improves the hydrophilicity of CD molecules. The HPBCD samples show a wide range of variability in which the product is substituted randomly. The average DS is influenced by reaction time, temperature and reactants [271].

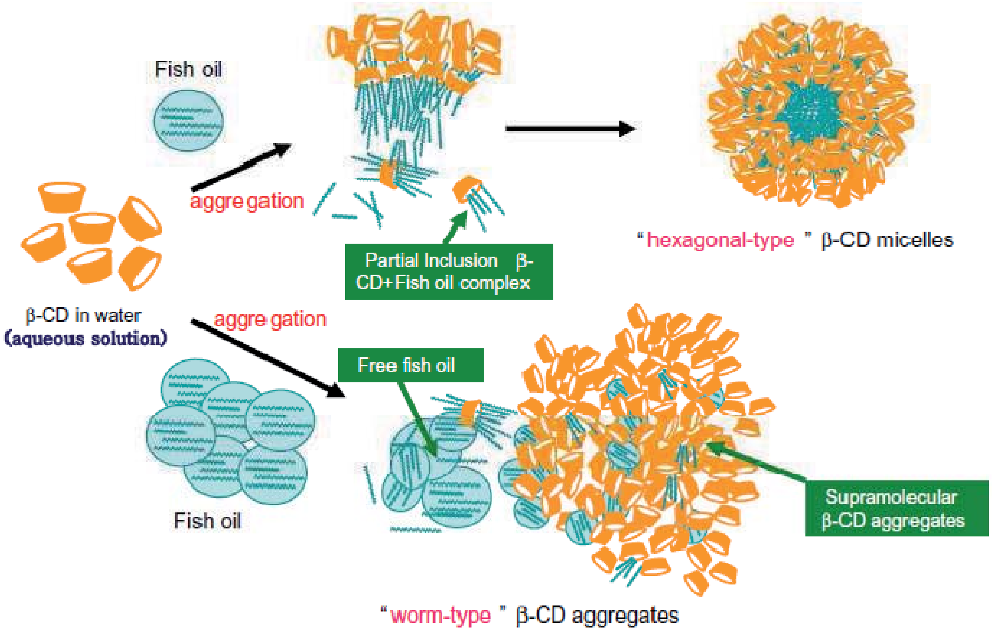

Fish oil
aggregation
Partial Inclusion β-CD+Fish oil complex
"hexagonal-type" β-CD micelles
β-CD in water
(aqueous solution)
aggregation
Free fish oil
Fish oil
Supramolecular β-CD aggregates
"worm-type" β-CD aggregates

Figure 2.17 Mechanism of self-assembly process of β-CD with fish oil, adapted from Choi et al. (2009) [35], reprinted with permission.

This CD derivative is a multicomponent substance, heterogeneous, and amorphous hygroscopic mixture. The DS of HPBCD is 2.7-3 or 5-7 hydroxypropyl groups/CD rings. However, though HPBCD is highly soluble in water, when fatty acids were mixed with methylated CDs (dimethyl-βCD = DIMEB, randomly methylated βCD = RAMEB,) and HPBCD, their solubility in water was superior if entrapped by DIMEB. Increasing the number of double bonds in fatty acid molecules resulted in a more stable inclusion complex. The reason for this phenomenon is the compact geometry of fatty acids, which lead to the better fitting into the CDs cavity than those of more linear long-chain fatty acids [272].

2.6.3 Soybean soluble polysaccharide

Soybean soluble polysaccharide (SSPS) is a substrate extracted by hot water from ‘okara’, which is the residue or by-product of soybean after oil and protein extraction. Initially, the soybean cotyledon is cleaned from hull and hypocotyls and further separated from fat (defatting process). From the defatted meal, protein and water-soluble substances are extracted by water. The SSPS is then extracted from the okara by water and heat treatment (100 ^{o}C) at pH 4.0 – 5.0 [202].

Chemically, SSPS is an acidic polysaccharide containing a protein fraction [171] and mainly consists of a rhamnogalacturonan backbone branched by b-1,4-galactan and a-1,3- or a-1,5-arabinan chains, and homogalacturonan [202-204]. Nakamura et al. (2004) reported that SSPS, at low concentration, can be used to stabilize oil-in-water emulsions over a wide range of pH.

The behavior of SSPS in a mixed system showed the superiority of this substrate to protect the oil droplets and stabilized the emulsion. The SSPS coverage of droplets reduced small molecular weight surfactants’ (Tween 20 and 80) access to the surface of oil droplets, and no destabilization was caused by addition of surfactants. Addition of SSPS to emulsion stabilized by caseinate did not affect the droplet size distribution. However, SSPS caused bridging of emulsion droplets stabilized by whey protein isolate (WPI). This means that the interaction between milk protein and SSPS occurs at oil-water interfaces, particularly at pH 4.0 [205].

SSPS is more surface active than gum Arabic (GA) and the reduction of interfacial tension was reported five times greater than that by GA [180]. However, pectin has a strong ability to scavenge free radicals due to the high amount of galacturonic acids (65%) compared to only 18% in SSPS [182]. The inhibitive effect on oxidation showed by SSPS cannot be attributed to its ability to increase emulsion viscosity. Moreover, SSPS also did not show a strong metal-chelating ability similar to gum arabic.

2.6.4 Maltodextrins

Maltodextrins are hydrolyzed products of starch with dextrose equivalent (DE) lower than 20. The DE value indicates the degree of hydrolysis of starch into glucose or dextrose and is expressed as percentage of total solids, on a dry weight basis, that is already converted into reducing sugars. Starch has a DE close to 0%, dextrin is between 1 and 10%, glucose or dextrose is 100%, and sucrose is 120%, i.e., is 1.2 times as sweet as dextrose. The higher the DE, the more sugars are present, and the hydrolytic conversion is higher while average molecular mass is lower. Dextrose equivalent and degree of polymerization (DP) are indicators of stability and functionality [198].

Maltodextrins are D-glucose polymers in which the individual D-glucopyranose unit is joined by (1 → 4) linkages. They are white powders containing linear amylase and branched amylopectin [37]. Typical maltodextrins production methods are acid conversion at 135 °C to 150 °C for 5 to 8 min, and enzymatic digestion. Alpha-amylase or pullulanase are used in which the starch slurry with enzyme is heated at 75 ^{o}C, hold for a fixed time, and heating continue at 105 °C or acidifying at pH 3.5 to inactivate the enzyme [2]. Maltodextrins have already been used in many food applications due to their solubility in water, and can be used as texture modifiers, fat replacers, crioprotectors, volume enhancers, gelling agents, and as an encapsulation matrix [3].

The physicochemical properties of maltodextrins are influenced by the variation of DE values. Increasing DE causes hygroscopicity, solubility, and osmolality to increase as well as improve the effectiveness regarding reduction of the the freezing point. On the other hand, decreasing DE enhances the viscosity, cohesiveness, and coarse crystal prevention [198]. Within similar or same DE values, the physicochemical properties of maltodextrins can be different if the proportion of high and low molecular weight saccharides is varied. The high-molecular-weight saccharides determine their solubility and solution stability whereas the sweetness, viscosity and crystallization are correlated with the amount of low-molecular-weight components [84]. The dextrinisation reaction progresses until the required DE product is obtained. Usually, after 1 hour, the DE is about 10-12, and by 2 hours it will be 15–16 [138].

The irregular maltodextrin aggregates from an acid-hydrolyzed corn starch have diameters of 3 to 5 µm and are estimated to be similar in size to the fat crystals [223]. Maltodextrins produced from whole grain rice derivatives are reported to have protein microparticles with a 1-5 µm diameter. Setser and Racette (1992) reported that amylodextrins, which have aggregates of submicron particles (about 0.02 µm), are able to form short and pseudoplastic texture of gel network. The network builds systems which are similar to the fat crystals in a continuous oil phase, and this network is mainly determined by the particle size of maltodextrins [81;223;254].

Low-DE maltodextrins are reported to have better functionality compared to high-DE ones. For drying sensitive ingredients, they can be used as non-browning carriers. They also have low humectants properties and better flowability at higher equilibrium relative humidity (ERH) [197]. However, low DE maltodextrins tend to retrograde in solution compared to those with higher DE, which form a less viscous solution but show increasing sweetness.

Takeiti et al. (2010) describes the relationship between the morphological and physicochemical characteristics of different maltodextrins. From three manufacturers and two botanical sources, the average particle size was in the range of 39.44 - 289.17 µm with a 2.82 – 6.47% moisture content. The morphology of particles varied from fractured, spheres, broken shell, non-broken particles, cylindrical, inflated, and collapsed. All these influence to a great extent the rate of wetting and dissolution of powder. Spherical, irregular, and filamentous shapes resulted in low wetting times. Increasing DE value within the same botanical source decreased the dissolution time [273]. This outcome underlines that variation in DE values results in maltodextrins with different physicochemical properties. Altering the condition of hydrolysis may produce maltodextrins with similar DE values but different properties. Also, variation in spray drying condition may obtain particles with a wide range of morphological dissimilarities.

Some studies reported that maltodextrins can form precipitation via a mechanism called, alignment of linear molecules, by hydrogen bonding, which cause aggregation and thereby precipitation. This is similar to the mechanisms which initiate retrogradation in amylase. If the maltodextrin chain contains amylopectin, its branched structures can interfere to the aggregates formation, prevent precipitation and thus stabilize the maltodextrin solution [140]. Factors that lead to an increase in the rate of precipitation of maltodextrin solution are extended hydrolysis time and temperature, and increased enzyme concentration particularly the α-amylase [139]. This enzyme tends to attack the branch parts to release linear oligosaccharides. Thus, extensive hydrolysis will cause more linear structures in the solution and lead to instability.

Previously, the DE provided by the manufacturer was used to predict maltodextrin properties. However, the DE values only are apparently not sufficient to determine the product performance and its application. Maltodextrins with the same DE value but produced by different manufacturers or from various botanical sources can have varied properties. Therefore, scientists have found that the molecular composition, particularly the saccharide content, is a better tool to evaluate the physical and biological properties of maltodextrin [37;139;175].

Composition of maltodextrin 20 is reported to be 0.8% glucose, 5.5% maltose, 11.0% maltotriose and 82.7% higher saccharides. Enzyme-hydrolyzed maltodextrins have a lower concentration of high-molecular-weight saccharides. Thus, these types of products are more soluble in water than acid-hydrolyzed products with equivalent DE [198]. The high viscosity of maltodextrins is caused by the high level of high-molecular-weight saccharides [138].

2.6.5 Modified starch

Starch is modified to fulfil the demand for desirable and unique starch properties for use in food. These involve chemical and physical alteration of native starch characteristics to increase starch functional properties. Starch modifications can be accomplished in many ways, e.g., (1) derivatization by etherification, esterification, cross-linking, and starch grafting, (2) decomposition via acid or enzymatic hydrolysis and starch oxidization, and (3) physical treatments using heat and moisture [265]. Starch functionality such as pasting, retrogradation, viscoelastic, and rheological properties depends on many factors. They include molecular weight, size, and structure of its components as well as the distribution of molecular weight and the molecular structure of amylose and amylopectin [305].

Two main components of normal corn starch are 25% amylose and 75% amylopectin. Amylose is a linear molecule of 1,4 linked α- D-glucopyranosyl units with slightly branched by α-1,6-linkages. Amylose properties are: 10^5 – 10^6 Da, DP (degree of polymerization) of 500 – 5000, and having 9 -20 branches per molecule, with the chain length (CL) of between 4 and 100 glucose units and greater. On the other hand, amylopectin, the highly branched component of starch of α- D-glucopyranosyl units linked by α-1,4 linkages, consist of only 5 – 6% of 1,6 bonds branch points [306]. Among corn starches, differences in granule swelling, peak viscosity, shear thinning during pasting, peak temperature, and gel firmness depend on the variation of amylopectin structure [9].

The basic physical properties of starch can be altered by biotechnological modification of the molecular structure of starch. In plant, the main molecular alterations of starch are amylose content, amylopectin chain structure, and phosphate content [25]. The amylose content is reported as the ingredient with the greatest contribution to the physical properties of starch. Increasing the amylose content in maize results the chain length of amylopectin molecules and starch crystallinity [29]. The swelling power and retrogradation behaviour are influenced to a great extent by the amylose content. The hydration capacity (swelling power) can be adjusted by controlling the levels of granule-bound starch synthase (GBSS) [211], while the retrogradation is also influenced by the amylopectin chain length and phosphate content [283].

The re-crystallisation properties of starch can be modified by increasing the chain length of the amylopectin constituent [126]. A high amylopectin content results in reversible re-crystallisation of starch, while the consequence of a high amylose content is irreversible starch crystallisation [191]. Since this study used modified starch from *waxy* maize, the literature review focuses on the chemical modification of *waxy* corn starch, particularly the succinylation.

2.6.5.1 Chemically modified waxy maize starch

Waxy corn is an essential material to produce modified starch and contains 100% amylopectin [316]. Thus, the starch granule obtained from this type of corn is devoid of amylose. The waxy corn is possible when the *wx* gene is introduced into any corn type, or the combination of any other mutant with the *wx* gene [302].

Chemical modification of starch aims to improve paste consistency, clarity and smoothness, and to impart the cold-storage and freeze-thaw stability [160]. The alteration by chemical modifications is done via addition of certain moieties on the starch linear chains of the glucose units to change the molecular size and viscosity characteristics. White and Tziotis (2000) described five chemical modifications that are commonly done in starch development, namely acetylation, hydroxypropylation, phosphorylation, cross-linking, and succinylation [306].

Acetylated starch can be obtained by esterification of native starch with acetic anhydrate in the presence of an alkaline catalyst. The hydroxypropylated starches are prepared by etherification of native starch with propylene oxide in the presence of an alkaline catalyst. The introduction of hydroxypropyl groups disrupts the inter- and intra-molecular hydrogen bonds causing a weakening of the starch granular structure thereby increasing the motional freedom of the starch chain in amorphous regions [252]. The cross-linking reaction is expected to insert intra- and inter-molecular bonds into the starch granule at random locations to strengthen and stabilize the granule [1]. Cross-linked starch forms pastes that are less susceptible to break down due to extended cooking time, increase acidity and shear [102]. Cross-linked starch can be used as the source of dietary fiber. This can be achieved by reacting granular starch with multifunctional reagents to produce ether or ester inter-molecular linkages between hydroxyl groups on starch molecules [309;310].

The degree of cross-linking can be changed, and the extent of swelling can be manipulated to control the thickening properties of starch [8]. The size of cross-linked amylose was reported to be stable even though its susceptibility toward sequential hydrolysis by isoamylase and β-amylase was decreased. A cross-linking reaction between amylopectin and amylose was also reported [125].

The swelling power of acetylated *waxy* maize starch increased because of the open structure of amylopectin which present dominantly thus allowing rapid penetration of water into the structure [160]. Cooke and Gidley (1992) reported ΔH_{gel} of hydroxypropyl *waxy* maize starch is decreased. The ΔH_{gel} measured the quantity and quality of crystallinity as an indicator of the loss of molecular order within the granules upon gelatinization. They suggested that the hydroxypropyl groups disrupt double helices within the amorphous region of the granules and caused the number of double helices that unravel and melt during gelatinization lowered than in unmodified maize starch [38].

A combination of substitution and cross-linking, also known as dual modification, produces starch with high stability against acid, mechanical and thermal degradation [309]. The physicochemical properties of *waxy* corn starch are influenced to a great extent by the sequence of chemical modification, hydroxypropylation or cross-linking. The hydroxypropylated-cross-linked starch exhibited higher Brabender viscosity, onset and peak gelanitization temperature, and β-amylolysis limit but lower retrogradation than the cross-linked-hydroxypropylated starch. Different distribution patterns of the modifying groups in these two starches are responsible for the above outcomes. From the structure analysis, the modification sequence determined the location of substitution, their susceptibility to enzymes and their physicochemical properties [301].

Succinylation of starch improves its properties, particularly decreasing gelatinization temperature, increasing freeze-thaw stability, viscosity stability, thickening power, ability to swell in cold water, stability in acid and salt, and reducing the tendency to retrograde. This modification involves addition of succinic anhydrate (dihydro-2,5-furandione) into starch molecules [22].This modified starch is permitted for food application with a maximum allowable content of 3% [23]. Such modification to produce octenyl succinic acid (OSA) starch has been approved by FDA to be used in food and has also been approved as a food additive by the EU (code number E 1450).

OSA starches are almost colourless and tasteless in solution and therefore have been used extensively for encapsulation purposes as well as for beverage emulsions. As an encapsulation matrix for valuable ingredients, this starch is able to protect the ingredients from oxidation and can act as a combination of surfactant and stabilizer [278]. The increase in swelling power is attributed to the ease of hydration as a consequence of an increasing number of hydrophilic groups added into the starch molecule [22]. The modified starch can be easily dispersed into oil/water mixtures without extensive agitation and has excellent emulsifying capacity, and thus is able to form homogenous emulsions without separation for a period of time at room temperature [161].

III Objectives

This study is divided into two main parts: the preliminary and the main research. This division is essential to carry out a systematical study by designing step-by-step experiments and to eliminate unimportant variables in the main research. The preliminary research is conducted to select fish oil from various sources and types that are currently available on the market, to investigate the best combination of walls from various food hydrocolloids, and to determine an appropriate method to be used in the main research. The outcomes from the preliminary research are applied in the main research and evaluated by comparison with other production processes and different coating materials.

Previous works have underlined that the best way to emulsify fish oil is to combine coating materials that function as a carrier matrix and as an emulsifier [260;261]. Hence, in this research, 7 biopolymers (β-Cyclodextrin (β-CD), maltodextrin, soybean soluble polysaccharide (SSPS), hydroxypropyl betacyclodextrin (Kleptose® HPBCD), corn starch, methyl cellulose, and modified starch or OSA-starch (Hi-Cap® 100)) are evaluated in combination as coating materials for fish oils. The selection is based on their superiority as reported in literature. Four matrices, i.e., β-CD, SSPS, maltodextrin, and HPBCD, are examined in the preliminary research, while corn starch, methyl cellulose, and OSA-starch are evaluated in the main research.

Beta-Cyclodextrin (β-CD) and hydroxypropyl betacyclodextrin (HPBCD) offer advantage by encapsulation in the molecular form (nanoencapsulation) [34]. Both have a unique molecular cavity for entrapping guest molecules, including fatty acids [58;314]. Maltodextrin and corn starch are filler matrices [244;245], which are cheap, highly soluble in water and able to form stable emulsions [3]. Soybean soluble polysaccharide (SSPS) is claimed to have excellent emulsifying properties and is better than gum acacia (gum arabic) [180]. Methyl cellulose is soluble in cold water, forming gels when heated, and the gels are thermo-reversible [67;250]. Modified starch has replaced the ordinary starch due to its better emulsifying capacity [265]. The ability of those coating materials to protect fish oil is evaluated in this study.

The objective of the preliminary research in particular is to investigate microencapsulation of fish oils by two processes: spray granulation (SG) and SG followed by fluid bed film coating (SG-FC). In the first process, the selection of three types of fish oil is based on the ratios of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (10/50, 33/22, and 18/12). Each type was emulsified with SSPS and maltodextrin to produce 25% oil powders. In the second process, 15% film coating of HPBCD was applied to the granules from the first process. To date, SG has never been used for microencapsulating fish oil. Very few studies have discussed the powder properties obtained by the fluid bed film coating process. Because of the high drying temperature used in spray drying, deterioration of sensitive ingredients caused by oxidation has been reported [104;147;148] . As spray granulation offers low drying temperature (max. 70 ºC) and product temperature (max. 40 ºC), it is very interesting to examine the ability of this method to produce microcapsules containing sensitive cores such as fish oil.

The aim of the film coating (FC) process is to protect non-encapsulated oil on the surface of microcapsules produced by SG. The findings of previous research clearly show that the non-encapsulated oil is responsible for the rancidity of fish oil powder. According to Thies 2004, microcapsules made by drying the emulsion can be categorized into type B structure, where the core is dispersed throughout the matrix. The protective barrier is distributed throughout the interior and is not present at the outer surface of the capsules. The thickness of the matrix can be very thin. Therefore, more layers probably would provide the secondary wall for the dispersed core [281].

Hydroxypropyl betacyclodextrin (HPBCD) was selected as the coating material due to the advantage offered by its molecular cavity for taste masking of unpleasant active ingredients, including fish oil. Gamma-Cyclodextrin (CD) has been used before to eliminate the unpleasant taste and smell of fish oil as well as to stabilize it against oxidation [236;270]. Recently, β-CD has also been used to encapsulate fish oil [35]. However, native β-CD has low solubility in water, and to overcome this problem HPBCD was chosen. The aqueous solubility of HPBCD of 65% at 25 ºC and 80% at 50 ºC allows this matrix to form more stable suspensions for coating, and it is expected to build better barriers than native β-CD with its aqueous solubility at 20 ºC, g/100 ml of only 1.85 [241]. Therefore, by enveloping the granules with HPBCD, it is expected that the microcapsule surface can be protected and at the same time the off-flavor absorbed. The ability of SG and SG-FC to produce stable products is assessed by measurement of the microcapsule stability during storage for 6 weeks at room temperature (± 21 ºC) and in a refrigerator (3 - 4 ºC).

In the main research, the ability of SG and SG-FC to microencapsulate fish oil is examined and compared to spray drying (SD) and freeze drying (FD). Spray drying is a common technique used for microencapsulation of food ingredients [44;261]. It is a simple inexpensive method in which either proteins or polysaccharides or a combination of both can be used to create the shell. However, SD has some drawbacks, e.g., high drying temperature (can be more than 200 ºC) and that it is only suitable for matrices that are highly soluble in water [77]. Spray drying an emulsion containing sensitive ingredients such as fish oil is risky.

Unlike SD, FD has been shown to be an attractive method for extending the shelf life of foods. Drying is carried out at temperatures lower than ambient temperatures, and the absence of air prevents product deterioration caused by oxidation or chemical modification. This method can minimize the product damage due to decomposition or changes in structure, texture, appearance and flavor, which can occur as a consequence of the high drying temperature used in spray drying [165].

For spray granulation (SG), low drying temperature and the formation of granules with the “onion-skin” structure has distinguished this method from the commonly used ones. As heat may trigger the oxidation, production where this factor is eliminated should increase powder stability.

Therefore, this research characterizes and compares the effect of (1) low to medium drying temperature but longer residence time of spray granulation, (2) secondary coating in the fluid bed film coating process, (3) high drying temperature but very short residence time of spray drying, and of (4) no heating but very long drying time of freeze drying on microcapsule stability. Several combinations of walls to encapsulate fish oil will be used in each of the drying processes. The results are compared based on product stability against oxidation, microencapsulation efficiency and microcapsule physical properties by particle size analysis, and scanning electron microscope (SEM) examinations. The oxidative stability is evaluated based on two indicators: peroxide values (PVs) and detection of volatile propanal measured by headspace gas chromatography upon storage at room temperature (± 21 ºC) and in the refrigerator (3 - 4 ºC) for 8 weeks.

IV Material and methods

4.1 Materials

4.1.1 Fish oil

The fish oils used in this study were selected based on different EPA:DHA ratios (Table 4.1). In the preliminary research, all three types were tested; however, for the main research, only C1 fish oil with a 33:22 ratio was chosen.

Table 4.1 Characteristics of fish oils as core substance

Fish oil	*EPA:DHA ratio*	*Omega-3 fatty acids (mg/g)*	*Initial PV (meq/kg)*	*Antioxidant (mg/g)*[a]	*Supplier*
Epax 10/50 (E)	10:50	670	3.0	3.7	Epax AS, Lysaker, Norway
Ultra refined (C1)	33:22	620	< 1	3 - 4.5	Cognis, Illertissen, Germany
Ultra refined (C2)	18:12	360	< 1	1-2	Cognis, Illertissen, Germany

[a] Antioxidant of mixed tocopherol (analysis certificates issued by producers)

4.1.2 Matrices

In order to obtain the best matrices, several coating materials were evaluated in the preliminary study, i.e., SSPS, maltodextrin, and β-cyclodextrin (β-CD). The matrices introduced in the main research are corn starch, methyl cellulose, hydroxypropyl betacyclodextrin (HPBCD), and modified starch.

The prelimary experiments showed that it was difficult to form stable emulsions with β-CD, both as a single matrix or in combination with SSPS and maltodextrin, due to its low solubility in water. Corn starch failed to be fluidized in the ProCell spouted bed and led to an inefficient spray granulation process. Methyl cellulose created lumps inside the spouted bed chamber possibly due to its high hygroscopicity, which resulted in a very thick emulsion. Therefore, the coating materials selected for the drying process were SSPS, maltodextrin, modified starch and hydroxypropyl betacyclodextrin as these did not cause any problems. They were tested in combination in the main research.

Table 4.2 Coating materials used in combination to disperse fish oil droplets

Material	***Type***	***Supplier***
Soybean soluble polysaccharide (SSPS)	Soyafibe-S-EN100	Fuji Oil, Osaka, Japan
Maltodextrin	Granadex M 20	Biesterfeld Spezialchemie, Hamburg
β-cyclodextrin	native	Roquette GmbH, Frankfurt, Germany
Corn starch		Provided by IPC Process Centre, Dresden
Methylcellulose		Provided by IPC Process Centre, Dresden
Hydroxypropyl betacyclodextrin	Kleptose® HPBCD	Roquette Freres, Frankfurt, Germany
Modified starch	Hicap®-100	National Starch Food Innovation, Hamburg

4.1.3 Chemicals

Three main measurements that need chemicals for the analysis: determination of peroxide value (PV), surface or non-encapsulated oil, total oil content and propanal determination by Headspace Gas Chromatography.

4.1.3.1 Peroxide value analysis

Chemical	*Specification*	*Supplier*
Acetic acid	rotipuran 100% p.a. glacial	Carl Roth
Chloroform	rotisolv > 99.8%	Carl Roth
Sodium thiosulfate solution	standardized	Sigma-Aldrich
Potassium iodide	> 99%	Carl Roth
Starch	puris p.a. from potato	Sigma-Aldrich
Water	distilled	IEL

4.1.3.2 Headspace gas chromatography analysis

Chemical	*Specification*	*Supplier*
Propionaldehyde (propanal)	reagent grade, 97%	Sigma-Aldrich
Trans-2-nonenal	97%	Sigma-Aldrich
Nonanal	≥ 95.0% (GC)	Fluka
cis-4-heptenal	≥98%, FCC, Kosher, FG	Aldrich
2,4-decadienal	Kosher, FG	Aldrich
α – tocopherol	type VI, from vegt.oil	Sigma-Aldrich
Ethylenediaminetetraacetic acid solution	pH 8.0, ~0.5 M in H_2O	Fluka

4.1.3.3 Analysis of surface oil and total oil content

Chemical	*Specification*	*Supplier*
Isohexan	rotisolv > 99.8%	Carl Roth
Sodium acetate buffer	in solution form, pH 4.65	Riedel-de Haën
Ethyl acetate	rotipuran ≥ 99.5 %, p.a.	Carl Roth
Pancreatin	from porcine pancreas	Sigma-Aldrich
α- Amylase	from *Bacillus licheniformis*	Fluka Biochemika

4.1.4 Equipment

4.1.4.1 Spouted bed (Glatt-ProCell-5 Lab System)

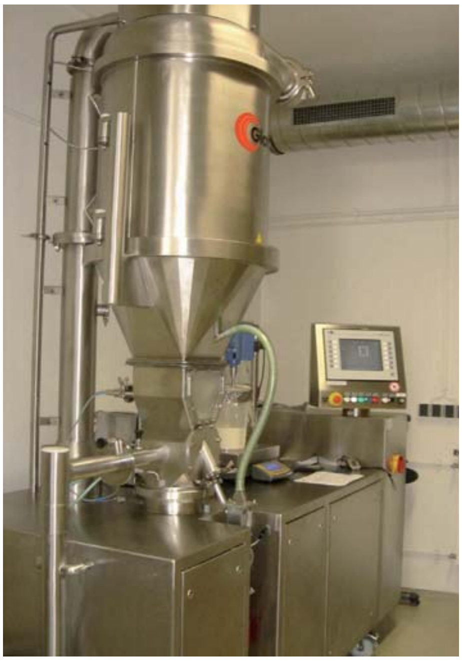


Fig. 4.1 Spouted Bed Equipment (Glatt-ProCell-5 Lab System)

Parameter	*Technical data*	*Operating conditions*
Spraying rate	2000 g/h	10 – 13 g/min
Inlet air temperature	max. 200°C	50 - 75°C
Outlet air temperature	n.a.	30 - 40°C
Product temperature	n.a.	30 - 40°C
Air flow	max. 250 m^3/h	85 – 120 m^3/h
Nozzle diameter	1.0 mm	1.0 mm
Spraying pressure	max. 6.0 bar	2.0 – 3.0 bar

4.1.4.2 Fluid bed film coating (Würster Process), GPCG-1

Parameter	*Operating conditions*
Spraying rate	7 – 8 g/min
Inlet air temperature	60 - 70°C
Outlet air temperature	30 - 40°C
Product temperature	30 - 40°C
Spraying pressure	2.0 bar
Air flow	27 – 29 m^3/h
Nozzle diameter	1.0 mm

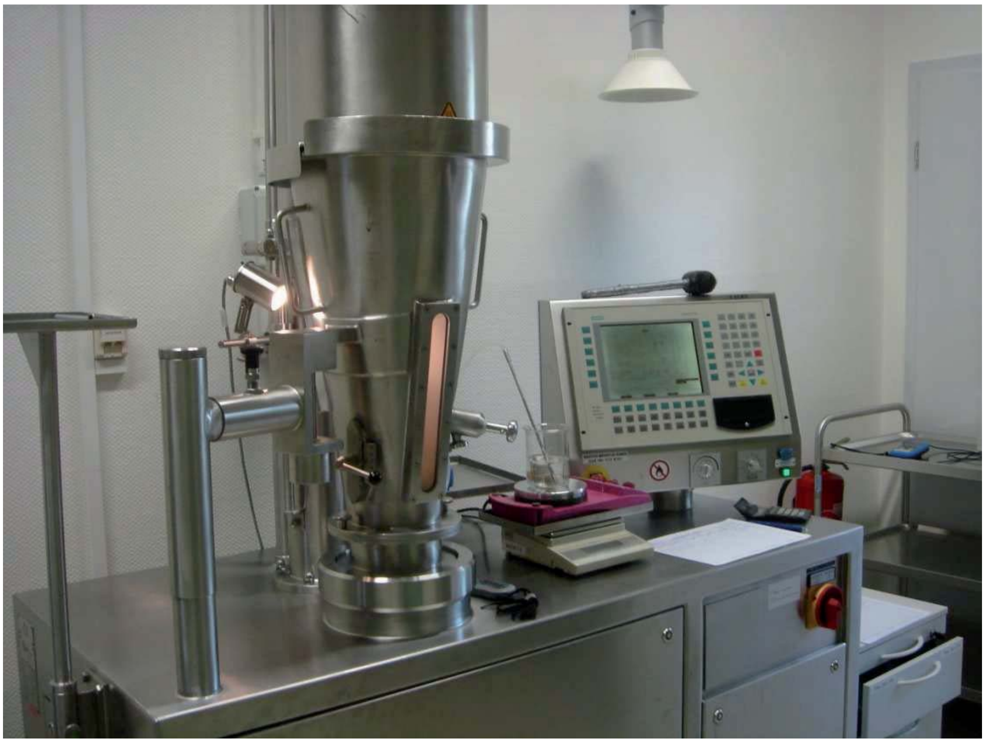


Fig. 4.2 Fluid bed film-coating apparatus (Würster Process-Insert), GPCG-1.1.

4.1.4.3 Spray dryer: Nubilosa AJM 014

Parameter	*Technical data*	*Operating conditions*
Capacity	1 – 5 kg/h H_2O	
Inlet temperature	max. 250°C	± 180°C
Outlet temperature	max. 130°C	85°C ± 5°C.
Pressure	max. 4,5 bar	
Nozzel diameter	1,5 mm	1,5 mm

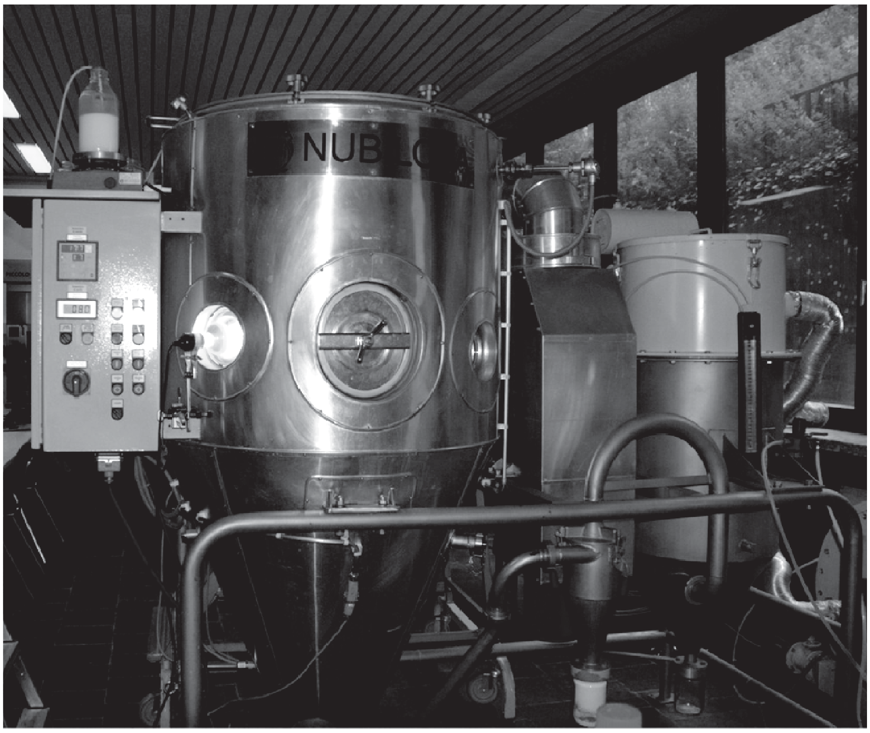


Fig. 4.3 Nubilosa AJM 014 spray dryer

4.1.4.4 Freeze dryer

Parameter	***Technical data***	***Operating conditions***
Ice condenser capacity	max. 4 kg	
Ice condenser temperature	approx. -85°C	- 50 °C
Shelf temperature during freezing and drying	approx. -50°C to +99 °C	-25 to 20 °C
Vaccum value	0.0010 – 4.020 mbar	0.120 - 0.770 mbar
Total processing time		approx. 40 h

Fig. 4.4. Christ Alpha 2-4 LSC freeze dryer

4.1.5 Analytical equipment

Equipment	***Type***	***Producer***
Aw analyzer	Novasina LabMaster-aw	Novasina AG, Switzerland
Centrifuge	Biofuge® primo R	Heraeus Instruments
Digital scale	AB 304-S / Version Fact	Mettler Toledo
Freezer	Temperature -70 °C	Störk Tronic, K.H. Spitzl

Equipment	***Type***	***Producer***
Gas Chromatography	Type 6890N	Agilent Technologies
	Software	Cycle composer
	Autosampler	CTC Analytics PAL
Headspace gas analyser	CheckMate 9900	PBI Dansensor
Heater & magnetic stirrer	IKAMAG ® RCT	Janke & Kunkel KG
Homogenizer	Ultra Turrax: type T45	Janke & Kunkel KG
	Ultra Turrax: type T18	Janke & Kunkel KG
	Ultra Turrax : type T50	IKA Labortechnik
	Gann, Type EIRoFE	Gann, Machinebau GmbH
	Panda 1K, NS1001L	GEA Niro Soavi S.p.A, Italy
Light microscope	Leitz Diaplan	Ernst Leitz Wetzlar
Mikroskop Camera	F10 CCD	Panasonic
Moisture analyzer	MA 100	Sartorius, Germany
Pipettes (various volume sizes)	Eppendorf Research	Eppendorf
Peristaltic pump	Pumpdrive PD 5001	Heidolph Instruments
Oven	T 5042	Heraeus
Refrigerator	Privileg de luxe	Privileg
Rotary evaporator	Rotavapor R-114	Büchi Laboratoriums-Technik AG
Scanning Electron Microscopy	SEM Philips XL 20	Philips
	FEG-SEM, JSM 6400F	Jeol, Japan
Shaker / agitator	Orbital shaker SSL-1	Stuart
Particle analyzer	Sympatec LF laser diffractometer	Sympatec, Clausthal

Equipment	*Type*	*Producer*
Vortex mixer	VF 2	Janke & Kunkel IKA-Labortechnik
Water bath heater	MP	Julabo Labortechnik
Water purifier	Synergy UV	Millipore
Vacuum machine	Vac-Star 2000	Verpackungsmachinen AG

4.1.6 Auxiliary and consumable materials

Material	*Producer*
Aluminum seals + septum	GC Mueller
Buret	DURAN
Beaker glass (50 ml - 500 ml)	Simax
Centrifuge tubes (50 ml)	Becton Dickinson
Cuvettes	BRAND
Erlenmeyer flask (50 ml - 1000 ml)	VWR
Filter	VWR
Filter paper, folded, 240 mm (d)	Macherey-Nagel GmbH
Forceps-Dumoxel	Fine Science Tools GmbH
Forceps-Inox style 5	Fine Science Tools GmbH
Measuring cylinder (25 ml - 1000 ml)	DURAN
Microscope slides (76 x 26 mm)	R. Langenbrinck
O-Rings for forceps	Fine Science Tools GmbH
Parafilm®	American National CanTM

Material	*Producer*
Pasteur's pipettes	Braun
Pipette tips	Greiner bio-one
Rotilabo-wide neck bottles, PVC	Carl Roth
Stirring Bars	VWR
Vinyl - Examination Gloves (latex-free, powder-free)	Top Glove

4.2 Methods

4.2.1 Preliminary research

4.2.1.1 Preparation of emulsions

Emulsions to be dried by each of drying method used (SG, SD, and FD) were prepared in similar ways. For spray granulation process, the emulsions were prepared at 50% total solids (SSPS and maltodextrin at a ratio of 1:6.5). Firstly, 65% (w/w) maltodextrin and 10% (w/w) SSPS were weighted into a 3 L plastic jar and dissolved in purified water using Ultra-Turrax (T50, IKA Labortechnik, Staufen, Germany) at 10,000 rpm for 2-3 min. The dispersion of matrices was immersed in a cold water bath with ice and cooled for 10-20 min until a temperature of 10-12 °C was reached. Fish oil at a ratio of 1:4 (core:wall) or 25% (w/w) was added into the dissolved coating materials and mixing was continued at 8,000 rpm for 2-3 min. There was no addition of antioxidants during preparation of emulsions. The coarse emulsions were then further homogenized using a laboratory homogenizer (Panda 1K, NS1001L, GEA Niro Soavi S.p.A., Parma, Italy) at 270/40 bar. The emulsion preparation was repeated three times, because 3 fish oils (Epax 10/50, Cognis 33/22 and Cognis 18/12) were tested in the preliminary research.

4.2.1.2 Spray granulation

The homogenized emulsion was collected in plastic jars that were covered with plastic sheets to avoid contact with oxygen, and immersed in an ice water bath. Before spraying the emulsion, 250 g maltodextrin was inserted into the Spouted Bed, ProCell 5 LabSystem (Glatt Ingenieurtechnik GmbH, Weimar, Germany) as the seed particles. When the spray granulation (SG) process started, the seeds were suspended by the fluidized gas into which the emulsion was sprayed and then the seeds entrained upward. The drying temperature was in a range of 50-70 °C, spraying pressure was 3.5 bars and spraying rate of ± 10 g/min. Product temperature was in a range of 30-40 °C. The residence time for granulation to obtain 350–650 µm granules was ± 60 min, after a steady state condition was achieved. The first 300 g granules were discarded because they contained seeds (maltodextrin). The granules containing fish oil only were then collected in a glass bottle and kept at 3 °C while waiting for storage and further analytical tests. The SG processes were conducted at the IPC Process Center, Dresden, Germany.

4.2.1.3 Fluid bed film-coating process

Granules from SG were coated with solution of 15% (w/w) hydroxypropyl betacyclodextrin using a bottom spray (Wurster) fluid-bed coating equipment (GPCG 1.1, Glatt Ingenieurtechnik GmbH, Weimar, Germany). The inlet and outlet air temperatures were 60-70 °C and 30-40 °C, respectively. The SG granules were placed in the chamber and as soon as the process was started, they were fluidized by the stream of gas. At the same time, the solution of 15% HPBCD was sprayed into the bed of granules. Spraying air pressure was 2.0 bar with a spraying rate of ± 8 g/min. The product temperature was in a range of 30-40 °C. The coated granules were collected in a glass bottle after ± 50 min and kept at 3 °C for storage and further analytical tests. The experiments were also conducted at the IPC Process Center, Dresden, Germany.

4.2.1.4 Peroxide value analysis

The peroxide value (PV) analysis of the microencapsulated fish oil was determined by iodometric titration [308] as described by Kolanowski et al. (2004, 2006) and according to Wrolstad (2005) [146-148]. This method is based on the titration of iodine released from potassium iodide by peroxide using a standardized thiosulfate solution as the titrant and a starch solution as the indicator. The method detects all substances that oxidize potassium iodide under the acidic condition. Sample representing 0.625 g of oil was placed into a 250-ml glass-stoppered Erlenmeyer flask and 30 ml of 3:2 acetic acid/chloroform solutions was added and swirled until dissolved completely. Then 0.5 ml saturated potassium iodide solution was added and the mixture was allowed to stand for exactly 1 min with occasional shaking following the addition of 30 ml distilled water.

The titration started by addition of standardized 0.1 N sodium thiosulfate solution to the flask gradually until the yellow color had almost disappeared; the flask was shaken constantly and vigorously. At this time, 0.5 ml of 1% starch indicator solution was added and titration was continued until the violet colour disappeared. While the solution of sodium thiosulafte added drop-wise, the flask must be shaken vigorously to liberate all iodine from the chloroform layer. The total sodium thiosulfate added was recorded. Determination of a blank also conducted to the sample without oil. All measurements were done in duplicate. The PV is calculated using the equation:

$$PV = [(S - B) \times N \times 1000)] / W$$

where S is the volume (ml) of sodium thiosulfate required to titrate the sample
B is the volume (ml) of sodium thiosulfate required for the blank
N is the calculated normality of the standardized sodium thiosulfate
W is the weight of the sample (g)

4.2.1.5 Headspace propanal

Propanal is a specific marker for the oxidation of *n*-3 PUFA and was therefore chosen as an indicator of the oxidative stability of fish oil microcapsules. As a secondary oxidation product of omega-3 fatty acids, propanal has been previously used by many others [7;52;54-57;63;69;73;107;248;259;307].

A capillary gas chromatograph (GC) fitted with an Optima delta-6 capillary column (0.35µm x 30m x 0.32mm), and a flame ionization detector (FID) was used for propanal headspace analysis. One gram of powder was weighed and dissolved in 4 mL of Milli-Q water, and sealed in a 10-mL vial before being equilibrated at 60 °C for 15 min in a CTC Analytics PAL autosampler. An aliquot of the headspace was then injected into an autosystem gas chromatograph (Agilent Technologies 6890N). The injector and detector temperatures were 180 and 220 °C, respectively. The oven temperature was set initially at 30 °C for 1 min and increase to 50 °C at the rate of 2.5°C/min, then to 90 °C at the rate of 10 °C/min. The propanal peak is detected between minute 3.7 – 3.8, as shown in GC chromatogram (Fig. 4.5). Propanal was quantified using standard propanal solution of known concentrations from which a standard curve of propanal concentrations and peak areas was then created.

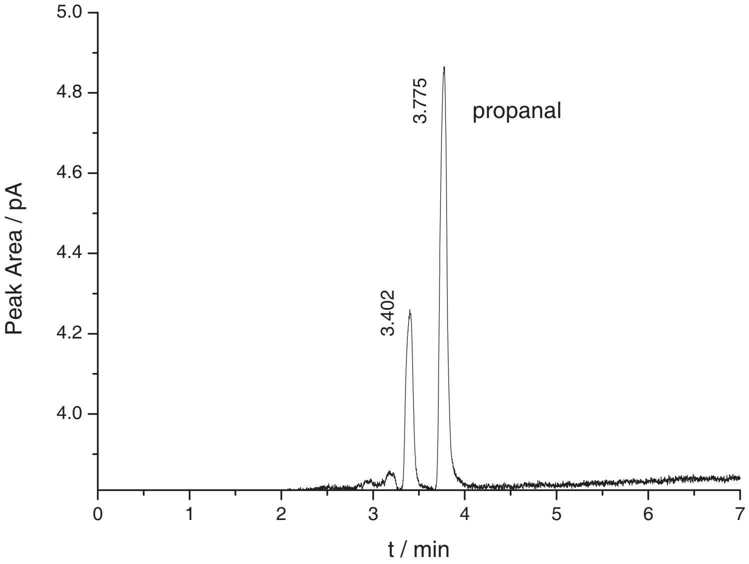


Fig. 4.5 Example of propanal peak detected from sample prepared with MC-4 formula (combination of SSPS, maltodextrin, and HPBCD)

4.2.1.6 Storage condition

Samples (10 g each) were placed in a narrow-mouth glass bottle and covered with aluminum crimp seal and rubber (septum). The bottle was tighten using a hand crimper to ensure proper sealing (as in GC vials), stored at room temperature (± 21 °C, 30% RH) in the absence of light and without nitrogen flush for six weeks. The same conditions were applied to samples stored in the refrigerator at 3-4 °C. The oxidative stability of microcapsules was monitored regularly, and experiments were carried out in duplicate.

4.2.1.7 Particle size analysis

The analysis of particle size was carried out using a Sympatec LF laser diffractometer (Sympatec, Clausthal-Zellerfeld, Germany). The equipment was operated at air pressure of 1 bar with focal length of 500 or 1000 mm. The particle size distribution is volume based and calculated by HRLD mode (Fraunhofer approximate calculation).

4.2.1.8 Total oil measurement

The total oil content was determined by an enzymatic digestion method as described by (Curtis et al. 2008). Briefly, 250 mg fish oil powder (W_{mc}) was weighed, and 30 mg porcine pancreatin was added. Then 10 mL of sodium phosphate buffer (W_{buff}) was pipetted into the vial, it was vortexed and placed in a shaking water bath at 37 °C, and shaken at 60 rpm for 1 hour. After cooling to room temperature, 10 mL of ethyl acetate (W_{sol}) was added and centrifuged at 1,000 rpm for 10 min. The organic layer (the top layer) was removed (1-2 mL) and added to a tared vial, and the weight (W_{ext}) recorded. The layer was flushed with nitrogen to remove the solvent and dried in the oven at 40 °C for 1 h to ensure complete evaporation. The final weight of extracted oil was then recorded (W_{oil}). The total oil extracted and the percentages of oil in the microcapsules were calculated as follows:

Total oil extracted, $W_{tot} = (W_{sol}/W_{ext}) \times W_{oil}$

Percentage of oil in the microcapsules $= (W_{tot}/W_{mc}) \times 100\%$

4.2.1.9 Surface oil content measurement

Non-encapsulated oil of fish oil powder was determined by the washing method described by Varavinit et al. (2001) and Tan et al. (2005) [294] [274] with modification. Iso-hexane (50 mL) was added to an accurately weight of 5 g powder in a 250-mL flask and shaken for 10 min at 225 rpm on an orbital shaker (Stuart SSL-1, Carl Roth, Karlsruhe, Germany). The slurry was then filtered through filter paper and each filter paper with solid particles was washed three times by passing 20 mL of iso-hexane through each filter paper each time. The filtrate caught in the 250 mL round-bottom flask was then clamped in the rotary evaporator (Rotavapor®, Büchi Labortechnik AG, Flawil, Switzerland) to evaporate iso-hexane for about 20-25 min at 40 °C. To complete solvent evaporation, the flask was dried in the oven at 90 °C for 30 min. The surface oil content was determined gravimetrically.

4.2.1.10 Calculation of encapsulation efficiency

The microencapsulation efficiency (ME) was calculated as follows:

$$\text{ME} = [(\text{Total oil} - \text{Non-encapsulated oil}) / \text{Total oil}] \times 100\%$$

4.2.1.11 Moisture content analysis

The moisture content was measured using Sartorius Moisture Analyzer, Model MA 100, Sartorius AG, Goettingen, Germany. A specific program was created to analyze sensitive powder containing fish oil. Briefly, 5.0 g powder was accurately placed into the aluminum pan and heated at 105 ºC. The measurement times were varied among samples. The moisture content (%) was recorded for each sample after stable weight was obtained.

4.2.1.12 Water activity (Aw)

The measurement of water activity was carried out using Novasina LabMaster-aw, Novasina AG, CH-8853 Lachen, Switzerland. Sample was carefully filled into a half of a sample dish. Each dish was tempered according to the adjusted temperature. The measurement was performed at ± 21 ºC. Analyzing times were varied from one sample to another and the results were recorded after the equilibrium has reached.

4.2.1.13 Scanning electron microscopy

Scanning electron microscopy to investigate the micro-structural properties of the microcapsules was performed using a SEM Philips XL 20 (Nijmegen, The Netherlands). The samples were placed on double-sided adhesive carbon tabs, mounted on SEM tubs and coated with gold/palladium in a sputter coater. The coated samples were then analyzed using the SEM operating at an accelerating voltage of 30 kV with magnification of 25 times x and/or 100 times.

For examination inside the particles, samples were mixed with an epoxy resin (Technovit 5000, Kulzer, Germany) containing Cu flakes to improve conductivity. After drying and hardening, the mixture was cut and polished to obtain a cross section of particles. The prepared samples were analyzed using SEM Philips XL 20 (Nijmegen, The Netherlands) and a high resolution field emission gun scanning electron microscope (FEG-SEM) JSM 6400F (Jeol, Japan).

4.2.1.14 Experimental design and statistical analysis

The experiments are based on a factorial design and the results represent the means of two replicates. The statistical analysis was done using SPSS version 17.0 and Data Analysis and Statistical Software (Stata 11). Some graphs were created using Stata 11, and the others using OriginPro 8G (OriginLab Corporation, Northampton, MA-USA).

4.2.2 Main research

In the main research, only fish oil 33:22 (EPA:DHA) was used as a core. Emulsions were prepared similar to the emulsification process in the preliminary research (Table 4.3). In four groups of formulas, each of the formula had a different matrices composition. The microcapsules were produced by three drying methods, i.e., spray granulation, spray drying and freeze drying. All results were treated similarly to that in the preliminary research, including storage test condition (however, storage time was 8 weeks), measurement of oxidative stability (PV and propanal by headspace GC), determination of microencapsulation efficiency (surface oil and total oil content), and observation of the physical properties of the powder by particle size, moisture content, water activity (Aw) analyses, and scanning electron microscopy. Parameters for spray granulation process were the same as in the preliminary experiment. Therefore, in the following, only the production of microcapsules by spray drying and freeze drying is described.

Table 4.3 Combination of coating materials used in the main research

Matrix Combination	*Process*	*Matrix combinations (%)*				*Type of fish oil (25%)*
		SSPS	*Maltodextrin*	*OSA-starch*	*HPBCD*	
MC-1	SG	12.5	62.5	-	-	33/22 ultra-refined
	SD	12.5	62.5	-	-	
	FD	12.5	62.5	-	-	
MC-2	SG	10	65	-	-	
	SD	10	65	-	-	
	FD	10	65	-	-	
MC-3	SG	10	-	65	-	
	SD	10	-	65	-	
	FD	10	-	65	-	
MC-4	SG	10	50	-	15	
	SD	10	50	-	15	
	FD	10	50	-	15	

4.2.2.1 Spray drying

Emulsions to be dried by SD and FD processes were prepared similarly to those dried by SG process. Matrices (as listed in Table 4.3) were firstly dissolved in purified water, in a 5 L plastic beaker, using Ultra Turrax (T 45, Janke & Kugel, K.G.) at 10,000 rpm for 2-3 min. Fish oil (25%) was added and mixing continued at 8,000 rpm for 2-3 min to produce 3 L emulsion with 50% solid content. The coarse emulsion was further homogenized using a laboratory homogenizer (Emulgor Etrofe, Gann) at 200 bar. The second homogenization was repeated two times to ensure a complete homogenization. The emulsion was then collected in 500 ml glass bottles and covered with plastic stoppers. Before spray drying process, all bottles were immersed in ice-water bath.

A pilot-plant spray dryer (Nubilosa AJM 014, Italy) was used to convert the emulsions into encapsulated powder. The emulsions were kept in glass bottles immersed in an ice-water bath. Immediately after the emulsification process, the drying of fish oil emulsion took place. The emulsion was pumped into the spray dryer chamber at a constant feeding speed and a very short drying time (just a few seconds). The inlet temperature was set ± 180°C and outlet temperature was 85°C± 5°C. Powder was collected in a glass jar and the each jar changed every 5 minutes to avoid prolonged exposure to heat. The fish oil powder obtained was transferred immediately into a cold glass jar and immersed as quickly as possible to an ice-water bath. All spray-dried powders were subjected to the "day zero analysis" to determine PVs and propanal content. These measurements were done on the same day immediately after the spray drying process and followed by storage tests at room temperature.

4.2.2.2 Freeze drying

Emulsion preparation was the same as described for spray drying process. The freeze drying method was initiated by a freezing process. The emulsion was divided, poured into aluminium plates with a thickness of ± 0.80 cm and frozen at -70°C for 24 hours. A Christ Alpha 2-4 LSC freeze dryer was used to freeze dry the emulsion. During the drying process, the ice condenser was set at lower than -50 °C. The frozen emulsion was dried for ± 40 hours. Cold and dried emulsion was collected and grounded to obtain a fine and fluffy powder.

V Results

This chapter presents the results of the laboratory work for the preliminary and main research. The preliminary research was conducted to examine microcapsule production by SG and SG-FC with two variables: type of fish oil, and combination of matrices (Fig. 5.1). The outcomes, which are based on (1) the best process and (2) type of fish oil and matrices that produce microcapsules with the highest oxidative stability, are applied in the main research.

In the main research, only one type of fish oil was tested, and the superiority of SSPS and maltodextrin was compared with other matrix combinations (Table 5.2.). Each formula was introduced into three production methods: SG, SD, and FD. All powders were subjected to evaluation through storage tests and determination of their physical properties before they were finally compared in terms of stability against oxidation.

5.1 Preliminary research

Each of fish oil was emulsified with a combination of (1) soybean soluble polysaccharide (SSPS) and maltodextrin, and (2) β-CD, SSPS and maltodextrin. From these two formulas, only the first could be used in the preliminary research, as the second one with β-CD failed to become homogenized and sprayed in into the ProCell 5 spouted bed. The β-CD increased the emulsion viscosity over time during and after emulsification and thus caused blockage of the homogenizer. When less than 5% of β-CD was used, homogenization was successful, but the spray granulation process was not efficient and produced agglomerated granules. Therefore, the results obtained in the preliminary research were fish oils which only coated by 10% (w/w) SSPS and 65% (w/w) maltodextrin to produce 25% fish oil powders by SG and SG-FC.

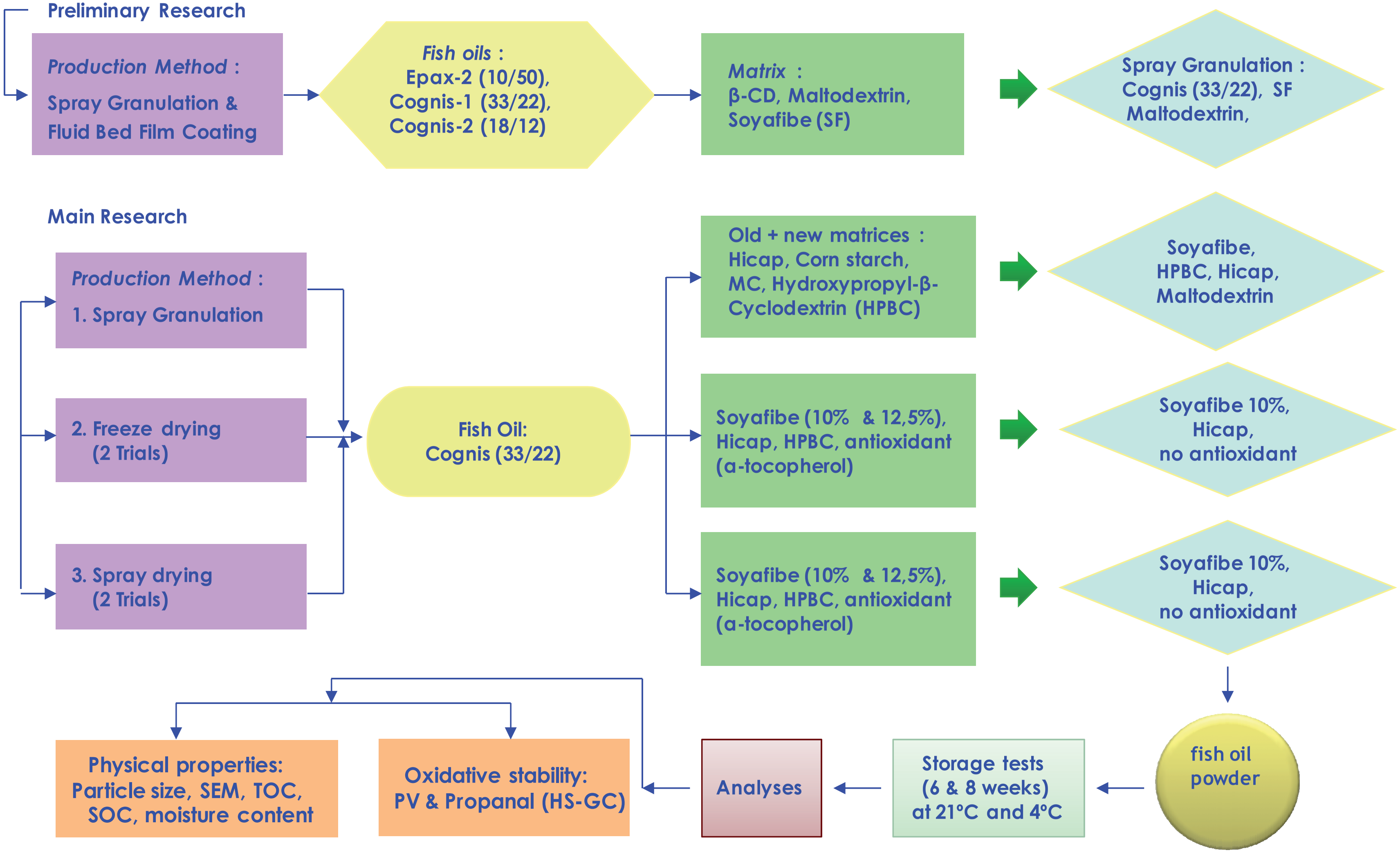

Preliminary Research
Production Method : Spray Granulation & Fluid Bed Film Coating
Fish oils : Epax-2 (10/50), Cognis-1 (33/22), Cognis-2 (18/12)
Matrix : β-CD, Maltodextrin, Soyafibe (SF)
Spray Granulation : Cognis (33/22), SF Maltodextrin,
Main Research
Production Method : 1. Spray Granulation
2. Freeze drying (2 Trials)
3. Spray drying (2 Trials)
Fish Oil: Cognis (33/22)
Old + new matrices : Hicap, Corn starch, MC, Hydroxypropyl-β-Cyclodextrin (HPBC)
Soyafibe, HPBC, Hicap, Maltodextrin
Soyafibe (10% & 12,5%), Hicap, HPBC, antioxidant (a-tocopherol)
Soyafibe 10%, Hicap, no antioxidant
Soyafibe (10% & 12,5%), Hicap, HPBC, antioxidant (a-tocopherol)
Soyafibe 10%, Hicap, no antioxidant
fish oil powder
Storage tests (6 & 8 weeks) at 21°C and 4°C
Analyses
Oxidative stability: PV & Propanal (HS-GC)
Physical properties: Particle size, SEM, TOC, SOC, moisture content

Fig. 5.1 The experimental design

5.1.1 Production of microcapsules by spray granulation

Granules from SG were stored 6 weeks both at room temperature and in a refrigerator. The oxidative stability was monitored weekly by measuring PV and propanal content. The physical properties of microcapsules were determined by (1) calculation of microencapsulation efficiency (ME), (2) measurement of particle size distribution, and (3) scanning electron microscope (SEM) examinations.

5.1.1.1 Storage at room temperature (± 21 °C)

The SG granules were kept in tightly closed glass bottles without nitrogen flush or additional antioxidant, and stored in the dark. The daily temperature of the storage room was ± 21°C with ± 30% RH without seasonal fluctuation. The containers were impermeable to oxygen. The powder stability figures based on PV and propanal were plotted against storage time (Fig. 5.2 and 5.3).

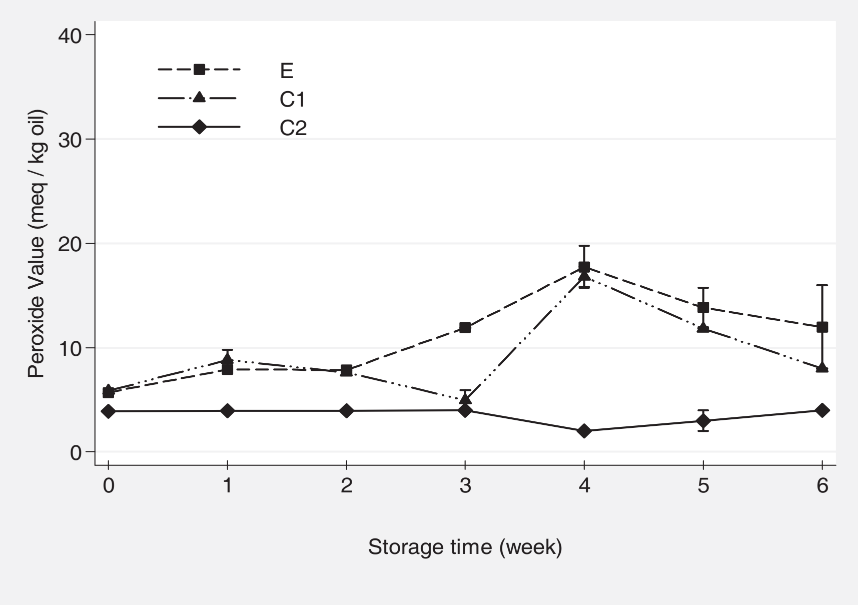


Fig. 5.2 Oxidative stability of fish oils with EPA/DHA ratios E (10/50), C1 (33/22), and C2 (18/12) at room temperature based on PV of uncoated powder.

The formation of peroxides in the fish oils E (10/50) and C1 (33/22) increased over the storage period. The induction period (IP) of C1 was 3 weeks, while it was only 2 weeks for E. The peroxides of these samples reached the highest peak at week 4 and then decreased for the remaining 2 weeks. A similar trend was observed for propanal (Fig. 5.3) where fish oil E produced significantly more propanal than C1 with an IP of about 2 and 3 weeks, respectively. Fish oil C2 (18/12), however, showed an incredibly low formation of hydroperoxides as well as propanal throughout the storage time.

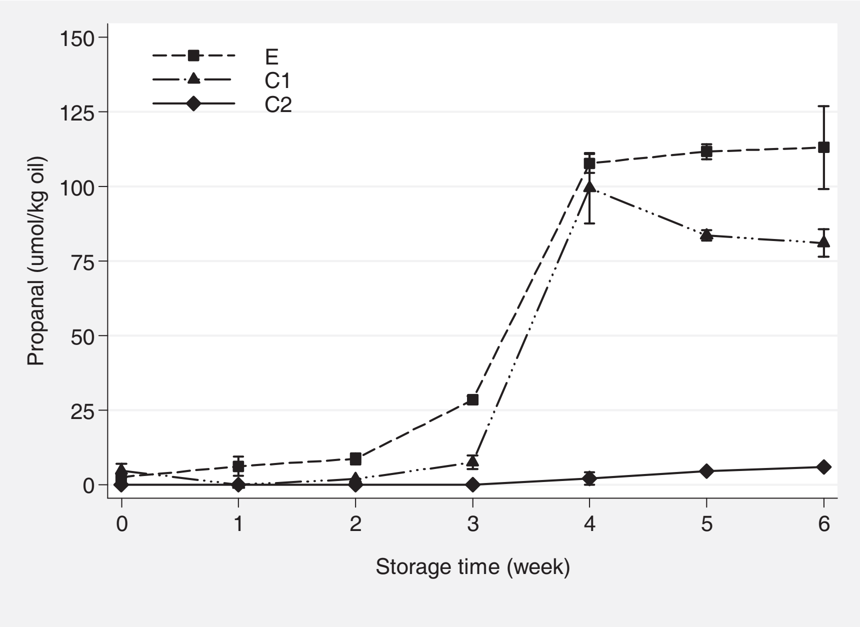


Fig. 5.3 Oxidative stability of fish oils with EPA/DHA ratios E (10/50), C1 (33/22), and C2 (18/12) at room temperature based on propanal of uncoated powder.

5.1.1.2 Storage in refrigerator (3-4 ºC)

Similar conditions were applied for storage at low temperature except that the powder stability was monitored every two weeks (Fig. 5.4 and 5.5). This decision was taken due to the very low formation of hydroperoxides measured by PV as well as the small amount of propanal in the pre-examinations.

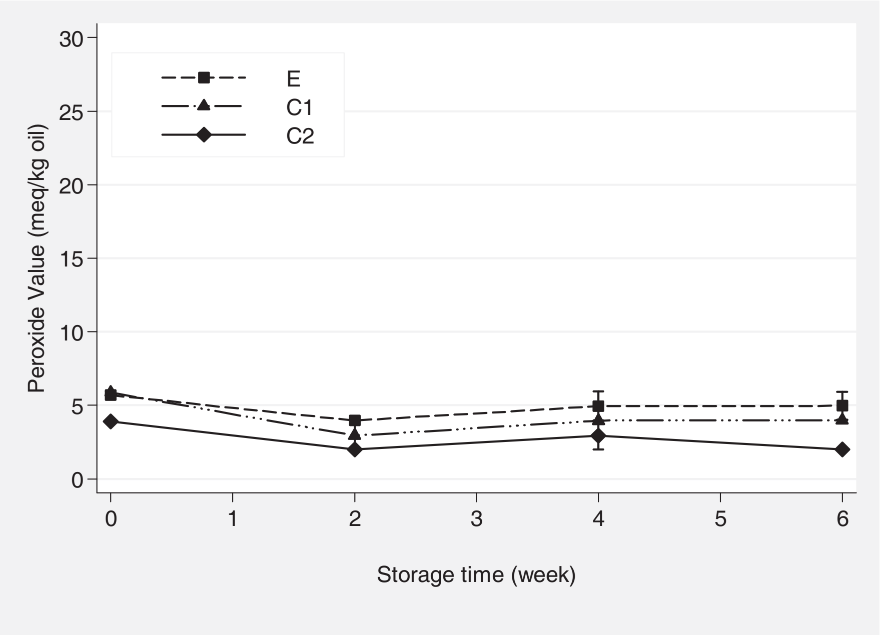


Fig. 5.4 Oxidative stability of fish oils with EPA/DHA ratios E (10/50), C1 (33/22), and C2 (18/12) at refrigerator (3-4 ºC) based on PV of uncoated powder.

Although the initial PVs of fish oils were not 0, i.e., ± 5 meq/kg oil, it can be said that the powders were stable and did not show any extreme formation of peroxides throughout the storage period. In average, the values were below 6.0 meq/kg oil. Powder prepared from C2 fish oil were the most stable with a very low formation of hydroperoxides.

The measurements of propanal by GC revealed about the same patterns as the PVs, where powder stability increased in the order E < C1 < C2. For E fish oil, as the storage time continued, the propanal slightly increased (Fig. 5.5). The C1 samples had higher initial propanal at week 0, which decreased after 2 weeks and rose again until the end of storage period. However, propanal was never detected in the sample prepared from C2 fish oil.

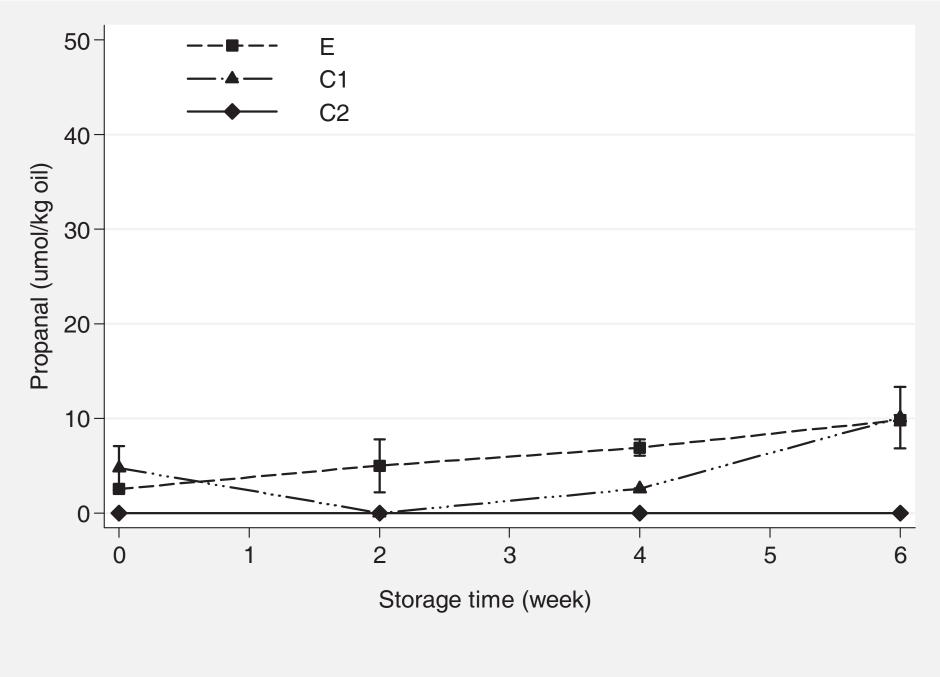


Fig. 5.5 Oxidative stability of fish oils with EPA/DHA ratios E (10/50), C1 (33/22), and C2 (18/12) at refrigerator (3-4 ºC) based on propanal of uncoated powder.

5.1.2 Production of microcapsules by fluid bed film coating

Films of hydroxypropyl betacyclodextrin (HPBCD) were delivered into granules from SG by a fluid bed equipment using the Würster process. The SG granules (± 400 – 600 µm) were immediately coated by a 15% solution of HPBC resulting in SG-FC powders, which were treated similarly to the SG powders. Therefore, E-Co (sample prepared from E fish oil and coated), C1-Co, and C2-Co were stored for 6 weeks at ± 21 ºC and at 3-4 ºC. The oxidative stability and the physical properties were also monitored.

5.1.2.1 Storage at room temperature (± 21 ºC)

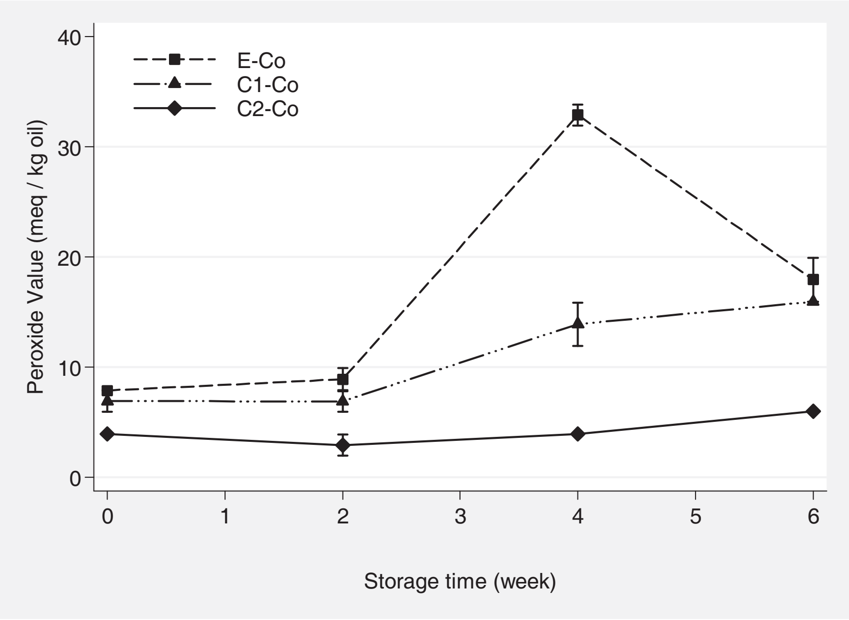


Fig. 5.6 Oxidative stability of fish oils with EPA/DHA ratios E (10/50), C1 (33/22), and C2 (18/12) at room temperature based on PV of coated powder.

High amounts of hydroperoxides were formed in the samples containing high amounts of omega-3 fatty acids (Fig. 5.6). In the first two weeks, the PVs were almost constant, but starting at week 2, the increments became very significant, particularly in the E-Co sample. The highest peak (32.90 ± 0.96 meq/kg oil) was observed at week 4 and then decreased. The C1-Co sample showed increased PVs but not as high as E-Co. The PV of the sample with the lowest EPA:DHA ratio (C2-Co) increased slightly from week 4 to 6.

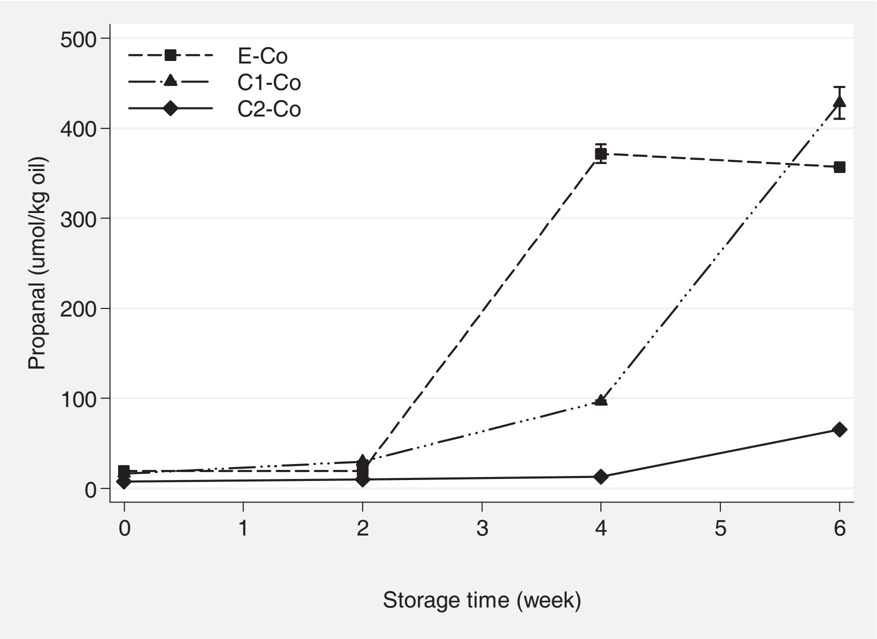


Fig. 5.7 Oxidative stability of fish oils with EPA/DHA ratios E (10/50), C1 (33/22), and C2 (18/12) at room temperature based on propanal of coated powder.

Development of propanal was in agreement with PVs for all coated samples stored at room temperature (Fig. 5.7). Starting at week 2, samples made with high amounts of PUFAs (E-Co and C1-Co) oxidized sharply and reached maximum propanal of 371.78 ± 10.26 µmol/kg oil and 428.32 ± 17.58 µmol/kg oil, respectively. The two SG-FC powders show different results (very significant amount of propanal and hydroperoxides) compared to uncoated powders (SG), but similar trends can be observed.

5.1.2.2 Storage in refrigerator (3-4 ºC)

Storage at low temperature prevents microcapsules from becoming oxidized after powder production (Fig. 5.8, and 5.9). However, the starting points both in the PV and in propanal graphs are elevated. Therefore, the maximum values are considerably higher than the values for the SG samples, where C1-Co remained stable over the storage period.

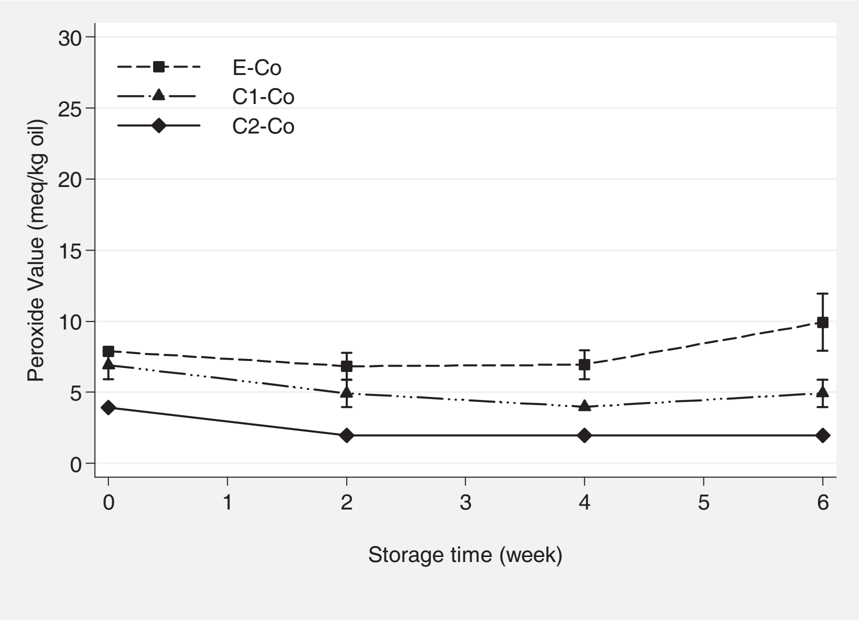


Fig 5.8 Oxidative stability based on PV of *coated powder* stored at 3-4 ºC, samples prepared from fish oils with EPA/DHA ratios: E (10/50), C1 (33/22), and C2 (18/12).

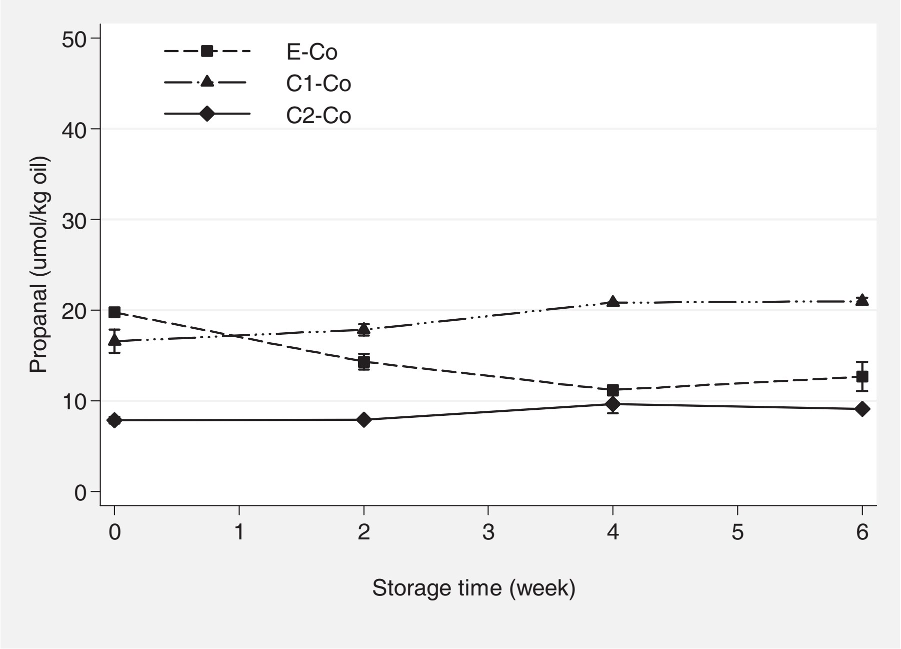


Fig. 5.9 Oxidative stability based on Propanal of *coated powder* stored at 3-4 ºC, prepared from fish oils with EPA/DHA ratios: E (10/50), C1 (33/22), and C2 (18/12).

5.1.3 Microencapsulation efficiency (ME)

The calculation of ME is based on the amount of fish oil retained in the microcapsules after extraction of non-encapsulated oil on the surface of microcapsules. The calculation is as follows:

$$ME\ (\%) = \frac{(Total\ Oil\ Content - Non\ Encapsulated\ Oil)}{Total\ Oil\ Content} * 100\%$$

The total oil content (TOC), non-encapsulated oil or surface oil content (SOC) and the ME values of all samples (SG and SG-FC) were calculated (Table 5.1). The TOC values are not as high as the theoretical content of oil load (the total amount is expected to be 25%). These variations can be caused by the fact that the oil is embedded very well in SSPS and maltodextrin. TOC analyses done by other methods including extraction and digestion by acids, extraction by organic solvents together with 10-hour refluxes, failed to separate the oil completely from the matrices. It is also possible that the enzyme was not able to totally extract the oil from the strong entrapment by the shells.

Another possibility can be attributed to the oil distribution. It is not impossible that the oil was not evenly spread out inside the walls. Some may be concentrated in the center of the granules, while some may be located near the surface of the granules. It is also possible that each granule contained a different amount of oil. Therefore, when the powder was weighed before the oil was extracted, only a small amount of it represented the whole batch. Powder with more oil resulted in higher TOC values, while powder with less revealed lower TOC values.

The non-encapsulated oil is the amount of oil that is extracted from the surface of granules or free oil. Since the amounts are considered to be low (less than 0.80%), the ME values for all powders are high. All microcapsules have more than 96% ME. However, the ME of SG (E, C1, and C2) was not significantly different from the ME of SG-FC (E-Co, C1-Co, and C2-Co).

Table 5.1 Total oil content, surface oil content and microencapsulation efficiency (ME)[*#]

Formula	*EPA:DHA ratio*	*Total oil content (%)*[**]	*Surface oil content (%)*	***ME (%)***
E	10:50	21.62	0.487^{b}	97.747^{a}
C1	33:22	21.55	$0.624^{a,b}$	97.104^{b}
C2	18:12	24.71	0.762^{a}	96.916^{b}
E-Co	10:50	19.95	0.532^{b}	97.333^{a}
C1-Co	33:22	19.44	$0.665^{a,b}$	96.579^{b}
C1-Co	18:12	20.09	0.716^{a}	96.436^{b}

[*] Ratio of SSPS and maltodextrin 1:6.5

[**] TOC, measured by enzymatic digestion method as described by Curtis et al. (2008)

[#] Means within the same column with different alphabet superscripts (a-b) are significantly different ($P < 0.05$)

5.1.4 Particle size distribution

Figure 5.10 illustrates the particle size of the granules from both processes, i.e., SG (uncoated) and SG-FC (coated). The graph is plotted according to the percentage of cumulative distribution and volume against the actual particle size.

Additional layers in the film coating process increased the diameter of the coated granules slightly compared to the uncoated ones. Uncoated granules with the size range of 400 – 500 µm represent 42 – 87% of the total population. The coated granules with the same size range correspond to 48 – 94%. About 5% granules in each group has a size of below 300 µm. Almost 100% or very few granules with diameter of more than 600 µm were found in the SG-FC samples and only about 1% in the SG samples.

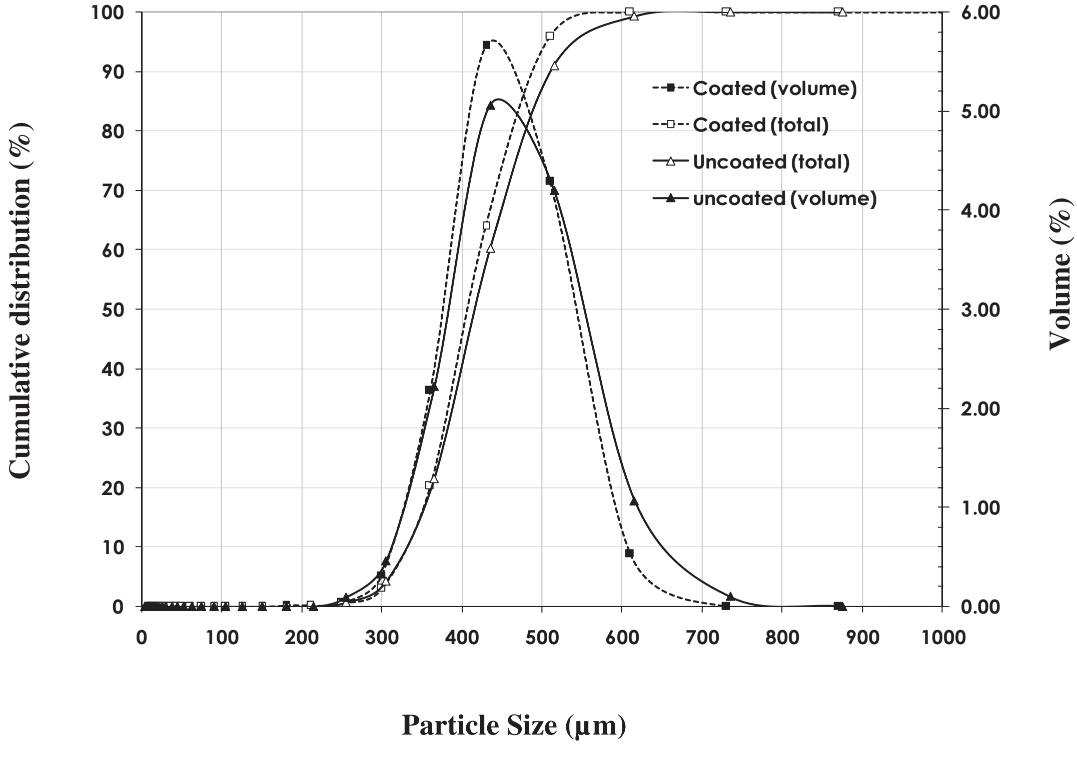


Fig. 5.10 Particle size distribution of uncoated and coated fish oil powder

5.1.5 Morphology of microcapsules

The scanning electron microscope (SEM) reveals the microstructure of SG granules (Fig. 5.11). The shape is not completely spherical and round, and the excess of emulsion drops are of evidence. The surface of each granule is not smooth but seems rough. The SG-FC granules have a similar shape (Fig. 5.12). The surface morphology obviously has imperfections, i.e., cracks, holes, and fissures.

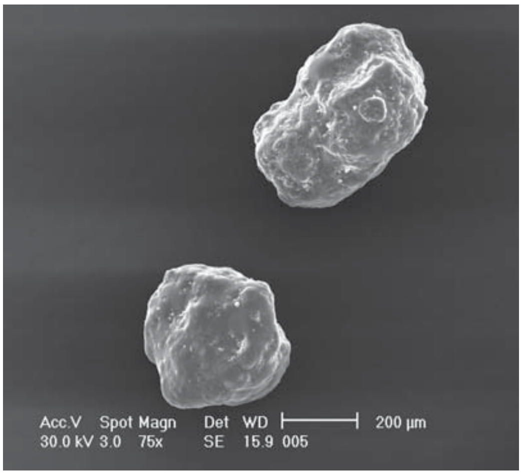


Fig. 5.11 SEM of microcapsules produced by spray granulation

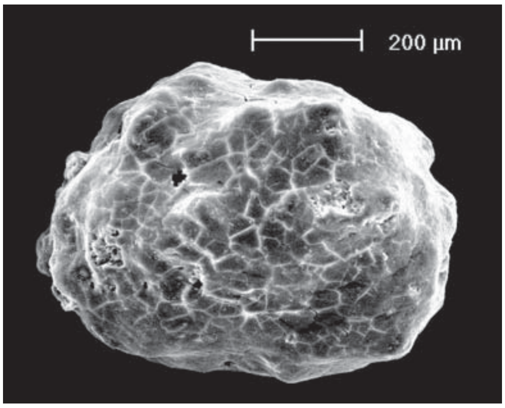


Fig. 5.12 SEM of microcapsules produced by SG-FC processes

5.2 Main research

The preliminary results show that microcapsules prepared from C2 fish oil (18/12 EPA/DHA) containing the lowest amount of omega-3 fatty acids (360 mg/g) exhibited the highest oxidative stability compared to the other two fish oils (E & C1) with 670 and 620 mg/g omega-3 fatty acids, respectively. Production by SG was better in terms of product stability compared to production by SG-FC. Storage at low temperature (3-4 ºC) successfully suppressed the rate of oxidation without any extreme formation of hydroperoxides and propanal for 6 weeks.

Although samples prepared from C1 fish oil (33/22 EPA/DHA) were not as stable as C2, C1 fish oil was used for the experiments in the main research. The decision was taken considering its EPA and DHA ratio. Since samples with 360 mg/g omega-3 fatty acids have proven to be stable, investigation of the higher amount of PUFAs was then needed to answer the question of the critical upper limit for the total fatty acid content. These challenges become more important especially when C1 fish oil is incorporated into different new matrices and the microcapsules are produced by other drying methods.
Therefore, the powders prepared from 33/22 fish oil were examined for stability after production by SG in comparison with powders produced by SD and FD. The oxidative stability of the fish oil microcapsules was only examined based on storage at room temperature for 8 weeks.

5.2.1 Production of microcapsules by spray granulation

In order to compare the ability of SSPS and maltodextrin to encapsulate fish oil, new matrices were tested in the main research, including corn starch, methyl cellulose, and modified starch. HPBCD was also used as the main matrix instead of as coating polymer applied previously in the SG-FC process.

Early trials were conducted to find the best compositions (percentage of solid content) of new matrices to be used in combination with SSPS. However, corn starch and methyl cellulose were not used further in the main research because emulsions made from corn starch or methyl cellulose with SSPS and/or maltodextrin were not efficiently fluidized in the spouted bed and caused the powder to agglomerate. Excessive sticky materials were found, thus these two polymers were excluded (Table 5.2).

Table 5.2 Matrices for encapsulation of fish oil (main research)

Code	*Matrices combination (%)*				*Fish oil (25%)*
	Soyafibe	*Maltodextrin*	*Hi-Cap 100*	*HPBCD*	
MC-1	12.5	62.5	-	-	Cognis-1 33/22
MC-2	10	65			
MC-3	10	50	-	15	
MC-4	10	-	65	-	

SG microcapsules obtained from four different formulas were stored similarly to those in the preliminary research. The PV values and propanal were plotted against storage time (Fig. 5.13 and 5.14). Increasing the amount of SSPS from 10% (in MC-2) to 12.5% (in MC-1) significantly improved powder stability. The trend shifted from the green line (with highest PV of 17.79 ± 1.95 meq / kg oil) toward the blue line (with highest PV of only 10.97 ± 1.00 meq / kg oil) (Fig. 5.13). Combination of SSPS, HPBCD and maltodextrin (MC-4) produced powder with the lowest stability (highest PV of 31.93 ± 0.02 meq / kg oil). Modified starch and SSPS (MC-3) showed almost the same capability as 12.5% SSPS and maltodextrin in retarding lipid oxidation.

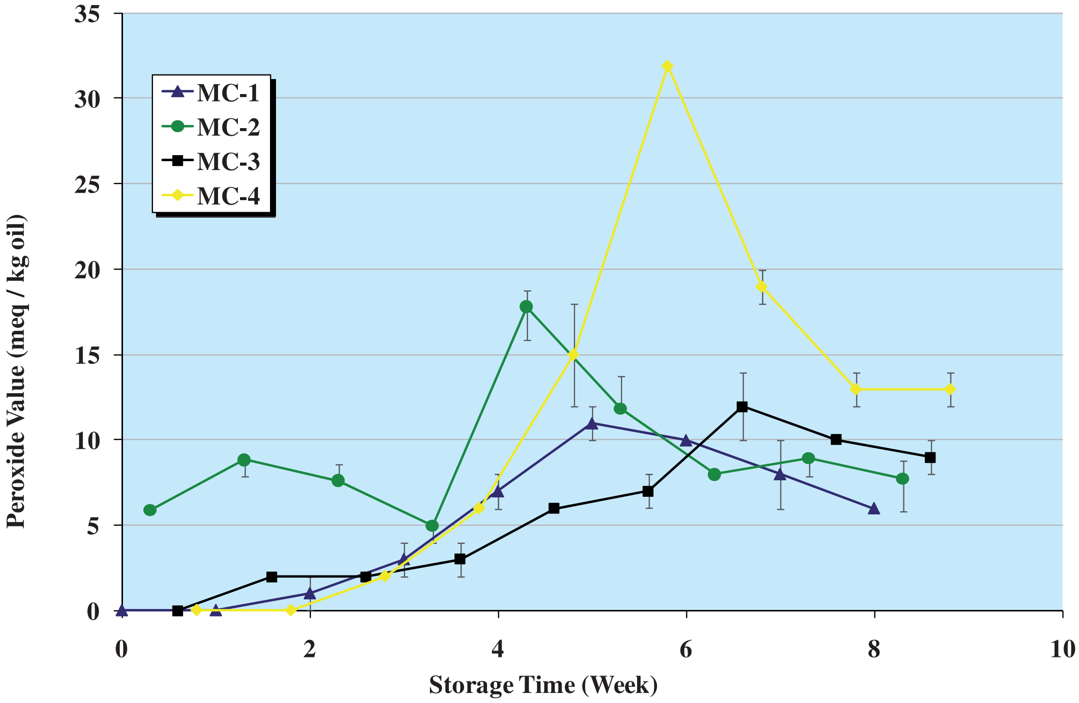


Fig 5.13 Oxidative stability based on PV of *spray granulated* microcapsules at room temperature. Samples prepared from MC-1 (12.5% SSPS + 62.5% maltodextrin), MC-2 (10% SSPS + 65% maltodextrin), MC-3 (10% SSPS + 65% modified starch), and MC-4 (10% SSPS + 15% HPBCD + 50% maltodextrin).

The propanal development in SG powder shows a similar trend (Fig. 5.14). There were distinct increments after week 3, 4, and 5 for MC-2, MC-4, and MC-3, respectively. At those weeks, the IP of each mentioned sample was started. Interestingly, before each of the IP point, the PVs of all samples from week 0 were stable, but then increased sharply. Likewise, the MC-2 sample oxidized earlier than the others as indicated by the IP that started at week 3, followed by MC-4, MC-3, and MC-1. The highest peak was 138.24 ± 1.67 µmol / kg oil in the MC-4 sample.

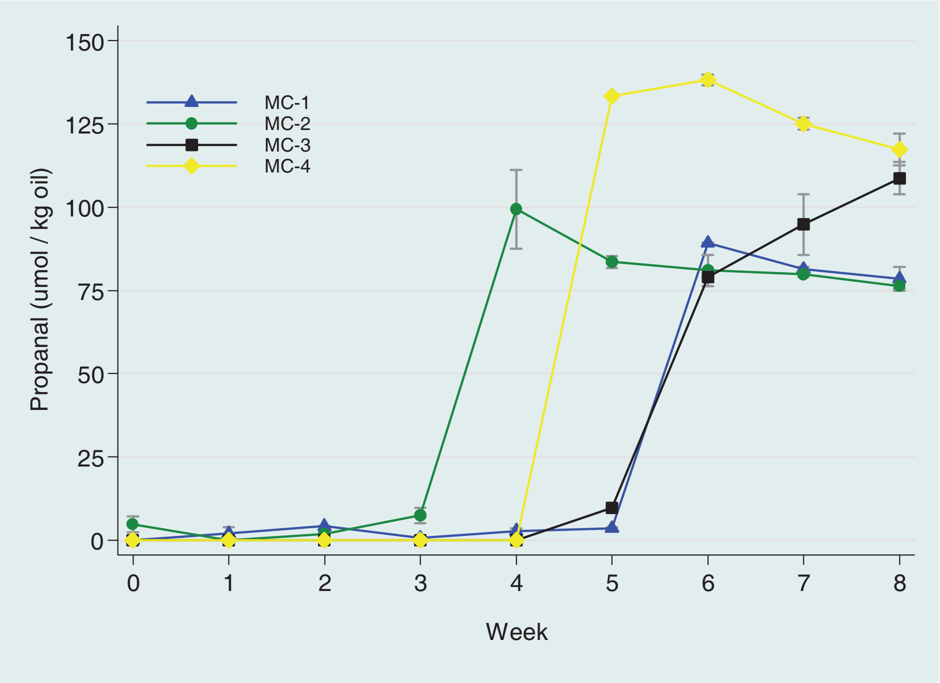


Fig. 5.14 Oxidative stability based on propanal of *spray granulated* microcapsules at room temperature. Samples prepared from MC-1 (12.5% SSPS + 62.5% maltodextrin), MC-2 (10% SSPS + 65% maltodextrin), MC-3 (10% SSPS + 65% modified starch), and MC-4 (10% SSPS + 15% HPBCD + 50% maltodextrin).

The patterns of propanal formation of MC-1 and MC-3 were similar. Both produced low propanal up to week 5, which then decreased (MC-1) and increased (MC-3). The MC-3 sample did not oxidize up to week 4, as no propanal could be detected by GC, however after the IP had started, the oxidation rate rose significantly.

5.2.1.1 Powder morphology and particle size distribution

Morphology, microstructure and particle size distribution of SG microcapsules are alike as described previously in subsection preliminary research. However, granules produced in the main research had a less spherical shape, and the raspberry-like structures were more obvious (Fig. 5.15).

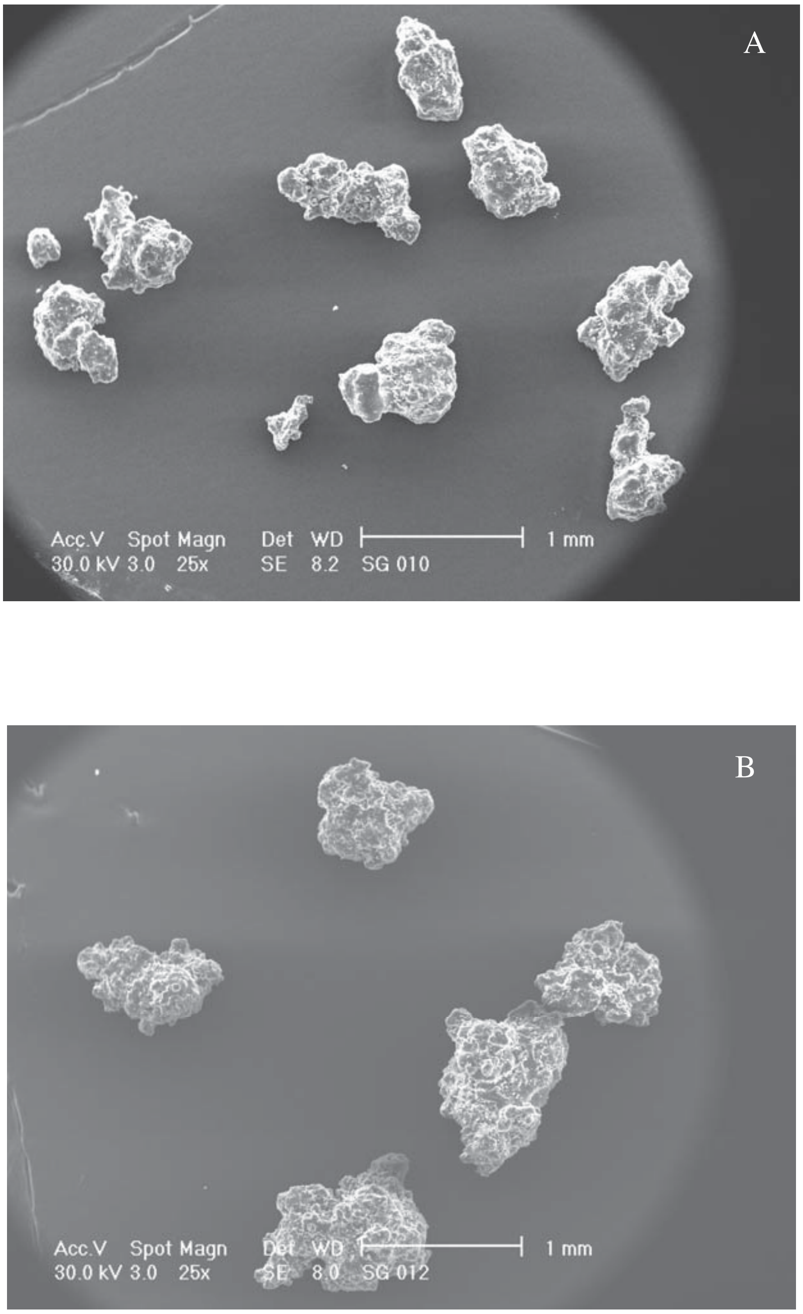

A
Acc.V Spot Magn Det WD 1 mm
30.0 kV 3.0 25x SE 8.2 SG 010
B
Acc.V Spot Magn Det WD 1 mm
30.0 kV 3.0 25x SE 8.0 SG 012

Fig. 5.15 SEM of microcapsules produced by *spray granulation*. (A) Granules prepared from SSPS and maltodextrin, and (B) Granules prepared from SSPS and modified starch

5.2.2 Production of microcapsules by spray drying

With spray drying, the hydroperoxides formation revealed that combination of SSPS, HPBCD and maltodextrin (MC-4) gave the lowest protection to fish oil (Fig. 5.16). The oxidation started at week 2 after production and increased sharply at week 3. The other three formulas retarded oxidation quite significantly in the first 2 weeks as shown by PV of below 5.0 meq / kg oil. However, the PVs of the MC-1 and MC-2 samples gradually increased up to week 6 and then decreased. Combination of SSPS and modified starch (MC-3) demonstrated the best inhibition toward oxidation in the first 6 weeks, as indicated by PVs below 10.0 meq / kg oil.

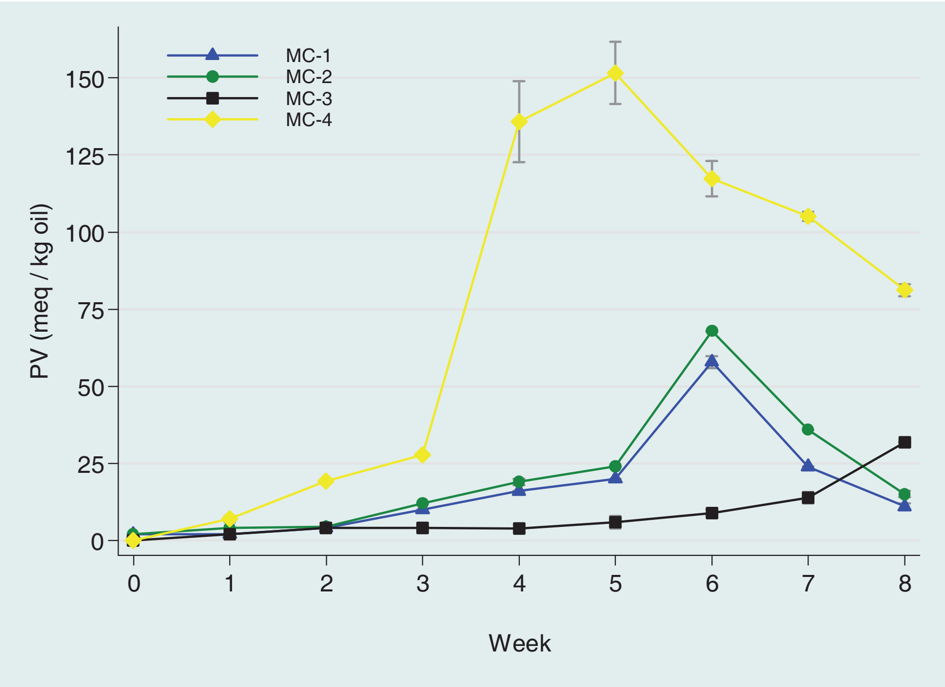


Fig. 5.16 Oxidative stability based on PV of *spray dried* microcapsules at room temperature. Samples prepared from MC-1 (12.5% SSPS + 62.5% maltodextrin), MC-2 (10% SSPS + 65% maltodextrin), MC-3 (10% SSPS + 65% modified starch), and MC-4 (10% SSPS + 15% HPBCD + 50% maltodextrin).

Propanal formation is in agreement with peroxide formation (Fig. 5.16 and Fig. 5.17). The MC-4 formula was not able to prevent oxidation as shown by the incredibly high formation of propanal, particularly at week 5, which reached 752.22 ± 18.35 µmol / kg oil. Extreme propanal values (more than 300.0 µmol / kg oil) were also observed for the MC-1 and MC-2 samples after 5 weeks of storage. Similarly, MC-3, which consist of SSPS and modified starch, proved to be the most effective matrices to suppress oxidation, although in the first 5 weeks the amount of oxidation product was still higher than that of SG granules.

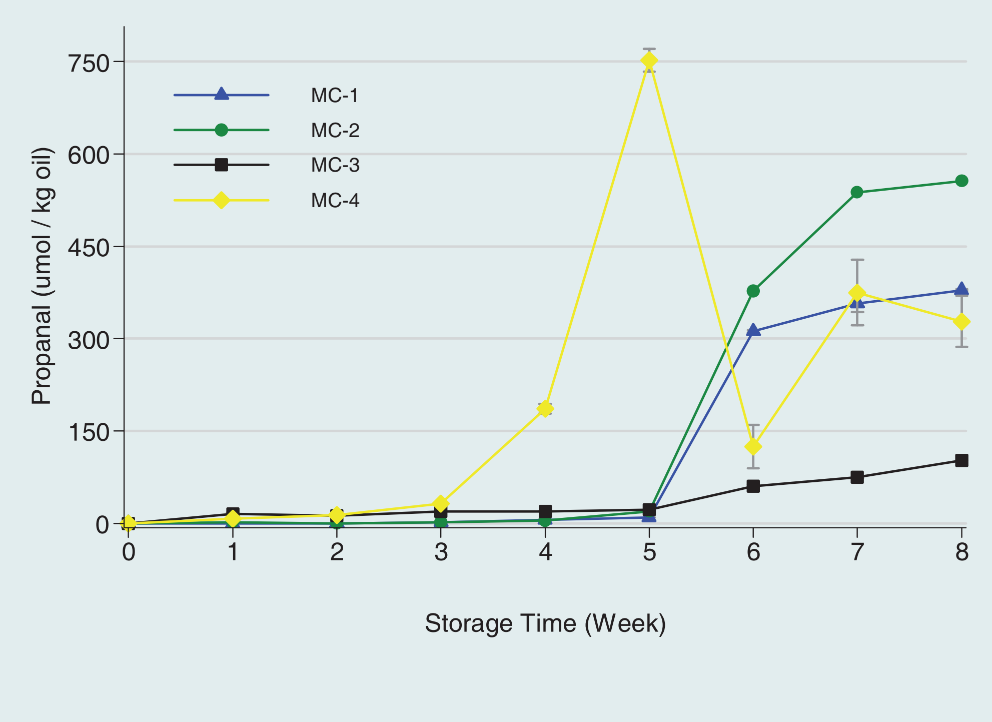


Fig. 5.17 Oxidative stability based on propanal of *spray dried* microcapsules at room temperature. Samples prepared from MC-1 (12.5% SSPS + 62.5% maltodextrin), MC-2 (10% SSPS + 65% maltodextrin), MC-3 (10% SSPS + 65% modified starch), and MC-4 (10% SSPS + 15% HPBCD + 50% maltodextrin).

5.2.2.1 Powder morphology and particle size distribution

The physical microstructure of SD powder is different from that of SG (Figs. 5.18A and 5.18B). The particles are round and spherical in shape with or without dents on the surface. Each particle has a hollow structure, and some are very small and attached to the bigger ones; particle size is in a range of 25 – 100 µm.

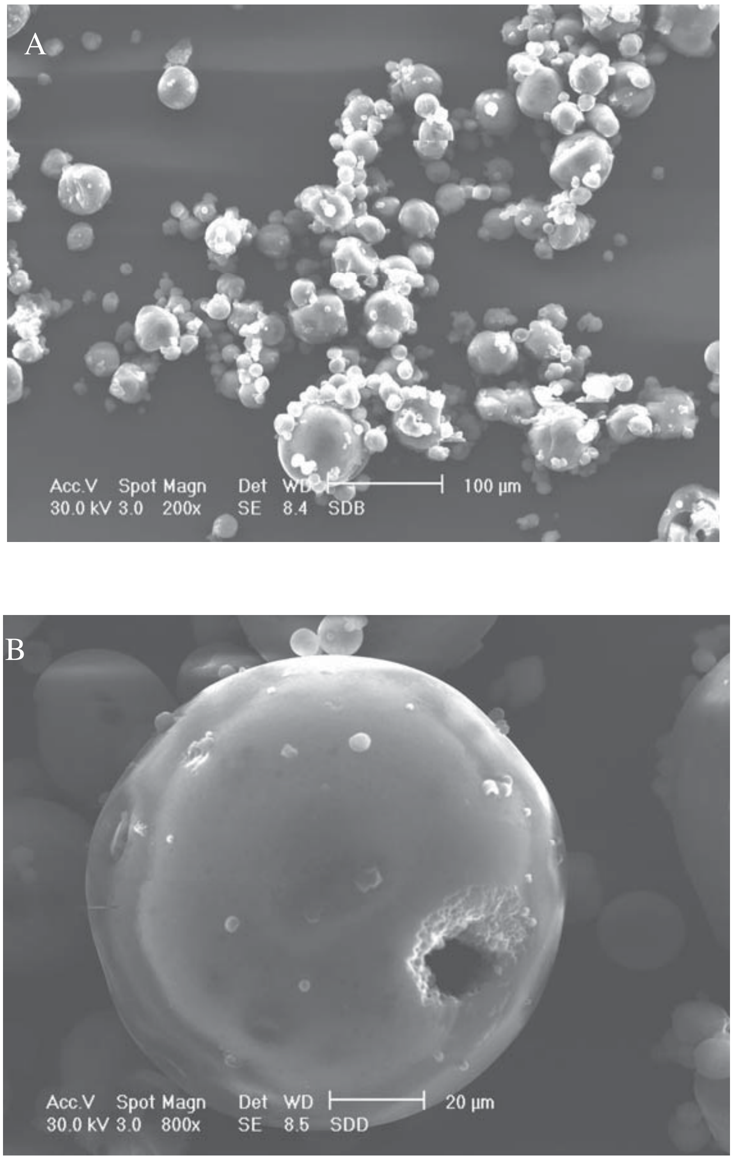

A
Acc.V Spot Magn Det WD 100 µm
30.0 kV 3.0 200x SE 8.4 SDB
B
Acc.V Spot Magn Det WD 20 µm
30.0 kV 3.0 800x SE 8.5 SDD

Fig. 5.18 SEM of microcapsules produced by *spray drying*. (A) Particles made by combination of SSPS and maltodextrin, and (B) Particles made by SSPS and Hi-Cap.

5.2.3 Production of microcapsules by freeze drying

Based on PVs, all FD samples oxidized at an extremely high rate compared to those obtained by SG and SD (Fig. 5.19). Starting from week 2, lipid degradation progressively increased until week 5 for MC-1 and MC-2 with a maximum of 390.38 ± 31.44 meq / kg oil for MC-4 at week 6. The PVs of MC-3 powder steadily increased until the end of the storage test.

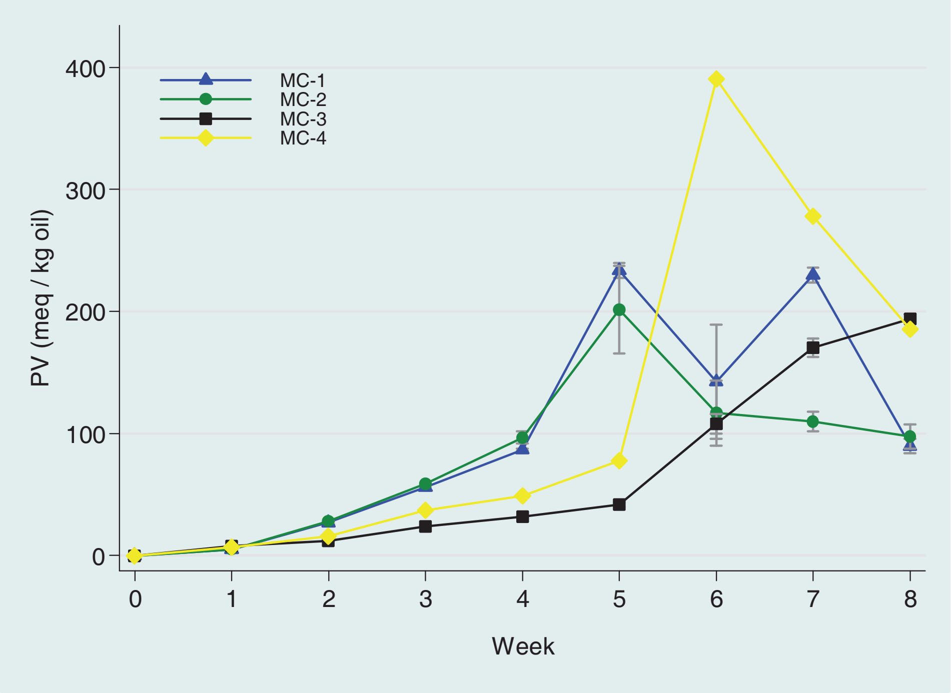


Fig. 5.19 Oxidative stability based on PV of *freeze dried* microcapsules at room temperature. Samples prepared from MC-1 (12.5% SSPS + 62.5% maltodextrin), MC-2 (10% SSPS + 65% maltodextrin), MC-3 (10% SSPS + 65% modified starch), and MC-4 (10% SSPS + 15% HPBCD + 50% maltodextrin).

In contrast to the PV results, propanal formation was slightly decelerated, at least in the first 4 weeks (Fig. 5.20). Combination of SSPS (either 10% or 12.5%) with maltodextrin ineffectively inhibited oil oxidation in powders after 4 weeks. As for the PVs, propanal developments also fluctuated in these samples, where they reached maximum values that reduced in the following weeks but then increased again. The powder made with the MC-3 formula confirmed the superiority of SSPS in combination with modified starch for reducing the rate of lipid oxidation. However, in contrast to the previous results, MC-4 microcapsules demonstrated a lower trend in propanal formation compared to MC-1 and MC-2. The IP of FD powders started one week earlier than that of SD powders.

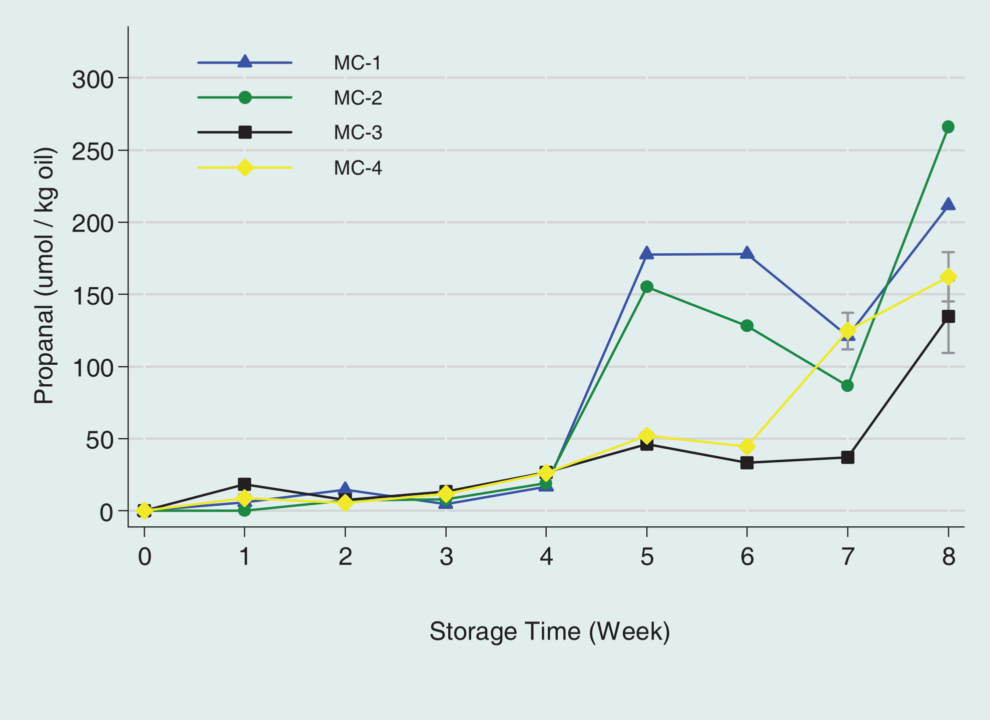


Fig. 5.20 Oxidative stability based on propanal of *freeze dried* microcapsules at room temperature. Samples prepared from MC-1 (12.5% SSPS + 62.5% maltodextrin), MC-2 (10% SSPS + 65% maltodextrin), MC-3 (10% SSPS + 65% modified starch), and MC-4 (10% SSPS + 15% HPBCD + 50% maltodextrin).

5.2.3.1 Powder morphology and particle size distribution

Freeze drying produced powder with a different microstructure as those of SG and SD powders. The shape is not uniform and irregular, some are flake-like, and some are porous or have a sponge-like structure (Fig. 5.21). Visual observation shows that FD powder is very light and fluffy. The particle size distribution is 50 – 300 µm.

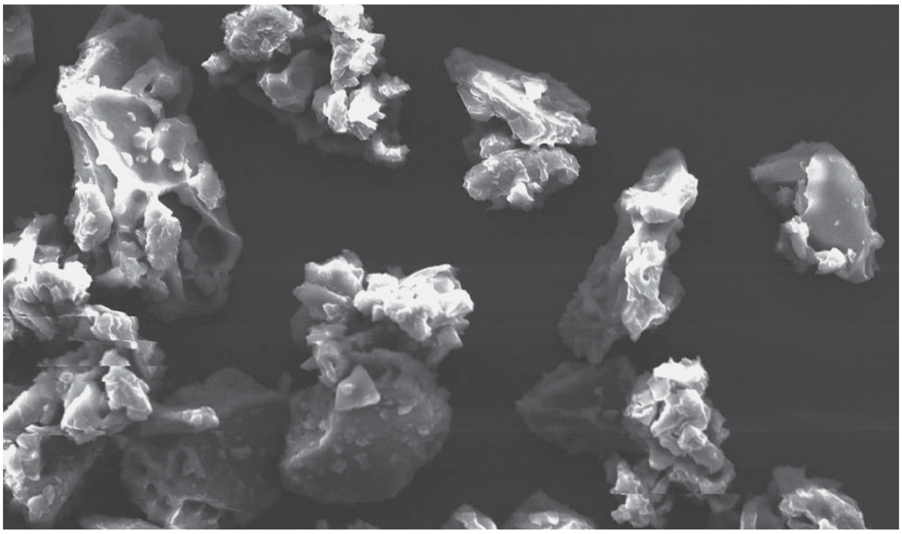


Fig. 5.21 SEM of microcapsules produced by *freeze drying*. Powder prepared by combination of SSPS and maltodextrin.

5.2.4 Moisture content and water activity (a_w)

The moisture content and a_w of each sample were measured after powder production, in the middle and at the end of storage test. The average values of the three measurements in each production method are summarized in Table 5.3.

Table 5.3 Moisture content and water activity

Production method	*Combination code*	*Moisture content (%)*	a_w
SG	MC-1	2.29	0.17
	MC-2	5.87	0.42
	MC-3	6.58	0.51
	MC-4	6.59	0.51
SD	MC-1	2.59	0.21
	MC-2	2.23	0.16
	MC-3	2.87	0.24
	MC-4	3.01	0.25
FD	MC-1	2.70	0.22
	MC-2	3.23	0.27
	MC-3	2.98	0.25
	MC-4	3.12	0.26

Overall, the powder moisture content is less than 6.60 % for all measurements, and low in SD and FD samples (only 2 – 3 %). The maximum observation is 6.59 % moisture content measured in MC-4 of SG granules and the minimum value is 2.21% observed in MC-2 sample dried by spray drying. The water activity is proportional to the moisture content. Samples containing more water exhibit higher water activity.

VI Discussion

6.1 Preliminary research

6.1.1 Effect of type of fish oil on powder stability

In the present study, different mechanisms of oxidation in three food systems (bulk oil, emulsion, and powder) are considered to determine powder stability. According to Jacobsen et al. (2008) [121], lipid oxidation in multiphase food systems and reaction of ingredients with lipid molecules as well as antioxidants are very complex. The extent of oxidation is driven by the physical properties of food itself, type of emulsifier, antioxidants and their concentration, pH, the existence of preformed hydroperoxides, other pro-oxidants, and metal ions.

Thus, the oxidative stability of fish oil powder in this research might be influenced by early oxidation in fish oils, and in emulsion, production methods, and the storage conditions. The bulk fish oils contained significantly high amount of unsaturated fatty acids with maximum initial PV of 1.0 meq/kg oil (Table 6.1). The amount of transition metal, particularly iron, was < 0.1 mg/kg. The initial PVs remained constant upon receiving the oils and before powder production, giving an indication that the oils were in good quality.

The quality of bulk oil and the amount of initial hydroperoxides are important to determine powder stability and the quality of the food product enriched with the powder. Contradicting reports exist in this regard. Let et al. (2004) [155] underlined that type of fish oil with very low initial PV was critically important to retard oxidation of milk enriched with fish oil. Nielsen et al. (2009) [209] highlighted that in some of their experiments, good quality fish oils with very low initial PVs deteriorated fish-oil-enriched milk within two weeks. However, in other cases, oils of lower quality (higher initial PVs) had been successfully incorporated into yoghurt. Oxidation was hardly detected in fish-oil-enriched strawberry yoghurt [120]. The discrepancies in the previously mentioned two systems might depend on the physical and chemical properties of milk and yoghurt thus resulting diverse oxidation rates.

Table 6.1 Specification of tested fish oils

Fish oil	*Ratio of EPA:DHA*	*Total omega-3 fatty acids*	*Initial PV (meq/kg)*	*Antioxidant of mixed tocopherols (mg/g)*[a]
10/50 TG (E)	10 : 50	670 mg/g	1.0	3.7
Ultra refined (C1)	33 : 22	620 mg/g	< 1	3 - 4.5
Ultra refined (C2)	18 : 12	360 mg/g	< 1	1 - 2

[a] According to the certificates of analysis issued by the producers

All fish oils used contained mixed tocopherols (TOHs) added by the producers (Table 6.1). It is a common practice that food industries add several thousand milligrams antioxidants per kilogram or less than 3 mg/g of mixture [53;151]. Many reports state that the tocopherol activity is related very much to their concentration upon addition, particularly α-tocopherol. In high amounts, it actions can changed to become pro-oxidants. This rule is different for δ-TOH and γ-TOH. Each tested fish oil contained a mixture of TOHs to prevent oxidation of unsaturated fatty acids, and the concentration depending on the PUFAs content. Addition of mixed tocopherols showed that the oils were well protected and stable until the experiments started. Therefore, it is presumed that significant oxidation did not occur in bulk oil, and that any changes in oxidation were caused by emulsification process, microcapsule production by SG and SG-FC, and storage conditions.

Three types of fish oils based on the ratio of EPA:DHA were tested. Production of microcapsules by SG and SG-FC obtained fish oil powders and was categorized into 2 groups, *uncoated* and *coated* powders. Samples were divided in tightly closed bottles (without nitrogen flush) and stored at room temperature in the absence of light and in a refrigerator (3 to 4 °C) for 6 weeks. Peroxide value (PV) analysis (to measure the primary oxidation products) and head-space propanal (to measure the secondary oxidation products) were performed every week except for samples stored in the refrigerator, where analyses were conducted every 2 weeks. This decision was taken because of the low formation of hydroperoxides observed during the preliminary research. Therefore, the discussion focuses on the powders stored at room temperature.

In general, the results show that the oxidative stability of fish oil microcapsules depends on *the total amount* of PUFAs contained in each type of oil and also on the type of fatty acids: EPA and DHA. High amounts of PUFAs in fish oils increased the development of PVs and propanal over the storage time. Frankel (2005), Nawar (1996), and Hamilton et al. (1997) emphasized that the location and number of double bonds in a lipid molecule govern its susceptibility to oxidation [71;92;208]. As the degree of unsaturation increases, the rate of fatty acid oxidation also rises.

Though good quality oils were used, and spray granulation was started immediately after oil emulsification, it was possible that free radicals might have already existed, even in a very small amount. The number of these accelerators was predicted to be linear with the amount of PUFAs and the degree of unsaturation. This assumption was based on the evidence of substantial increments of PVs and propanal after microcapsules were produced by SG at week 0, before the storage tests were started (Table 6.2.). Formation of PVs and propanal were higher in E and C1 samples (both contained 670 and 620 mg/g omega-3 fatty acids) than in C2 (360 mg/g omega-3 fatty acids). Further process by SG-FC confirmed significant detection of propanal as volatile decomposition product of preformed peroxides, particularly in E-Co and C1-Co samples. Among these samples, PV and propanal in E-Co (with 10/50 of EPA/DHA) was higher than in C1-Co (with 33/22 of EPA/DHA). This indicates that despite the total content of omega-3 PUFAs, type of fatty acid (EPA or DHA) in fish oil also determined the rate of oxidation.

As the storage tests proceeded, the extent of oxidation progressively increased, particularly after week 3 of storage at room temperature (Fig. 5.2). In the first 3 weeks, PV was below 9 meq/kg oil except sample E where PV had increased at week 2. The highest peaks were reached after 4 weeks of storage in powder E and C1 and then decreased. The increments (from weeks 2 to 4) were 9.86 ± 1.98 and 9.20 ± 0.79 meq/kg oil for powder E and C1, respectively, which were very significant ($P < 0.01$) with 95% confidence intervals of [6.82; 12.95] meq/kg oil and [6.25; 12.14] meq/kg oil. Similar trends were found for PV values of coated powder, E-Co, and C1-Co (Fig. 5.6).

Table 6.2 Formation of hydroperoxides before the storage test at Week 0

Fish oil composition	*Initial PV (meq/kg oil)*	*PV (meq/kg oil)*	*Propanal (µmol/kg oil)*
Epax, EPA/DHA = 10/50			
E	1	5.69 ± 0.04	2.54 ± 0.14
E-Co	1	7.89 ± 0.02	19.76 ± 0.03
Cognis-1, EPA/DHA = 33/22			
C1	1	5.87 ± 0.09	4.75 ± 2.32
C1-Co	1	6.91± 0.98	16.57 ± 1.29
Cognis-2, EPA/DHA = 18/12			
C2	1	3.89 ± 0.01	0 ± 0
C2-Co	1	3.93 ± 0.01	7.85 ± 0.34

These findings can be explained by the existence of bis-allylic methylene groups in PUFAs. Frankel (2005) [71] reported that the oxidizability of polyunsaturated fatty acids, such as 18:2, 18:3, 20:4, and 22:6, was related to the number of bis-allylic positions present in the fatty esters. The oxidizability of each PUFA increased approximately two-fold for each active bis-allylic methylene group (Table 6.3). Therefore, the rate of oxidation of DHA (22:6) was 5 times higher than that of linoleic acids (18:2).

This is in agreement with the results of the present study. Powder prepared from E fish oil (EPA/DHA or 10/50) oxidized more rapidly than powder prepared from C1 fish oil (33/22) because the first oil contained 2 times more DHA than the 2nd oil. DHA consists of 5 bis-allylic methylene groups, whereas EPA has only 3. Therefore, the oxidizability of sample E was approximately 3-fold than sample C1. Samples E produced more hydroperoxides and propanal than samples C1 (Fig. 5.2 – 5.5).

Table 6.3 The rate of autoxidation of unsaturated fatty esters, adapted from [71] (Frankel 2005, p.21): rewrite with permission.

Fatty esters	*Number of -CH_2-*	*Mole O_2 per 100 hr* [a]	*Relative rate* [a]	*Oxidizability $M^{-1/2}sec^{-1/2}$* [b]	***Relative rate*** [b]
18:1	0	0.04	1		
18:2	1	1.63	41	0.020	1
18:3	2	3.90	98	0.041	2.1
20:4	3	7.78	195	0.058	2.9
22:6	5			0.102	5.1

[a] neat methyl or ethyl esters were autoxidized at 37°C and rates were measured by oxygen absorption with a Warburg respirometer [106]

[b] chlorobenzene solution were oxidized at 37°C in the presence of 2,2'-azobis (2-methylpropionitrile) and rates were measured by oxygen absorption with a pressure transducer in an automated recording gas apparatus [39]

A previous study reported that the oxidative stability of PUFAs in an aqueous solution was reversed from that in the bulk phase and in the emulsion form in which DHA (22:6n-3) was found to have the highest oxidative stability followed by EPA (20:5n-3), ALA (18:3n-3), GLA (18:3n-6), and LA (18:2n-6) [195]. The authors described that the oxidative stability increased with the increasing degree of unsaturation. PUFAs have a tight pack conformation in an aqueous solution [133] and it may cause difficulties for free radicals and/or oxygen to attack the substrates in such conformation. This finding was the opposite from what have been found in this study where powder prepared from fish oil containing more DHA was less stable than powder made from fish oil containing more EPA.

There are two possible reasons for the discrepancy: initial oxidation during emulsification and powder production. The ease to oxidation in oil-in-water emulsion is attributed to the relatively harsh condition during homogenization [121]. Homogenization results in an increased surface area in the emulsion, and thus lipid interactions with aqueous pro-oxidants, metal ions, and oxygen were also increased [27]. Despite their existence in bulk fish oils, trace levels of metal ions might be included in other ingredients, such as in coating materials. Thus, the sources of metal accelerators in emulsion could be from fish oils and coating materials. Metal catalyzed decomposition of peroxide has been reported as the major cause of lipid oxidation in multiphase food systems, including in emulsion [73;184]. It is believed that the chance of early oxidation in emulsion was small, due to stabilizing properties exhibited by SSPS.

SSPS is reported to have covalently attached peptide moieties, which play a key role in inhibiting lipid oxidation in emulsion. The mechanisms of suppressing lipid oxidation by SSPS are based on the surface activity possessed by its molecules leading to a reduction of the interfacial tension at the oil-water interface. This ability is can be attributed to peptides, which are covalently attached to the rhamnogalacturonan main back-bone. These protein moieties facilitate SSPS molecules to be adsorbed and act as anchors on the surface of oil droplets for the carbohydrate regions. The carbohydrate moieties help in stabilizing the oil droplets by preventing aggregation via steric repulsion. Physically, SSPS protects the oil droplets from radical attacks by membranes built from the extension of the carbohydrate chains from the droplet surface into the aqueous phase [180;205].

Though SSPS is reported to be an excellent matrix for suppressing lipid oxidation, early hydroperoxides formation was always possible during emulsification. Therefore, if the oxidation has started at this stage, its rate might be driven by the interaction of iron present in the aqueous phase with the preformed radicals or peroxides in the oil-water interface [184]. In addition, because of the property of the fish oils, the membranes built by SSPS might not have been able to completely prevent the oil droplets from being attacked. The numbers of the double bonds were incredibly high, thus the chances of alkoxyl radicals to be formed were also multiplied. Most importantly, emulsion exposure to elevated drying temperature, oxygen and light during powder production might have induced further reactions of the preformed hydroperoxides and have caused the decomposition products to be accumulated in powder throughout the storage time.

6.1.2 Effect of drying process on powder stability

The present study used a spouted bed apparatus for the spray granulation (SG) process. SG by a spouted bed is a special processing option in particle technology. Unlike the standard fluidized beds, the fluidizing gas in the ProCell spouted bed enters the process chamber through two parallel gaps. The streams are diverted upward and combined centrally to fluidize particles upward [88;119]. The process started with the introduction of "seed particles" (the same coating material as used for microencapsulation) into the drying chamber. These particles were suspended by the air stream, and the emulsion was sprayed in with a regular speed and rate. With the ongoing process, the layers were built up surrounding the seed particles and the water was evaporated (drying). Despite the advantage of using a low drying temperature (± 60 °C), layers encapsulating the granules protected the fish oil from immediate oxidation during the process. This cannot be achieved by other drying methods such as spray drying.

Fish oil powder from SG with lower amount of omega-3 fatty acids (C2 - 360 mg/g and EPA:DHA ratio of 18/12) was found to be stable over six weeks of storage with a maximum PV of 3.99 ± 0.001 meq/kg oil (Fig. 5.2). The propanal development was only 5.89 ± 0.02 µmol/kg oil from production up to the end of the storage test (Fig. 5.3). The hydroperoxide formation and propanal development were not significant ($P < 0.05$) for all samples stored at 3-4 °C (Fig. 5.4, 5.5, 5.8, and 5.9).

Compared to the SG-FC samples, the powders produced by SG showed lower PVs and propanal during storage. Low drying temperature and granules microstructure are responsible for powder stability. The dispersed fish oil undergoes double protection. First, it is embedded inside matrices and second covered by layers in the SG process. Berquin (1961) [17] described the principles of SG to produce uniform granules by a mechanism of layer-by-layer growth, also known as the 'onion-skin' structure [158;159;318;319]. The deposition of successive layers on the surface of the seeds is possible because of the regular cyclic motion of particles in the bed that pass through the liquid spray. The system provides enough residence time in the annulus zone for each layer to dry before the next layer is deposited again in the spout zone [17;18;179]. As mentioned earlier, low drying temperature of SG is critical, particularly for the sensitive fish oil. Though the jet gas might be warm enough when entering the apparatus, but since the position of the nozzle and gas inlet is very close and ‘ideal’, the spray liquid will evaporate quickly in this zone and allow the gas temperature to decrease significantly before it comes into contact with the bed particles. In addition, the high velocity of the inlet gas prevents particle agglomeration during layer deposition [178;179].

Fluid bed film coating, as the secondary process for enveloping the granules investigated in this study aims to ensure the protection of fish oil from oxidation. The term film coating implies to the encapsulation technology that utilizes a spray process to deliver film material onto a core. The thin film is applied over an existing core to ensure that each of the particles is uniformly coated by an evenly distributed layer [291;304]. The technique is based on the use of fluidizing air to provide a uniform circulation of particles past an atomizing nozzle. As atomized coating materials contact particles, fluidizing air evaporates solvent or solidifies coating solids on the particles as part of a developing film [74].

The film coating process was conducted in a Würster insert in the fluid-bed equipment. There are 4 regions in a Würster chamber: (1) the spouting zone or central zone where the particles are wetted by sprayed coating liquid, (2) the inner cylinder zone where the drying process takes place, (3) the annular zone where particles move downward to the bottom of the fluidizing chamber, and (4) the tampon zone where the particles move gradually and subsequently enter back to the central zone for the new cycle [279]. This system involves the wetting and drying processes in close combination with particle movement. When particles move from the central zone, suspend and eventually fall through the annular zone, the coating layers of particles are built up [59].

The storage tests showed that uncoated powder from SG oxidized more slowly than the coated powder from the combination process (SG-FC). The maximum PVs and propanal values for uncoated powder (fish oil E) were 17.73 ± 1.99 meq/kg oil and 113.10 ± 13.87 µmol/kg oil, respectively. The maximum values for the coated powder were 32.90 ± 0.96 meq/kg oil (PV) and 371.78 ± 10.26 µmol/kg (propanal) (Fig. 5.6 and 5.7). The coated powder had higher initial values compared with the uncoated ones. In the first 2 weeks, the primary and secondary oxidation products developed slowly before they rose sharply.

The induction period (IP) developed one week earlier, i.e., at week 2 and not at week 3 as observed for the uncoated powder.

The lowest rate of oxidation in the SG-FC process was observed for fish oil with a lower amount of PUFAs (C2 fish oil). The maximum amounts of PV and propanal were only 5.98 ± 0.004 meq/kg oil and 65.27 ± 1.74 µmol/kg oil, respectively. For this type of fish oil, SG-FC induced development of propanal significantly compared to SG, where the maximum amount of propanal that could be detected was only 5.89 ± 0.02 µmol/kg oil.

The ease of oxidation of powders in SG-FC might be linked to the double exposure of the powder to heat during the processes. The first exposure occurred when the emulsion was dried by SG and the second when the powder was introduced into the coating process. Although the inlet temperature of the fluid bed was relatively low (60-70 °C) compared with spray drying, the coating process took approximately 50 min. These conditions might have hastened the oxidation. At week 4, although samples E and C1 contained almost the same amount of omega-3 fatty acids (670 and 620 mg/g, respectively), sample E produced more hydroperoxides and remarkably higher propanal than sample C1 (Fig. 5.6 and 5.7).

The particles heating inside the fluid bed itself might also explain these findings. Less stability of powder obtained by this process might be due to the high temperature in the annular zone and in the tampon zone at the bottom of the chamber. The temperature can be more than 60 °C. In the annular zone, as particles have to be dried to avoid agglomeration, no evaporation compensates the heating effects as in the central zone where particles received sprayed-liquid droplets [221]. If the powder passed through annular zone and reside at the bottom of the chamber several times because of the recirculation process, the oxidation rate might have increased at this time.

An investigation was done to examine the effect of temperature in these regions by El Mafadi et al. (2005) [60]. They modified the design of the Würster process by introducing an insert (a hollow conical disc) to avoid air flow through the annular zone. This design was then tested for drying and coating thermo-sensitive materials (*Lactobacillus casei Lcl* and *Lactobacillus acidophilus R0052*). In the new system, the temperature was more homogeneous in all zones. The temperature difference between the annular bed and the drying chamber was only 2 °C compared with the conventional equipment where the variation was approximately 15 °C. No air was circulated into the annular zone, and therefore, no overheating was observed. The results proved that the insert had eliminated the overheating, and the bacterial survival was improved by a factor of up to 2 [60].

Another factor that might be related to the extent of oxidation in SG-FC is the quality of the film. According to Krochta et al. (1994) [149], a film can be developed if the polymer has significant solubility in the solvent. The formation of a continuous and coherent film is crucial in the entire particle surface so that the coating layer can be functioned as a diffusion barrier. In this case, the quality of films might be related with the solubility of HPBCD in water, which is not as high as the solubility of modified starch. Less continuous and coherent films might have formed and because of this, less protection by layers was measured.

Therefore, despite the higher oxidizability of DHA compared with EPA, the remarkable increments of oxidation observed in samples from the SG-FC process were related to double exposure of PUFAs to heat, longer processing time of SG-FC-samples, the quality of film coating, and possibility of particles overheating in the fluid bed chamber. Those factors contributed to the accumulation of primary and secondary oxidation products, particularly in samples E and C1.

6.1.3 Microencapsulation efficiency

Microencapsulation efficiency (ME) reflects not only the non-encapsulated oil present on the surface of microcapsules but also the proportion of oil extracted from near the surface of the capsules [248]. Because the solvent-extractable oil in the powder was less than 1% (w/w), the ME was more than 96% (w/w) for all samples prepared from the three types of fish oils (Fig. 6.1).

The high ME obtained in the present study might be related to the properties of matrices used as wall materials, i.e., SSPS and maltodextrin. As reported previously, SSPS is a proteinous polysaccharide with emulsifying ability and is used to stabilize oil-in-water emulsions over a wide range of pH [171;207]. SSPS was adsorbed to the surface of the oil droplets and acted as a stabilizer. The protein content helps in embedding the carbohydrate moieties onto the oil-water interface [181;193]. The hydrolyzed starch offers protection to the encapsulated core. The degree of protection depends on the dextrose equivalent (DE) of the hydrolyzed starch. Higher DE systems are less permeable to oxygen and result in powders with higher encapsulation efficiency [104]. Maltodextrin with a DE of 20 was used in combination with SSPS. Their properties might have protected the fish oil and played an important role in the stabilization of the fish oil emulsion.

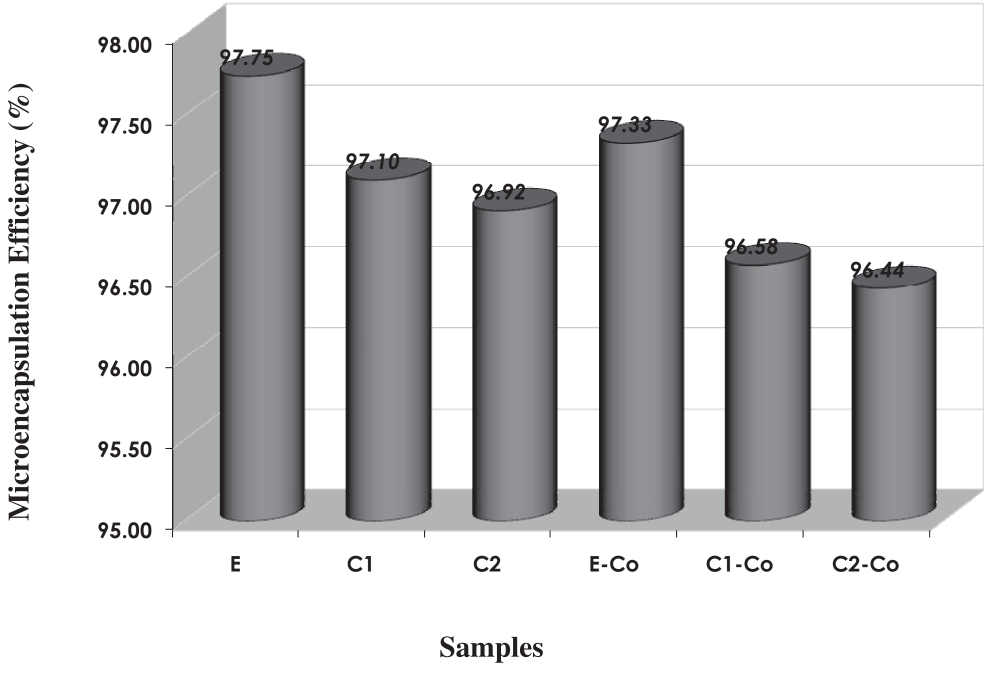


Fig. 6.1 Microencapsulation efficiency for all samples; uncoated powder (E, C1, C2) and coated powder (E-Co, C1-Co and C2-Co).

The ME values obtained in this study are comparable to those obtained by Rusli et al. (2006). For 20% and 40% oil loads, whey protein isolate-based and soy protein isolate-based microcapsules exhibited ME greater than 90%. The authors suggested that the proteins in the mixture of wall materials have the ability to form films around the oil droplets, which accounted for high ME [248].

Although the ME values in this research are relatively high compared with other results, it was expected that the coated samples would have higher ME than the uncoated ones. It was assumed that the film coating process would give better results because an additional layer would protect the non-encapsulated oil on the surface of the granules. However, there were no significant differences in ME values for coated and uncoated samples (Fig. 6.1). This unexpected outcome might be related to the film properties formed by the HPBCD.

As a coating material, HPBCD might be good to entrap the off-flavor, but the SEM showed that the coating did not cover all areas on the surface of particle. This might be influenced by the coating morphology and quality of HPBCD. Turton et al. (1999) reported that the coating morphology refers to variation of the physical properties of coating in each particle that receives the same amount of coating material. It is correlated with (a) unevenly distributed coating mass, (b) appearance of coating, which can be in amorphous or crystalline structures, and (c) existing porosity and fissures [291].

In the present case, the same ME values in uncoated and coated granules can be explained by the impact of HPBCD droplet collision and adherence to the SG particles. As the shape of SG particles was not completely round or as spherical as the glass beads, the droplets might be difficult to spread evenly. Each particle probably received a different amount of coating mass with unequal distribution. Fissures were evidence that the coating layer was too thin, thus at this part, particle surface might be exposed to the environment. This condition most likely unlocked the way for the solvent to enter the surface structure during the oil extraction process and resulted in about the same values of non-encapsulated oil for both uncoated and coated powders.

6.1.4 Particle size distribution and powder morphology

The particle sizes obtained were based on volume mean diameter and in a range of 300-700 µm (Fig. 5.11 and 5.12). Only 5% coated powder had particle sizes of more than 500 µm and approximately 12% of the uncoated powder. In other words, more than 50% particles were between 400 and 500 µm. The particle volume after the re-coating process was higher than that before recoating. This was because of the additional layers formed during fluid bed coating process.

Microcapsule microstructure and morphology are important to determine their stability, functionality, and flowability. The occurrence of dents, cracks, and pores on the surface of microcapsules unfavorably affects their functionality and reconstitution properties and also eliminates the protection function given by the carrier matrix [242;256].

The SG granules were not completely spherical and round (Fig. 5.11). Unlike the spray dried powders with a hollow inner structure, this process obtains solid particles where each particle consists of layers surrounding the seed. The surface of the microcapsules is mostly without cracks. Independent of the production process, composition of the wall is critical to determine powder quality. In this study, combination of SSPS and maltodextrin might be the reason for the minimized surface cracks. The absence of cracks is critically important to wall functionality in limiting fish oil deterioration and/or oxidation during storage.

The results are in line with those of previous studies by Sheu and Rosenberg (1998) [261], who reported that whey protein improved surface smoothness. In another works, WPI-based microcapsules have been reported to be free of surface dents. The protein content functioned as emulsifying and film-forming agents [313]. In this study, SSPS functioned as emulsifier and has film-forming properties that contributed to the production of microcapsules with minimum cracks in combination with maltodextrin, which forms low viscosity emulsion at high solid contents.

Knezevic et al. (1998) [145] classified the film coating as a complex process, which involves about 20 different process variables. Despite type of coating materials, drying temperature, particles motion and cycles, droplet and particle behavior during the process will determine the coating mass uniformity. The coating mass uniformity is determined by the amount of coating material received by each of the particles and can vary depending on: (1) the number of the particles passing through the spray zone, and (2) the amount of material deposited per particle per pass through the spray zone [30]. Due to the complexity of the coating process, Guignonet al. (2002) [89] underlined that the physical properties of the final coating structure is difficult to be predicted.

In this study, different surface morphologies can be observed for granules from the fluid bed film-coating process. Unexpectedly, the imperfections (fissures and cracks) were found in the coating layer (Fig. 5.12). According to Dewettinck et al. (1999) [47], these can be caused by the shrinkage of the film during drying. If the coating layers were too rigid or less elastic, drying might have caused the film to break. To envelope the microcapsules, 15% (w/w) of HPBCD was used, which might be too low and thereby thin films were formed. These films might be fragile and might decrease the protection on the surface of the granules.

Link and Schluender (1997b) [159] also found fractures on the coating surface in their experiment where they coated a single particle with polyvinylpyrrolidone (PVP). Similar results were observed by Dewettinck et al. (1998; 1999) [45;47] when they used hydrolysed gelatine and carboxymethylcellulose (CMC) to coat glass beads. However, they noticed uniform coating without cracks and holes when maltodextrin DE18 was applied at low spray rate.

Droplet-particle behavior during the coating process such as collision, impact and adherence, spreading, film formation and layering might have contributed to the formation fissures. The existing of holes in the coating might be caused by the improper droplet spreading after the adherence. According to Uhlemann (1990) and Werner et al. (2007b) [293;303], there are some possibilities in regards to the formation of droplet deposition after impingement. Droplets might have rebounded before adherence, spread unevenly and formed thick pancake on the surface, splashed (when droplet impact velocity is too high during impingement), or might recoil or be ejected.

Voids found on the coating of HPBCD were most likely caused by the occurrence of non-uniform coating mass distribution and leaving a deposit of 'thick pancake', thus some parts of surface area were not well covered. Beside the layer thickness, the existence of fissures, cracks and holes had decreased surface protection for the SG granules which should be provided by the outer layers formed by the secondary coating.

6.2 Main research

The experiments in the main research were designed to further examine the ability of spray granulation method to produce stable microcapsules. The 33/22 DHA/EPA fish oil (25% w/w) was emulsified with 4 combinations of matrices (MC-1, MC-2, MC-3, and MC-4) and subsequently dried by 3 production methods, i.e., spray granulation (SG), spray drying (SD) and freeze drying (FD).

The MC-2 formula consists of 10% (w/w) SSPS and 65% (w/w) maltodextrin. This combination has proved to be a good wall mixture for encapsulating fish oil. To further evaluate the role of SSPS in stabilizing fish oil emulsion, the percentage of SSPS is increased to 12.5% (w/w) in the MC-1 formula, and accordingly the amount of maltodextrin is reduced (62.5% w/w). To compare the outstanding properties of SSPS and maltodextrin, the role of modified starch (65% w/w) in combination with SSPS (10% w/w) is investigated in MC-3. Finally, the ability of hydroxypropyl betacyclodextrin (HPBCD) to reduce oxidation is tested in MC-4, not as a secondary matrix, but as one of the main matrices (15% w/w) along with SSPS (10% w/w) and maltodextrin (50% w/w).

In the following, the results of each process of SG, SD, and FD are discussed, particularly to examine the protection effect provided by coating materials to retard lipid oxidation. The evaluation is based on peroxide values (PV) and formation of propanal. Finally, comparison of each drying method is evaluated according to: (1) fundamentals of each drying process (time and temperature) and how are they related to product stability, (2) particle microstructure, (3) particle size distribution, (4) surface oil content which led to the calculation of microencapsulation efficiency.

6.2.1 Results of oxidative stability based on peroxide value vs propanal

Measurement of PV to monitor lipid oxidation is reported to be less sensitive, and empirical, particularly for samples which have been exposed to light or thermally abused. However, the precision of PV determination is relatively high [71]. Because of PV limitations, measurement of secondary oxidation products is needed in order to predict the powder shelf life more accurately. For that purpose, in this study, propanal as the main decomposition product of omega-3 PUFAs is analyzed by headspace GC. According to Frankel (2005) [71], analysis of volatiles by GC is the most useful and objective test to estimate powder stability because detected volatile products are closely related with development of rancidity.

The PV measurements in this work are determined by iodometric titration. The rationale of PV analysis by this method is to quantify how much peroxide is present in the oxidized sample. If the oil in the microcapsules has started to form peroxides due to the autoxidation process, in the presence of excess iodide, these hydroperoxides liberate iodine. Thus, the amount of iodine present is determined by titration with a standard sodium thiosulfate solution using a starch indicator [216].

In general, the mechanism of lipid oxidation involves three steps: (1) initiation, the formation of free radicals, (2) propagation, the free-radical chain reactions, and (3) termination, the formation of non-radical products. These three stages can be summarized as follows [71;208]:

$$
\begin{array}{lllll}
\text{Initiation:} & In^{\bullet} + LH & \rightarrow & InH + L^{\bullet} & (1) \\
\text{Propagation:} & L^{\bullet} + O_2 & \rightarrow & LOO^{\bullet} & (2) \\
 & LOO^{\bullet} + LH & \rightarrow & LOOH + L^{\bullet} & (3) \\
\text{Termination:} & LOO^{\bullet} + LOO^{\bullet} & \rightarrow & LOOL + O_2 & (4) \\
 & L^{\bullet} + LOO^{\bullet} & \rightarrow & LOOL & (5) \\
 & L^{\bullet} + L^{\bullet} & \rightarrow & LL & (6)
\end{array}
$$

In the presence of an initiator ($In^{\bullet}$), abstraction of hydrogen from a fatty acid (LH) will form an alkyl radical ($L^{\bullet}$) in which its reaction with oxygen will develop peroxyl radicals ($LOO^{\bullet}$), which have higher energy than the alkyl radical. If the peroxyl radical reacts with hydrogen from another fatty acid, thus a lipid *hydroperoxide* (LOOH) and new alkyl radical will be produced.

As can be seen from above equations, the primary oxidation products from autoxidation are *hydroperoxides* or simply referred to as ***peroxides***. Peroxides are labile species that are odorless and colorless. Due to its instability, peroxide can easily undergo enzymatic and nonenzymatic degradation to form complex secondary oxidation products, such as aldehydes, alcohols, ketones, and hydrocarbons [216]. Experts in lipid oxidation underline that the peroxides are not directly related to the actual sensory quality of the product. The presence of peroxides may indicate that some objectionable compounds, which are responsible for rancidity in food, are potentially formed in a later stage. Peroxide values are only applicable to peroxide formation during the early stages of oxidation because peroxides are vulnerable to further reaction. An increasing peroxide content can be observed if the rate of peroxide formation exceeds that of its degradation.

Peroxides (LOOH) (equation [1]) measured by the PV iodometric titration method are based on their oxidation potential to oxidize iodide (I^-) to iodine (I_2). The mechanisms of reactions are as follows:

$$L\cdot + O_2 + H\cdot \quad ----- \quad L\cdot OO\cdot H \qquad [1]$$

$$2\,KI + 2\,CH_3COOH \quad ----- \quad 2\,HI + 2\,CH_3COO^-K^+ \qquad [2]$$

$$L\cdot OO\cdot H + 2\,HI \quad ----- \quad LOH + H_2O + I_2 \qquad [3]$$

+ starch indicator

$$I_2 + 2\,Na_2S_2O_3 \quad ----- \quad Na_2S_4O_6 + 2\,NaI \qquad [4]$$

Purple colorless colorless

In this study, values illustrating the oxidative stability of powder based on PV inform that at the early stage of storage the PVs are low, thus PVs reach a maximum and then decrease. After the induction period, high PVs increments are observed. This may be related to the formation rate of peroxides which is exceeded by its decomposition rate. However, when the trend is reversed at the later stage, the PVs decrease. As previously reported by many others [71;216], a low PV can represent two oxidation rates, i.e., early or advanced oxidation. This is confirmed in all PVs in this study. Therefore, low PVs in the last few weeks of storage indicate that the peroxides have decomposed to form secondary oxidation products, including propanal.

Propanal measurement by GC aims to detect simultaneously secondary lipid oxidation products beside the primary oxidation products and to reveal a complete oxidation history of fish oil powder. The secondary oxidation products are produced when hydroperoxides (LOOH) are decomposed to form low molecular weight or volatile compounds and cause rancidity. Chemically, rancidity is initiated by the cleavage of hydroperoxide (LOOH) bonds and yields an alkoxyl radical ($LO^{\bullet}$) and a hydroxyl radical ($^{\bullet}OH$). The alkoxyl radical is more energetic than alkyl ($L^{\bullet}$) or peroxyl radicals ($LOO^{\bullet}$) and attacks the covalent bonds adjacent to the alkoxyl radical. This reaction results in radicals, which will interact with a variety of compounds to produce ketones, aldehydes, furans, alcohols, acids and hydrocarbonds, which cause the formation of undesirable off-flavors [68;69;71].

Determination of the propanal content in this study is based on the static headspace method. One important step in sample preparation is heating the sample at 60 °C to diffuse and vaporize propanal into the gas phase for 15 min., so that the equilibrium of volatile in the vial's headspace can be reached. After equilibration, the volatile concentration in the sample is estimated proportional to the volatile in the headspace. Frankel (2005) describes that the quantification is based on the volatile distribution between the sample matrix and the static gas phase under the condition that the gas extraction is discontinuous, thus the sample phase volume and gas phase volume are constant.

6.2.2 Role of combination of coating materials in retarding lipid oxidation

6.2.2.1 Oxidative stability based on peroxide values (PVs)

The PVs demonstrate that oxidation gradually increases as storage time progresses. In general (Fig. 5.13, 5.16, and 5.19), the hydroperoxides rise to reach peaks and then decrease. These patterns apply to all formulas except MC-3. The oxidation of fish oil in MC-3 samples increases until the end of the storage time, with no apparent peak in the middle of the storage period as observed in the other 3 samples (Fig. 6.2).

The stability of all samples (in SG, SD, and FD) is influenced to a great extent by the coating materials used. Generally, increasing the amount of SSPS in the emulsions lowered the rate of oxidation in the MC-2 and MC-1 samples. It is obvious that incorporation of 2.5% (w/w) more SSPS in MC-1 has reduce the overall oxidation rate. This trend, however, is not applicable for freeze dried powder, where the MC-1 samples exhibited higher PVs than the MC-2, especially after week 5 (Fig. 6.2). However, SSPS definitely has a significant role to prevent oxidation (Fig. 6.3).

The role of SSPS begins when the fish oil is emulsified with this matrix. According to Nakamura et al. (2004b), the protein content in SSPS is responsible to stabilize the O/W emulsion by anchoring the carbohydrate moieties onto the interface of the oil-water. The oil droplets are kept apart by steric repulsion. The long hydrophilic sugar chain of SSPS has the ability to create a thick layer (about 30 nm), thus preventing the droplets from coming together and inhibiting coalescence [206].

Moreover, Matsumura et al. (2003) described in detail the composition of SSPS, which is similar to that of gum arabic. SSPS contains galactose (46%), arabinose (23%), galacturonic acid (18%), rhamnose (5%), fucose (3%) xylose (4%) and glucose (1%). Based on this, SSPS has covalently attached peptide moieties, which play a key role to inhibit lipid oxidation in emulsion. The mechanisms of suppressing lipid oxidation by SSPS are based on the surface activity of its molecules, which reduces the interfacial tension at the oil-water interface. This ability is attributable to peptides which are covalently attached to the rhamnogalacturonan main back-bone. These protein moieties facilitate SSPS molecules to be adsorbed onto the surface of the oil droplets. The carbohydrate moieties help in stabilizing the oil droplets by preventing aggregation via steric repulsion and their chains (carbohydrate blocks) extend from the droplet surface into the aqueous phase, thus physically protecting the oil from radical attack [180;207].

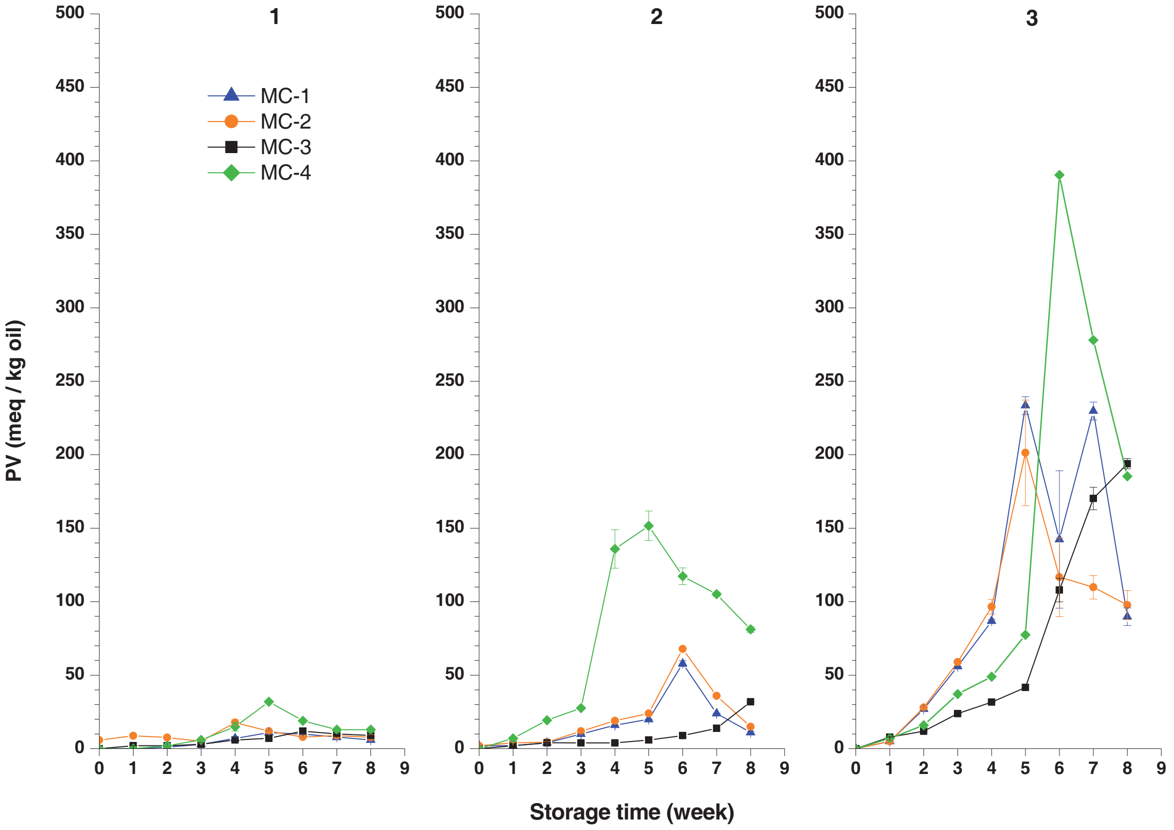


Fig. 6.2 Comparison of oxidative stability of fish oil microcapsules at room temperature based on peroxide value in (1) spray granulation, (2) spray drying, and (3) freeze drying. Formula 1, 2, 3, and 4 = MC-1, MC-2, MC-3, and MC-4.

SSPS has also demonstrated significant ability to scavenge free radicals by binding them into the reactive sugar component (galacturonic acids), which are localized on the droplet surface to inhibit lipid oxidation [78]. Low-molecular-weight (LMW) component contains more proteinous or peptidyl constituents than the whole SSPS and is responsible for the high-radical scavenging activity. On the other hand, the high-molecular-weight (HMW) component mainly consists of saccharides, rhamnogalacturonan and homogalacturonan, thus it is important for the stabilizing effect of SSPS [62]. The small amount of soy protein isolate present in carbohydrate chains attached by the covalent bond also contributes to the antioxidative activity of SSPS. This is in agreement with the amino-acid composition of SSPS, which is similar to the content of soy protein isolate reported in literature. There is also the possibility of an inhibitory effect by the action of LMW antioxidants such as polyphenols [75].

Although SSPS and gum arabic have similarities in preventing lipid oxidation, their combination with other matrices such as maltodextrin exhibited different effects. The mixture of SSPS and maltodextrin resulted in decreasing median diameter of the emulsion droplets. Microencapsulation efficiency (ME) is reported to increase as the weight fraction of SSPS in the mixture is raised. This mixture helped to stabilize emulsion at any weight fraction, whereas the maltodextrin and gum arabic mixture did not affect ME and had no emulsion stabilizing ability at a weight fraction of 0.25. When the weight fraction of SSPS and gum arabic decreased in the emulsion containing maltodextrin, the oxidative stability of encapsulated linoleic acid was decreased [192;193].

6.2.2.2 Oxidative stability based on formation of propanal

The powder stability based on propanal is determined by measuring the secondary oxidation products or aldehydes released by samples and captured by the GC column. The results of the experiments are similar to the PV measurements, where SG and SD samples are alike in terms of their oxidative stability and the protection effect given by coating materials (Fig. 6.3). For these two drying methods, increasing SSPS in the emulsion prevents oxidation at least in the first 5 weeks. Combination of SSPS, HPBCD, and maltodextrin (MC-4) is not effective in preventing oxidation, particularly in SD samples. Propanal formations in FD powders are different from those in SG and SD, in which combination of SSPS and maltodextrin (MC-1 and MC-2) were only effective for the first 4 weeks.

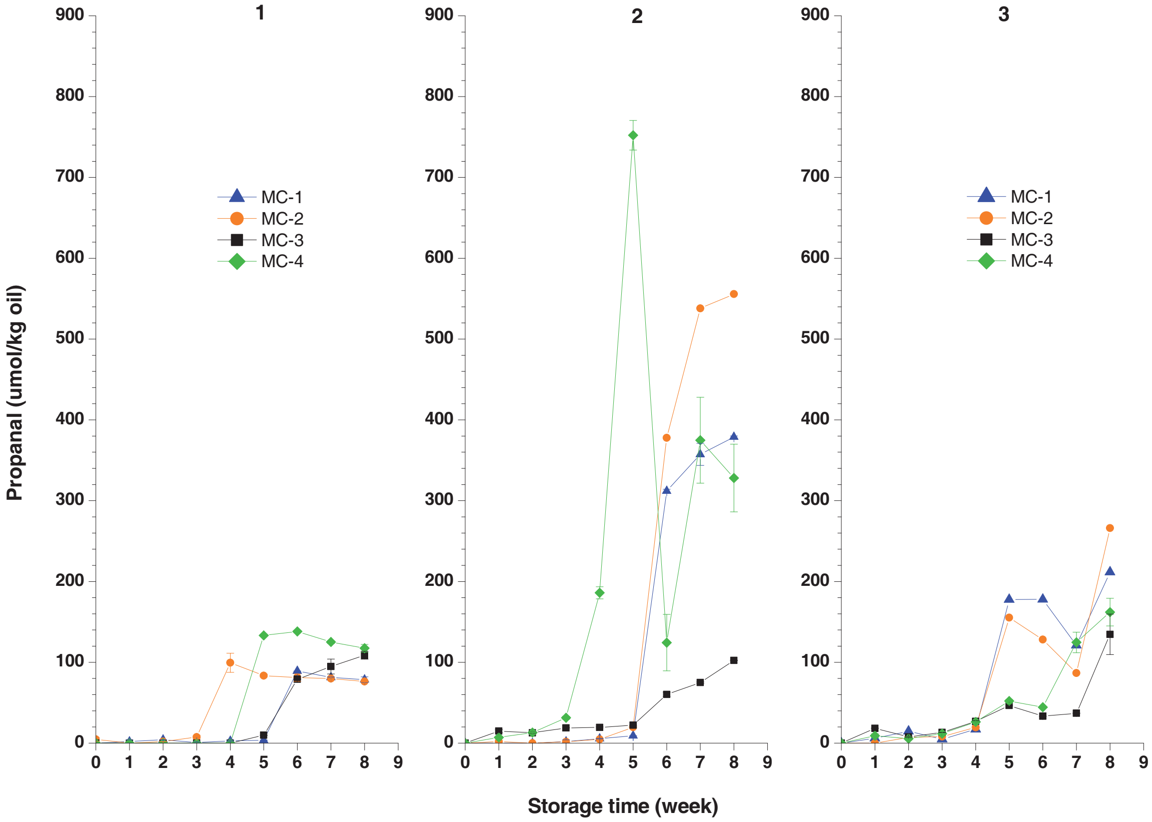


Fig. 6.3 Comparison of oxidative stability of fish oil microcapsules at room temperature, based on propanal of method: (1) spray granulation, (2) spray drying, and (3) freeze drying-FD.

Powder stability based either on PV or propanal, show that a combination of SSPS and modified starch (MC-3 formula) is the most effective matrix in preventing lipid oxidation in microcapsules. Modified starch used in this study is a chemically modified (E 1450) starch from refined *waxy* maize. *Waxy* corn starch is able to form smooth, stable, and high-viscosity colloidal suspension on cooling, while for unmodified starch, the formation of gel is observed [286].

The modified starch (trade name HI-CAP 100) was kindly provided by the National Starch Food Innovation. This starch contains less than 1% amylose and more than 99% amylopectin. E 1450 modification produces octenyl succinic anhydride (OSA) starches.

Chemical modification of starch with octenyl succinic anhydride is made by esterification of starch and anhydrous octenylsuccinic acid under alkaline conditions [288]. This modification involves addition of succinic anhydrate (dihydro-2,5-furandione) into starch molecules [22].

The superiority of this type of starch observed in this research is attributed to its properties. The OSA starch contains both hydrophobic and hydrophilic groups [23;24], where the octenyl succinate is attached to the starch chain via an ester linkage. The OSA-modified starch is able to form strong films at the oil-water interface. The residual of starch (glucose moiety) binds to the water and the octenyl part binds to the oil. Therefore, the separation of oil and water can be prevented, and the emulsion stability increases [200]. OSA starch is also reported to have surface-active properties [288], and excellent capability of lowering surface and interfacial tension [222].

OSA starches are able to protect the ingredients from oxidation and can act as a combination of surfactant and stabilizer [278]. This starch can be easily dispersed into oil/water mixture without extensive agitation, has excellent emulsifying capacity and is thus able to form a homogenous emulsion without separation for a period of time at room temperature [161]. Tesch et al. (2002) reported that OSA starches are surface-active substances, as proved by the measurement of the interfacial tension. They are able to be adsorbed at the interface, and able to stabilize droplets against coalescence. The emulsification properties of OSA starch are independent of pH value and ion valence as the main mechanism of stabilization is via steric hindrance. This is in contrast to the emulsion stabilization by protein via electrostatic repulsive force, where stabilization depends strongly on pH [278].

Another important remark from Fig.6.2 and 6.3 is powder instability when encapsulated by combination of 10% SSPS, 15% HPBCD, and 50% maltodextrin (Fig. 6.2 and 6.3; MC-4 formula), except for propanal in the freeze-dried sample (Fig. 6.3-3). Interestingly, this combination of matrices is actually similar to MC-1 and MC-2 formulas that do not include an additional of 15% HPBCD. However, the outcomes vary greatly, especially for the lowest protection of microcapsules produced by spray drying. Initially, incorporation of HPBCD into the formula was expected to provide a better oxidation inhibition because of the molecular encapsulation possibly given by the cyclodextrin cavities, but this is not the case.

The roles of cyclodextrins in stabilizing food emulsions as well as their mechanisms of action have been evaluated by Duchene et al. (2003) and Yu et al. (2001) [58;314]. They underlined that the hydrophobic cavity of cyclodextrin is able to include at least in part fatty acid chains. The inclusion characteristics are determined significantly by the molecular structure of cyclodextrin (α-, β-, or γ-cyclodextrin and or their derivatives) and fatty acid (chain length, and number of double bonds).

Fatty acid chains of C_4-C_8 interact and included well in the cavity of α- cyclodextrin [167]. In general, short-chain fatty acids can make an inclusion complex with α-cyclodextrin; the intermediate fatty acids chain ($\approx C_{10}$) suit better with β- cyclodextrin though part of the chain will be outside of the cavity. Fatty acids inclusion in the cavity of cyclodextrin gives an increase of their solubility in water. Formation of the inclusion complex between fatty acids and cyclodextrin can occur by (1) development of hydrogen bonds among the hydroxyls in position 6 of the cyclodextrin with the carboxyl of fatty acid chain, and (2) development of hydrophobic interaction between the cyclodextrin cavity and the chain of fatty acid hydrocarbon [166].

As with free fatty acids, cyclodextrins are reported to be able to form an inclusion complex with fatty acids of the glycerides. The complex stability depends on the acylation degree and decreases in the order free fatty acids > monoglyceride > diglyceride > triglyceride. Cyclodextrin complexes with triglycerides, however, are insoluble while those with free fatty acids and monoglycerides are soluble [272].

The inclusion complexes of cyclodextrin molecules with the triglyceride were formed according to the length of the fatty acid chain at the interface. Complexes of CDs and fatty acids can act as surface-active agents, because in the o/w interface, the CD molecules of the complexes are oriented to the aqueous phase, whereas the fatty acid residues to the oil phase. However, if the CD molecules entrap more than two fatty acids in a triglyceride, the surface-active property may disappeared due to the migration of the resulting hydrophilic complexes from the interface into the water phase [262].

Szente et al. (1993) underlined that five to six CH_2 units of a fatty acid need one CD capsule [272]. Jyothirmayi et al. (1991) [128], studied the molecular structures of linoneic acid and arachidonic acid with α- and β-CDs. The carboxyl arms of both fatty acids were embedded inside the CD cavity, but the double bonds were partly exposed or buried. The double bond at position 12-13 of linoleic acid was exposed, and the one at position 9-10 was entrapped. In arachidonic acid, the double bond at position 5-6 was buried while that at 8-9 was exposed. For these two fatty acids, the carboxyl arms were completely entrapped into the CDs cavity, however the methyl arms were not included.

In a study of fish oil nano-encapsulation with β-CD, He et al., (2008) revealed the self-assembly process of interaction between fish oil and β-CD. From the morphological observations, they concluded that the self-assembly aggregation depends on the concentration of the guest molecules, the ratio of host and guest, and the type of CD aggregates (crystal form or aqueous solution) [96].

The hexagonal aggregates of β-CD and fish oil were found by Choi et al., (2009) at mixing ratio of 10:1. However, at a higher mixing rate (10:10), this type of aggregation was not evident, instead, the worm-type aggregation was observed. The worm-type of β-CD aggregates adopted the crystal form with fish oil without clear interfacial barrier between fish oil and β-CD. It was possible that fish oil (in the form of triglycerides) was physically blended with b-CD without being entrapped or partially included inside the b-CDs cavity. The β-CD might precipitate and the fish oil might exist randomly among the precipitated β-CD, thus promoting the formation of supramolecules (as shown in Fig. 2.5) [35].

Choi et al., (2010) examined the complexes obtained by fish oil and β-CD self-assembly and aggregation mechanisms. The molecular inclusion of β-CD is apparently not possible to entrap fish oil as the ‘guest’ because of the complexity of fish oil components. The encapsulation of fish oil by β-CDs occurred by mean of the self-assembly mechanism only [34].

Yuan et al. (2007) investigated the ability of HPBCD to form inclusion and was determined by the DS, the substitution pattern and the character of the guest molecules.

HPBCD was reported to have a lower ability to form an inclusion complex with large spheriform guest molecules than the native β-CD. However, the ability of HPBCD to entrap smaller linear guest molecules is better than its parent β-CD [315].

Despite the fact that HPBCD has low ability to make inclusion complex with large guest molecules and may be able to form aggregates with fish oil, the rate to develop aggregates itself is also determined the role of HPBCD in stabilizing the emulsion. Messner et al. (2010) reports that the ability of CDs derivatives, including 2-hydroxypropyl CDs, to form aggregates decreased compared to the parent CDs. This decreased ability is related to the hydrogen binding capability. Due to the replacement of the hydrogen atom in the hydroxyl of native CDs by non-polar hydrocarbon groups, the HPBCDs lose their proton-donating properties [190]. This is confirmed by other researchers, who state that HPBCD has a weak tendency to self-assemble and form aggregates [83;95].

Therefore, the lowest protection given by MC-4 matrices might be related with the possibility of formation of non-inclusion complexes between HPBCD and fish oil instead of the 'guest-cavity' interaction as expected before. The cohesion forces involved in self-assemble aggregates among fish oil and HPBCD are weaker than the native CDs and easily to collapse under unfavorable conditions such as high temperature, intensive shaking and sonication [320]. In addition, it is not impossible that there is a competition between fish oil and SSPS to be captured by HPBCD cavities. As explained above, since fish oil is considered as large guest molecules then it is easier for SSPS to make inclusion complexes due to its linear molecular shape. If this assumption occurs, the amount of important emulsifier may be decreased substantially and thereby the droplet protection can be reduced accordingly. Minimum protection is observed particularly for spray-dried powders emulsified with these matrices, where volatile propanal is significantly high. Another possibility that might explain the low stability is that less maltodextrin is involved, as 15% has been substituted by HPBCD.

The particle size of maltodextrins is believed to play a key role in the formation of the three-dimensional network of particles in structured water layers. The network is loosely associated, and the gel structures are able to immobilize large amounts of water due to the large surface area and through hydrogen bonding. Despite decreasing the mobility of aqueous phase, this network also hinders heat transfer, and decelerates the movement of solutes away from water during storage [254].

The main advantage of using maltodextrins for food application is for ‘bodying effect’ through the relatively high viscosity. High DE maltodextrins are reported to have solubility, bulking and bodying characteristics similar to the corn syrup sweeteners [138]. In this research, maltodextrin with DE of 20 was used. Therefore, 50% w/w of maltodextrin incorporated in MC-4 formula may lead to a decreased in giving enough bodying structure to the emulsion.

6.2.3 Comparison of encapsulations methods

6.2.3.1 Microcapsule production by spray granulation

The state of art of SG as a method for producing microcapsules was described in section 6.1.2 (preliminary research). In the main research, only 33/22 fish oil was used, and 3 new combinations of matrices were tested (MC-1, MC-3, MC-4) and compared to the formula used in the preliminary research (MC-2: 10% SSPS + 65% maltodextrin).

The storage stability tests (Fig. 5.13) demonstrate that MC-1, MC-2, and MC-3 exhibited low formation of hydroperoxides (below 20.0 meq/kg oil) throughout 8 week period, and that only MC-4 reached the highest peak of 31.93 ± 0.02 meq/kg oil, at week 5. The propanal results (Fig. 5.14) show evidence of better oxidative stability than SD and FD, especially for samples prepared from MC-1 and MC-3 formulas. In the first 5 week, a maximum of only 5.0 µmol/kg oil propanal was detected in the MC-1 sample, whereas there was none in the MC-3 samples up to week 4 of storage. However, at week 5 propanal increased in both formulas.

Thus it can be confirmed that the SG process is able to produce microcapsules which can withstand storage at room temperature (± 21 ^{o}C) for at least 5 weeks for 33/22 fish oil containing 620 mg/g omega-3 fatty acids, and for more than 6 weeks in the case of 18/12 fish oil with 360 mg/g omega-3 fatty acids. This can be achieved if the fish oils are microencapsulated with a combination of 12.5% SSPS and 62.5% maltodextrin (MC-1), or 10% SSPS and 65% modified starch (MC-3).

The granulation process is indeed a very complex process, involving a large number of variables. As early as 1961, Berquin [17] proposed granulation mechanisms in the field of fertilizer. Uhlemann (1990), as well as Link and Schlunder (1997) presented more comphrehensive theories of spray granulation [159;293]. Some terms are used interchangeably, such as granulation, spray granulation, and wet granulation.

In early 2001, Iveson et al. [117] reviewed wet granulation processes and described new approach of the processes. They oberserved that fundamentally there are only 3 sets of rate processes that are important in determining wet granulation (1) wetting and nucleation, (2) concolidation and growth, and (3) breakage and attrition [277]. In the first process, the liquid binder is brought into contact and distributed with dry powder bed resulted nuclei granules. In the second step, granules compaction and growth take place due to collision between two granules, granules and seed powder, and/or granules with equipment. Finally, dried granules may break because of abrasion, and/or impact during processing in granulator.

Recently, Gruenewald et al. (2010) described another theory for granulation mechanism. They distinguished 4 different terms involved in granulation steps, namely primary dust, seed particles, nuclei, and granules. The primary dust is generated by overspray (spray-dried droplets) and by abrasion of granules. The dust can agglomerate with spray droplets in the spray zone and form seed particles. As the seeds become larger, the growth may shifted to layering in which the agglomerated dust is surrounded by the layers and called nuclei. After significant layer by layer formation, the granules are produced [85].

In order to understand how the SG process takes place in this study, the microstructure of cross-sectional granules was examined carefully by SEM examinations. The results confirm that the formation of fish oil granules is actually based on encapsulation of multiple cores by droplet depositions. Initially, the existence of very fine particles resulted from spray-dried emulsion droplets (which can be less than 5 µm in size), and the external seed (which was introduced before the SG process was started) (Fig. 6.4A), were bind together by the droplet drop to form seed particles (Fig. 6.4B). The seed particles (±25 µm) were then agglomerated and developed 'nuclei' (Fig. 6.4C). It is likely that the nuclei (>100 µm) were becoming the cores for a granule. The particle growth is then governed by the mechanism of layer by layer growth (Fig. 6.4D). Therefore, the SG granule is actually consisted of more than one core and they are covered by the successive layers. Finally, very fine particles were deposited on the surface of granules and led to the formation of raspberry-like microcapsules.

The rough surface observed in the raspberry-like granules (Fig. 6.4D) might be caused by the variation in the rate of evaporation. Becher and Schlunder (1998) observed that high solvent evaporation rates (due to a higher drying temperature) during the SG process resulted in more compact granules. The particle growth is based on the formation of thin layers of solid, thus leading to a more spherical shape. On the other hand, the low evaporation rate caused agglomeration of wet granules and resulted in a coarse morphology [13]. In addition, the emulsion viscosity might have a signifacnt effect on drop deposition and coating efficiency [50].

The nucleation rate is also important to achieve a steady state condition and affects to a great extent to the final granules morphology. In this case, the SG method needs an external supply of seeds before the process can be started. Each granule that has reached a desired particle size is removed automatically and must be replaced by a small nucleus. Ideally, the nucleation rate has to be the same as the production rate. If the nucleation rate falls below the rate of production, the system will run out of particles [293]. The imbalance of nucleation rate and granules production might have disturbed the steady state condition, hence the continuous granulation process inside the chamber might change.

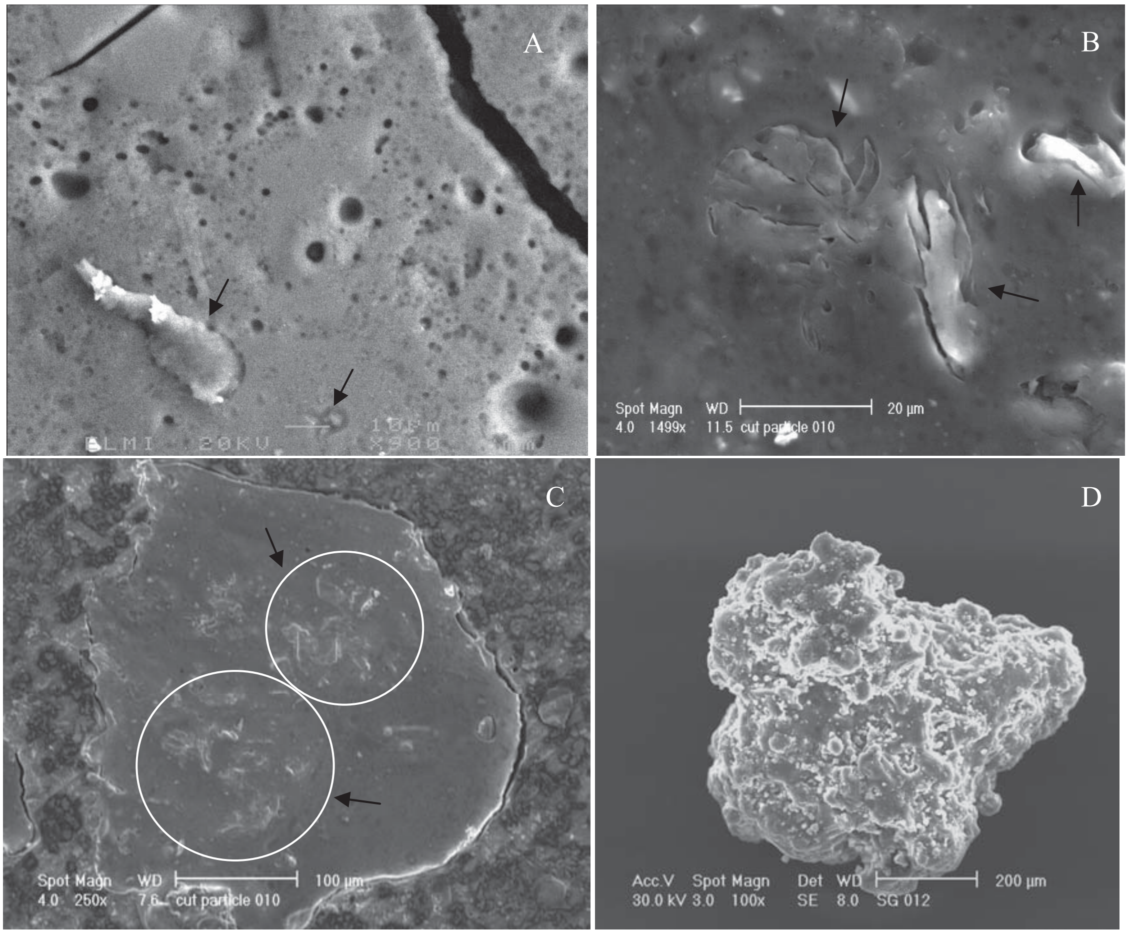

A
BLMI 20KV 10µm X900
B
Spot Magn WD 20 µm
4.0 1499x 11.5 cut particle 010
C
Spot Magn WD 100 µm
4.0 250x 7.6 cut particle 010
D
Acc.V Spot Magn Det WD 200 µm
30.0 kV 3.0 100x SE 8.0 SG 012

Fig. 6.4 Microstructure of a cross-sectional spray granulated granule to explain the mechanism of a granule formation: (A) a very fine particle and an external-supplied seed are exist as shown by the arrows, (B) very fine particles and external-supplied seed are bridged together to generate seed particles, (C) these particles are agglomerated to form nuclei as signed by the two circles, (D) a final granule is formed by combination of layer by layer growth and deposition of very fine particles on the surface of granule.

Based on SEM micrographs (Fig. 6.4A – 6.4D) and the theory proposed by Gruenewald et al., 2010, Figs 6.5 and 6.6 are created to illustrate the mechanism of a granule formation in SG process. Firstly, the fish oil is dispersed and embedded into matrices in the form of *emulsion*. Secondly, the minuscule of emulsion (spray drops) containing oil generate *fine particles* which then agglomerated and bridged together by spray drops to form *agglomerated particles* or *core*. Uniform depositions of spray droplets thus create thin films enveloping the core, and so-called *multiple cores* are then developed. Finally, *granules* are produced following a mechanism of layer by layer growth and by fine particles deposition onto the wet surface of multiple cores. Agglomeration and multiple layers protected fish oil significantly although the fish oil is exposed to medium heat treatment (40 – 70 ^{o}C) during the SG process.

Therefore, the oxidative stability of SG powder can be linked to its morphology. Spray granulated granules were found to be superior in stability toward oxidation at room temperature for both parameters, i.e., PV and propanal, compared to SD and FD powders. It can be said that the SG process produces granules which can be considered as “multiple encapsulation” granules.

It is important to note that prediction of how granulation is taking place inside the chamber is difficult. Material properties (ratio of core and walls, emulsion stability, viscosity, and solid content) as well as processing parameters (rate of spray drops, processing temperature, the chamber height, and the gas flow rate) are closely interrelated. All factors are interdependent to determine primary dust and nuclei formation, collision of drops to form a thin film, growth particle by layer by layer mechanism, and morphology of the end product. If the nucleation rate is equal to the granules collection, appropriate emulsion viscosity generates droplets distribution evenly. Thus, thin films are formed, and compact and smooth granules with onion-skin structure might be produced.

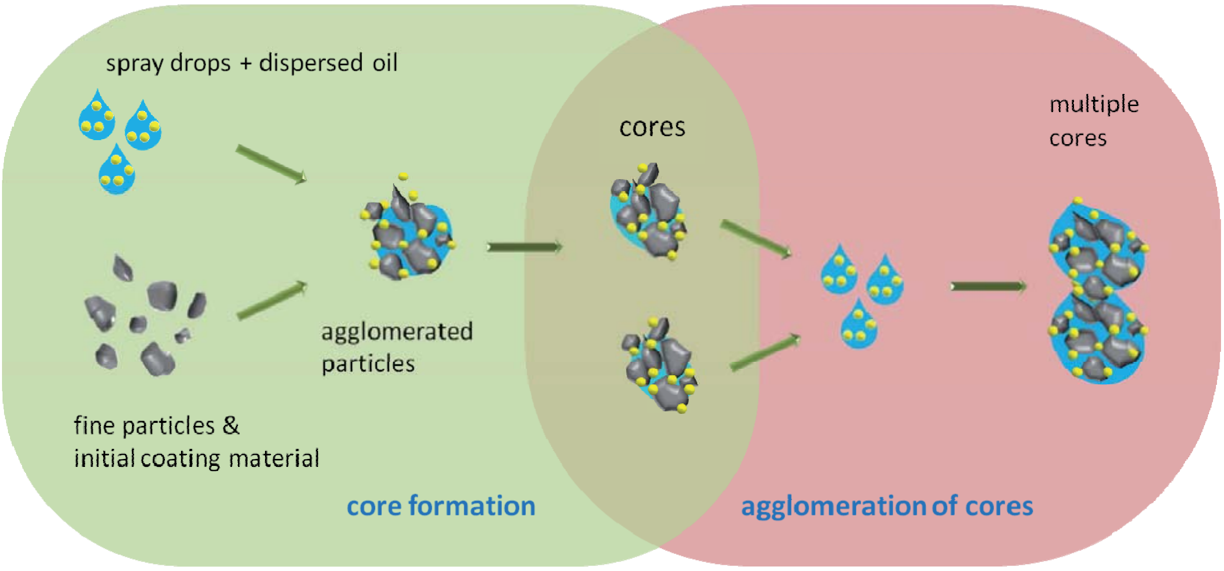

spray drops + dispersed oil
agglomerated particles
fine particles & initial coating material
core formation
cores
multiple cores
agglomeration of cores

Fig. 6.5 Mechanism of a granule formation in spray granulation process: (1) formation of multiple cores from agglomeration of seeds

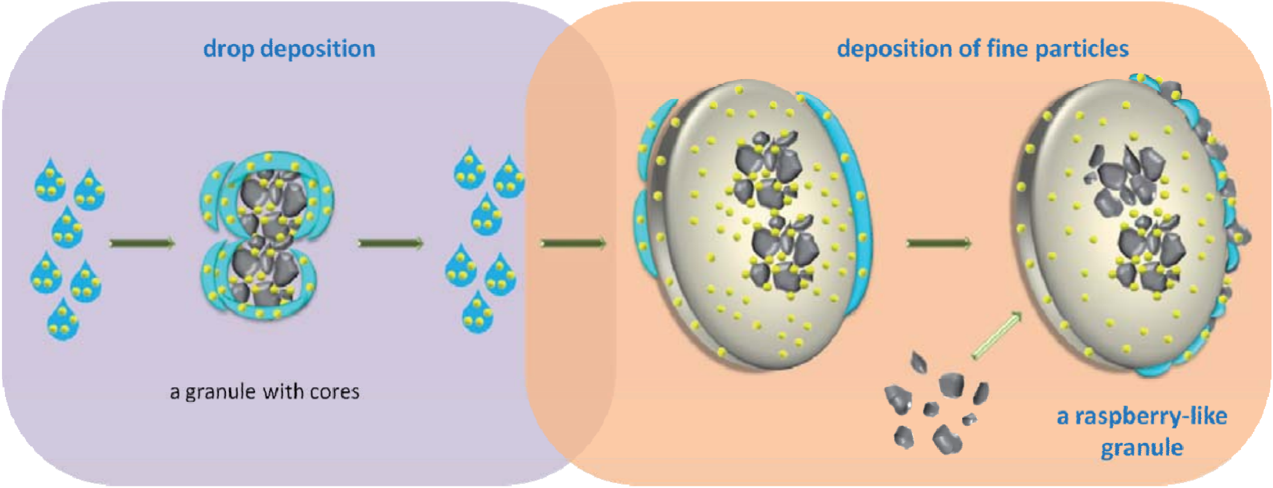

drop deposition
a granule with cores
deposition of fine particles
a raspberry-like granule

Fig. 6.6 Mechanism of a granule formation in spray granulation process: (2) granule enlargement formed by drop deposition, the layer growth, and deposition of fine particles onto the wet surface

6.2.3.2 Microcapsule production by spray drying

Spray drying of emulsion involves two main principles, i.e., formation of spray droplets and contact of spray with hot air. Spray droplets are produced from atomization as a result of breakdown of liquid (emulsion) and aimed for optimum evaporation. The air intake is the atmospheric air, which it is heated and used as a drying medium, cleaned by means of cyclones and scrubbers and then released again into the environment [295].

The process is initiated by the droplets – hot air contact in which the balances of vapor partial pressure and temperature are established between liquid and gas phases. The heat and mass exchange starts just after the droplet has been released from the atomizer, and the process progresses continuously while this droplet travels in the hot air [11]. As a consequence of temperature differences, heat transfer is carried out from air towards the droplets. The water evaporates from the opposite direction due to the vapor pressure difference [77]. Finally, the droplet begins to lose water in the form of vapor and subsequently the formation of particles begins.

Spray drying uses high drying temperatures, but the product temperature is low due to particles exposure to heat only for a few seconds [232]. This was considered to be important in the research design and the decision to apply spray drying as one of the methods to produce fish oil microcapsules. This is in agreement with what has been proposed by Bhandari and Adhikari [19], who suggest that spray drying can be used to dry heat-sensitive materials because the product temperature usually remains well below 100°C.

'Heat treatment' was understood as the key to determine powder stability in the preliminary study. Likewise, the time needed to dry the emulsion is also crucial. Accordingly, combination of temperature and time is critical in each drying method for ensuring product stability, particularly for drying sensitive ingredients such as fish oil.

The results of the storage tests have demonstrated that the SD powders were obviously less stable than the SG powders. The pattern is different for FD powders. While PVs are lower than for FD, results based on propanal content tend to be higher than for FD, especially after 5-week storage. Due to emulsion exposure to a very high drying temperature (± 180 °C), hydroperoxides formation is high. Chaiyasit et al. (2008) emphasized that the presence of pro-oxidants at high temperature can cause rapid decomposition of hydroperoxides after their formation, and thereby lead to difficult detection of hydroperoxides. Due to this detection difficulty, sometime it is reported that there is no accumulation during use and storage [27]. In the present study, it is assumed that the peroxides as the reactive substances were easily decomposed into secondary oxidation products during storage. As a consequence of rapid peroxide degradation, high amount of volatile propanal were detected.

This discrepancy is in agreement with what has been found by Frankel (1993) [69]. He investigated the oxidative stability of vegetable and two fish oils, menhaden and sardine oils based on PVs and gas chromatographic headspace analyses. He found that at 40 °C and 50 °C, the oxidative stability based on PV was not significantly different between the two fish oils ($P > 0.05$). However, at the same temperatures, the stability based on propanal formation was significantly different. The headspace GC analysis indicated that variation of oxidation at only 10 °C difference showed marked differences in the results.

Therefore, incredibly high propanal formation in SD powders (prepared from MC-1, MC-2, and MC-4 formulas) was accelerated by the high drying temperature. The inlet temperature used was ± 180 °C, and the drying time was very short, i.e., less than 2 min. The powders were collected and transferred into a new bottle every 5 min, and the bottle was immediately immersed in an ice-water bath. Although the drying time was extremely short, the exposure to high drying temperature critically accelerated the oxidation.

The inlet air temperature has been reported as a critical aspect in spray drying. The optimum inlet air temperature is reported to be in a range of 160 - 220 °C. A high enough inlet air temperature is required to allow rapid formation of a semipermeable membrane on the droplet surface [56]. However, if the temperature is set too high, it will cause oxidation acceleration.

The most stable product obtained by SD is the one that encapsulated by SSPS and modified starch. The key roles of these matrices have been discussed previously. Based on the outcomes, there might be other factors involved than only temperature differences and type of matrices in determining powder stability. Two possible factors are particle size and morphology. The particle size of SD powder is in a range of 50 – 150 µm (Fig. 6.7). This typical size of SD powder is relatively small compared to the size of SG granules, which are on average around 500 – 600 µm. Regarding the morphology of SD particles (Fig. 5.18), round and spherical particles are produced; some are quite small, but large particles are also formed.

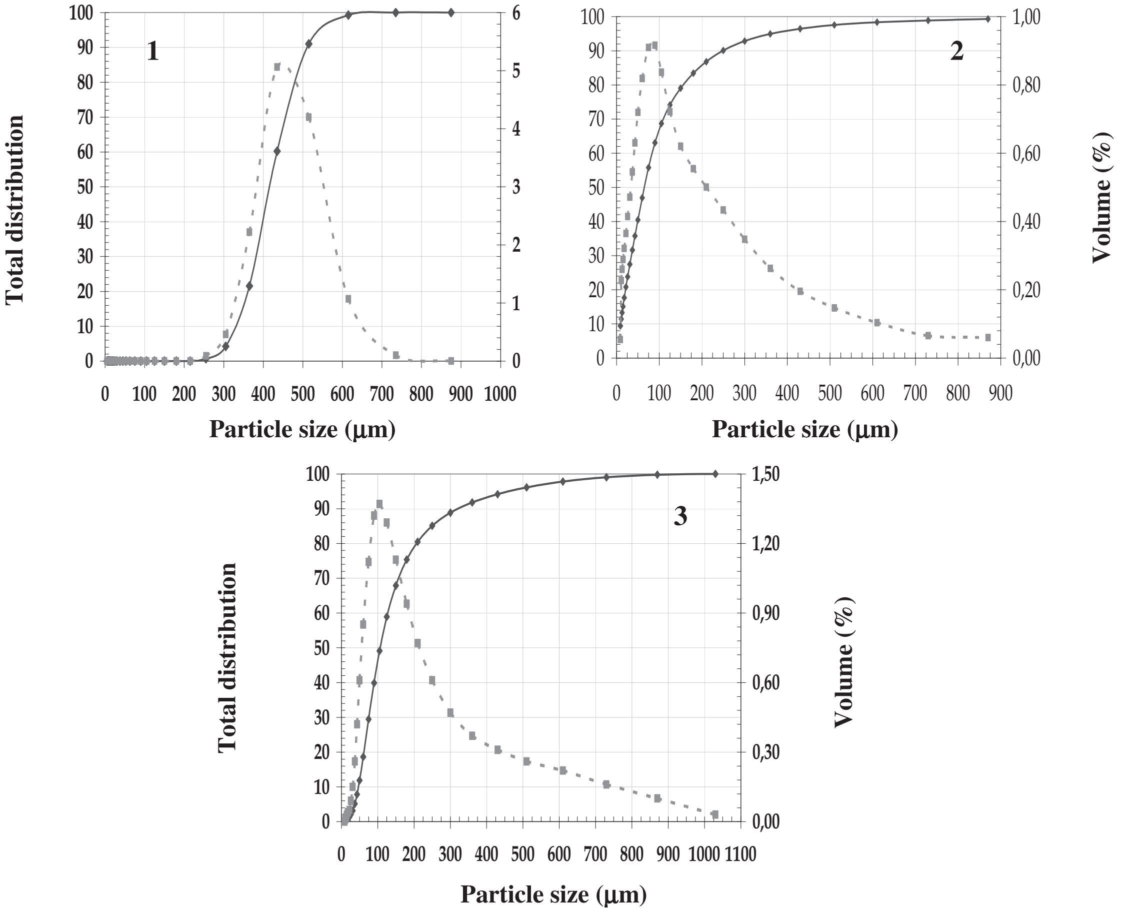


Fig. 6.7 Comparison of particle size distribution of microcapsules produced by (1) spray granulation, (2) spray drying, and (3) freeze drying.

High drying temperatures in SD can cause heat damage to the dry product or 'ballooning' to the drying droplet [56]. Ballooning occurs as the steam is formed in the interior of the drying droplet when the high inlet air temperature is applied. Formation of steam makes droplets puff (or balloon), and produces a thin-walled hollow particle [233].

Smaller particle size implies a large surface area, which may lead to a higher amount of non-encapsulated oil. Figure 6.8 illustrates a comparison of surface oil content (SOC) using three production methods. The amount of SOC obtained follows the order of SG < SD < FD. Factors involved and determining the amount of SOC in spray-dried powder have been studied intensively. The review of literatures shows that it is closely related to many aspects, including preparation of emulsion, emulsion viscosity and stability, as well as emulsion droplets size. Moreover, the amount of SOC extracted is proportionally linked to the total oil content and thereby to microencapsulation efficiency (ME).

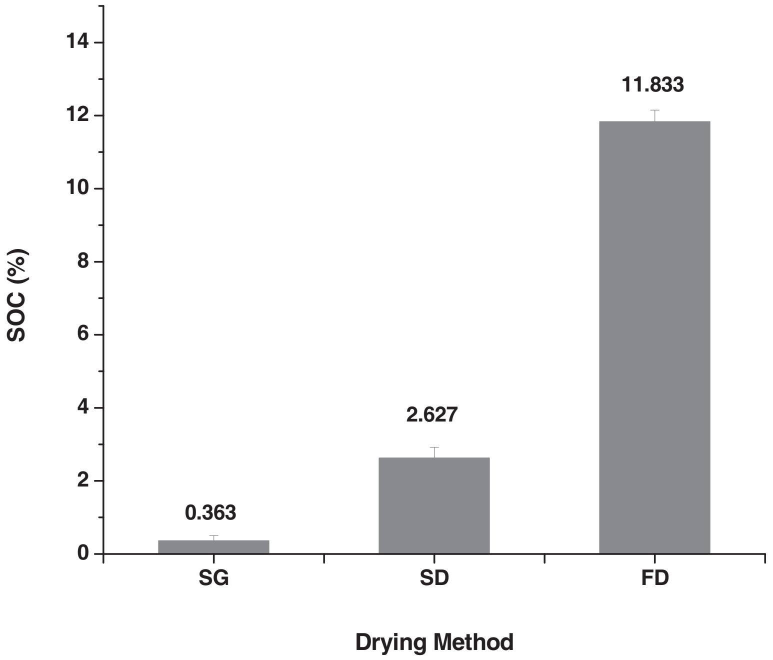


Fig. 6.8 Surface oil content (SOC) in spray granulation (SG), spray-dried (SD), and freeze-dried (FD) powders

Jafari et al. (2008) underline that emulsion plays an important role in determining the core retention and SOC of encapsulated powder [122]. Unstable emulsion will cause a large portion of the fat or oil on the surface of the powder. High amount of surface oil will result in: (1) poor dryer yields because most of the powders stick to the dryer chamber, (2) shorter powder shelf life due to rapid oxidation of surface oil, and (3) less efficient of wetability in final applications. An emulsion that meets these criteria is not necessarily stable over a long time period but long enough to be spray dried [233].

Previous studies described that the encapsulation efficiency of sensitive core materials is improved with decreased emulsion size [268]. Finer emulsion results in higher emulsion stability and affects the final powder characteristics (surface oil and total oil content of microcapsules) [122]. For example, Minemoto et al. (2002) reported for the encapsulation of linoleic acids that smaller emulsion size produced powder with slower oxidation rates than the powder produced from a larger emulsion size. This was due to the lower amount of non-encapsulated oil on the surface of spray dried particles [193]. However, the opposite was also the case, where microencapsulation efficiency was not affected by the homogenization pressure or smaller emulsion size [97-99;103].

Soottitantawat et al. (2003) claimed that the amount of surface oil increased with the increasing emulsion droplet size. The possible explanation for the higher remaining oil on the surface of particles was the breakdown of the large emulsion droplets during atomization. This problem led to lower stability of the particles during storage, since there was no protection against oxidation, and the hydroperoxides were easily decomposed and formed off-flavor products [268]. This is in line with a critical review done by Reineccius (2004) [234]. Finer emulsion size does not necessarily correspond to a longer product shelf life. The more the surface area of the oil droplets embedded inside the capsule wall, the greater the possibility for oxidation once the oxygen has entered into the particle.

Contradicting reports exist regarding the effect of particle size on encapsulation efficiency. Some researchers claimed that larger particle size results in better flavor retention and lower surface oil content, while others observed no effect of particle size on retention [65]. Jafari et al. (2007) [124] reported that medium-size particles have the highest encapsulation efficiency.

Despite emulsion viscosity, stability and emulsion droplet size, the ratio of core to wall material also affects ME. Hogan et al. (2001) found that microencapsulation efficiency by spray drying decreased sharply from 89.2% to 18.8% when the soy oil/sodium caseinate ratio was increased from 0.25 to 3.0, respectively. This tendency is related to the greater proportion of core materials close to the drying surface reducing the diffusion path length to the air/particle interface [122].

6.2.3.3 Microcapsule production by freeze drying

The principle of freeze drying is dehydration by sublimation of the ice fraction of a frozen product [230]. The process consists of three main steps: freezing, primary drying (sublimation) and secondary drying (desorption) [12;230;275]. Primary drying (ice sublimation) begins when the chamber pressure is reduced. The pressure is well below the vapor pressure of ice, and the ice is transferred from the product to the condenser by sublimation and crystallization onto cold coils (< -50°C). The sublimation process can take place because of the difference in pressure between the water vapor pressure at the ice interphase and the partial pressure of water vapor in the drying chamber [189]. The ice sublimes when the energy for the latent heat is supplied by radiation and conduction through the frozen product. The rate of sublimation is controlled by the vacuum level and heat input [100;189;230]. At this stage, the shelf temperature is raised to supply the heat removed by ice sublimation [276].

The experiments to produce microcapsules by freeze drying were designed to gain information on microcapsule stability when the process does not involve heat treatment. The results are compared with those from other two production processes, i.e., SG and SD, in order to answer the question: ‘to what extent would higher drying temperatures affect the stability of fish oil microcapsules?’ The review of literatures has confirmed that contrary findings exist regarding the effect of heat on product stability.

The oxidative stability of FD powder based on PVs and propanal was analyzed (Figure 5.19 and 5.20). The amount of hydroperoxides measured in a greater amount in all FD lines than that formed in SG and SD. Unlike in SG and SD, the PVs increased gradually after week 1 of storage in the four formulas tested. The MC-1, MC-2, and MC-4 samples showed fluctuating trends. MC-1 (233.46 ± 6.13 meq/kg oil) and MC-2 (201.33 ± 35.89 meq/kg oil) reached peaks at week 5 and MC-4 (390.38 ± 31.44 meq/kg oil) at week 6. After this period, decreasing patterns can be observed.

These findings can be explained by the morphology of FD powder, which is irregular in structure, very light, highly porous, and accordingly has a large surface area (Fig. 5.21). Based on its microstructure, the FD microcapsules can facilitate diffusion of oxygen from the air onto the particle surface, which gets into the inner part of the particle due to the amorphous state of the matrix. Because of this accessibility, oxygen can easily decompose lipids that are not protected by matrices and those on the particle surface. This is particularly relevant for the high surface oil content of the FD powder (11.83%) compared to SD (2.63%) and SG with only 0.36% (Fig. 6.8). Surface or non-encapsulated oil or free oil might be responsible for high formation of peroxides in freeze-dried samples in many studies.

Gejl-Hansen and Flink (1977) found that the microstructure of free oil on the freeze-dried oil-in-water emulsions was comprised of discontinuous oil deposits, caused by emulsion destabilization during freezing and drying, rather than in the form of individual oil globules. As a consequence, the rate of oxidation of surface oil followed the mechanism of lipids in continuous phase [76].

This is in line with the findings of Velasco et al. (2003, 2006, and 2009). The distribution of oil droplets in dried microcapsules is reported to have a significant effect on the powder stability. The lipid fractions are usually distributed in two different locations, i.e., on the surface and in the inner part of particles. Velasco et al. (2010) found that the intermediate RH (32%) exerted the highest effect on lipid oxidation in the free oil fraction, whereas a negligible or no effect was observed for the encapsulated oil fraction. The overall result has shown that for samples without antioxidants, the oxidation was faster for the free oil than for the encapsulated oil, as the oxygen diffusion through the matrix became a determining factor of the oxidation rate in the encapsulated oil fraction [298]. They concluded that lipid oxidation in the free oil fraction of freeze-dried samples oxidized as lipids in the bulk oil phase, while the encapsulated fraction oxidized as mechanism in individual oil droplets with different reaction rates [297-299].

Food microstructure determines the quality and level of food deterioration. One important example is the rate of oxidation in the porous product. The relationship between food microstructure and oxidative stability is underlined by Rahman et al. (2002, 2009) [227;228]. Freeze-dried tuna meat was less stable against oxidation compared to the same products which were dried in air drying and vacuum drying processes. The porosity of freeze-dried samples was found to be higher than that of air-dried and vacuum-dried samples, therefore the diffusion of oxygen into food structure was easier [227].

The porous structure may not only account for easy oxygen access onto the particle surface, but also correlates with the access into the inner part of particles. Desobry et al. (1997) showed that the microstructure of freeze-dried β-carotene with large pores extended into the interior of particles [44]. The typical properties of freeze-dried powder are influenced by freezing and drying steps in this process. Fast freezing develops numerous nucleation sites and forms a large number of relatively small ice crystals [132]. This type of ice formation will cause minimal damage to cellular and tissue structures [100] and will produce a fine structure of foods [66]. This is caused by minimal transport of water from inside the cells to the surrounding extracellular spaces, and the nucleation inside the cells can be minimized. After freezing, drying a frozen emulsion is started where pockets are created due to sublimation of the ice crystals [100], thus initiating the formation of a highly porous solid matrix of the freeze dried material [170].

Despite the porosity structure, the amount and location of radicals may play an important role to the rate of oxidation. Orlien et al. (2000) explained that the development of primary oxidation products in glassy food models depend on the presence of radical generators. Moreover, the rate of oxidation significantly affected the localization of pro-oxidants [210]. Therefore, as all samples may contain pre-form radicals that might already have existed since emulsification, and they are located mostly on the powder surface. Thus, if the radicals concentration in SG, SD, and FD are equal, then the limiting factor determining the oxidation rate is the oxygen diffusion into the matrix. Because FD powder has a large surface area, and the oxygen easily penetrates and comes into contact with the preform radicals, formation of peroxides is surely the highest one.

The SEM images of SG, SD, and FD powders are compared (Fig. 6.9). The microstructure of SD and FD powders in particular are similar to those reported in literature. Haque and Roos (2006) [94] investigated the differences in the physical state of spray-dried and freeze-dried lactose and lactose/protein mixtures. The results indicated that freeze-dried and spray-dried materials have different microstructures, and that the product properties obtained are varied depending on the drying method. Spray-dried particles were spherical, sometimes with dents, whereas freeze-dried lactose and lactose/protein mixtures had structures like pieces of broken glass or flake-like.

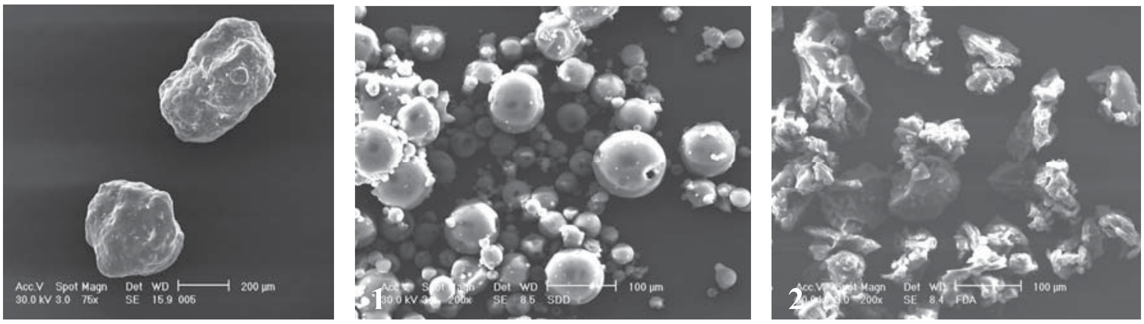


Fig. 6.9 SEM micrographs of fish oil microcapsules from (1) spray granulation, (2) spray drying, and (3) freeze drying

The highest volatile propanal was found in SD samples: MC-4 after week 3, and MC-1 and MC-2 after week 5 (Fig. 6.3). Generally, except MC-4, propanal development in SD was actually lower than that of FD, in the first 5 week of storage. The IP of MC-1, MC-2, and MC-3 of SD started at week 5, which was one week later than the IP at week 4 of all FD powders. However, after week 5, propanal in SD samples was extremely high (more than 378 µmol/kg oil), except for MC-3 formula.

This can be explained by the fact that the SD process uses very high drying temperatures, which function as a determining factor for lipid oxidation in the SD system. The lower PV trend of SD compared to FD can be caused by the high amount of peroxides which already accumulated in the powder and decomposed to become off-flavor products after 5 weeks of storage. During SD, the radicals might have formed significantly because of emulsion exposure to ± 180 °C. Frankel (2005) emphasized that hyroperoxides are rapidly decomposed at temperatures above 100 °C [71]. Accumulation of intermediate products (peroxides) was later transformed into propanal during storage. This hypothesis is confirmed by the higher propanal detection by GC in SD than that in SG and FD after week 5 (Fig. 6.3).

The trend of propanal formation in SD powder in this study is similar to the trend observed by Rahman et al. (2009). An investigation on lipid oxidation of freeze-dried grouper showed that there was an exponential increase of PV in samples stored at -20 °C and 5 °C. At 25 °C, PV increased in the beginning of storage test and then decreased. Interestingly, at 40 °C, PVs were exponentially decreased without any peak. This indicates that at high storage temperature, a high amount of peroxides was produced that then decomposed rapidly into secondary oxidation products [228]. The high storage temperature in this case is analogue to high drying temperature in the current research.

Oxygen diffusivity in relation to FD and SD stabilities might be explained by the glass transition temperature (T_g) approach. After the liquid feed is atomized into cloud droplets, the concentration of solute as a result of rapid water evaporation will transform droplets into syrup and finally to glassy particulates. The glassy state determines the physical characteristic of a material which has the structure of a liquid but the property of a solid [19-21].

Although spray drying uses high temperatures to evaporate water, the core product temperature can be kept below 100 °C due to the rapid evaporation of water from the droplets during the surface film solidification [19;232]. According to Soottitantawat et al.

(2004), the *Tg* of SSPS and maltodextrin mixture was 73.4 °C [267] and the *Tg* of OSA-starch was reported to be 56.35 °C [311]. These mean that during the spray drying process, the temperature of the particles is higher than the *Tg*. Levi and Karel (1995) explained that the collapse structure of spray-dried particles occurs during the drying process itself [157]. Moreover, Roozen et al. (1991) underlined that molecule diffusivity in carbohydrate systems increases dramatically at temperatures close to the glass transition temperature [240]. Therefore, based on the above theories, it can be concluded that oxidation reactions might have been induced during spray drying. Since the collapse structure occurs during the SD process, as a consequence of higher drying temperature than *Tg,* this condition may lead to the release of sensitive PUFAs, and free radicals may immediately be formed. The amount of accumulated free radicals can be extremely high due to the high ratio of the surface/volume of spray-dried spheres and the high number of small spheres.

At this stage, the oxidation products developed are in the initial stage and might be still in the form of oxidation precursors or free radicals only. This assumption is based on the fact that the particle exposure to heat is in the range of a few seconds at the most [232], and when the drying process has finished, the product is in a solid state where the reaction rate is slow. In addition, when the droplet water content reaches a critical value and the drying rate rapidly decreases, a dry crust is formed on the droplet surface and can function as a physical barrier to oxygen. That is why, except for MC-4 samples, the PVs and propanal of SD are low, at least in the first 4 – 5 weeks of storage.

However, as the storage tests at ± 21 ^{o}C proceed, the preformed radicals might progressively be changed into hydperoxides as indicated by significant PVs in the SD samples. Eventually, all these intermediate and unstable oxidation products are then converted to form stable volatile off-flavor products and indicated by decreasing PVs after week 6 (Fig. 6.2b) but increasing propanal (Fig.6.3b).

On the other hand, in freeze drying, the glass transition temperatures (*Tg*) of freeze-dried materials are higher than that of the spray dried, and they are susceptible to post-drying collapse [157]. More importantly, sublimation results in the replacement of the ice layer by air, and the droplets remain entrapped in the matrix (Fig. 2.8). The entrapment of oil is retained as long as the material storage conditions are kept below T_g [132]. Since FD applies a very low drying temperature, the product temperature will never exceed its T_g. Thus, during drying and the storage tests, the powders all remained in the glassy state. This could explain why the propanal development in the 3 FD formulas remained below that in the SD ones, especially at week 5 and later.

In an investigation of highly oxygen-sensitive products, Andersen et al. (2000) indicates that it is difficult to remove oxygen during the freeze drying process. Since the oxygen is able to permeate to the glassy matrices, the oil encapsulated in the matrices may not be completely protected from oxidation though the powder is in the glassy state and the matrix integrity is preserved. In such systems, some oil droplets might be oxidized rapidly and some might be oxidized slowly due to inhomogeneity in the degree of encapsulation [4].

Therefore, with respect to rapid decomposition of hydroperoxides, given that FD uses no heat treatment, the heat factor can be eliminated. In freeze drying, however, oxygen diffusion or oxygen permeation process into matrices becomes a limiting factor in determining the rate of oxidation. Both oxygen diffusivity and heat are critical to govern lipid oxidation, particularly for particles with a high ratio of surface/volume (such as in SD) and particles with a large surface area (in FD).

6.2.3.4 Moisture content, water activity and their relation to oxidation

The results of moisture content and water activity measurements show that the relationship of both parameters to the oxidation of microcapsules remains unclear (table 5.3 in section 5.2.4). Literatures suggest that the diffusion of oxygen may vary with different water content and water activity. The rate of diffusivity depends on the porosity of matrices and adsorbed water may form a protective layer against oxidation. Thus the physical, chemical, and microbial stability of food depends highly on the water content. The concept of water activity has been used as a reliable assessment for microbial growth, lipid oxidation, non-enzymatic as well as enzymatic activities in food [229].

According to the mentioned theory, three of SG samples (MC-2, MC-3, and MC-4) which have the highest moisture contents (5.87%, 6.58%, and 6.59%) and a_w (0.42, 0.51, and 0.51) should demonstrate better stabilities than other samples. In fact, one of the SG sample prepared from MC-1 formula shows similar stability to MC-2, MC-3, and MC-4 though the moisture content is only 2.29% and the water activity is relatively low (0.17). Moreover, all SD and FD samples are also have lower moisture content and a_w (similarly to MC-1 of SG) but they show less stability than MC-1 of SG, except MC-3 of SD and MC-3 of FD. The stability of MC-3 samples prepared by any drying method is thus related to matrix combination and the superiority of SG is controlled by the granule formation mechanism. Therefore this study suggests that higher water content and a_w in a sample do not necessarily correlate with better stability against oxidation.

This finding is in agreement with Bell et al. (2002). The authors emphasized that the effect of water on food chemical stability in solids is multidimensional and depend on the type of reactions and the physical characteristic of the system. No single explanation that currently exist on how water affects chemical reactions in reduced-moisture solids [14]. Recently, Rahman (2010) underlined that the water activity concept has limitation with respect to the state of equilibrium, it can be shifted due to heat treatment, pH, temperature, etc., the nature of the solute involved, and many physical changes, such as crystallization, caking, stickiness, gelatinization, and diffusivity could not be explained based on water activity alone. Based on those facts, the limitations of water activity concept could not make the concept invalid but it universal application is difficult [226].

6.2.3.5 Microencapsulation efficiency

Microencapsulation efficiency (ME) reflects the real amount of fish oil that is encapsulated inside the matrix. The dispersed oil droplets are not affected by extraction and repeated washing during the analysis of the surface oil content (Fig. 6.10). The highest ME is obtained by SG, with all values above 96%, followed by SD (83.62%) and FD (less than 50%). The ME values are determined based on the drying method used to produce the microcapsules, the amount of free oil (SOC), and type of coating materials used. The FD process yields powder with the highest SOC and thus the lowest ME. Among the 4 formulas, MC-4 exhibited the lowest ME values. These matrices did not provide enough protection for the oil droplets in the emulsion, which is evidence for the low oxidative stability. The ME values were lowest for all samples prepared with this formula and dried by any drying processes.

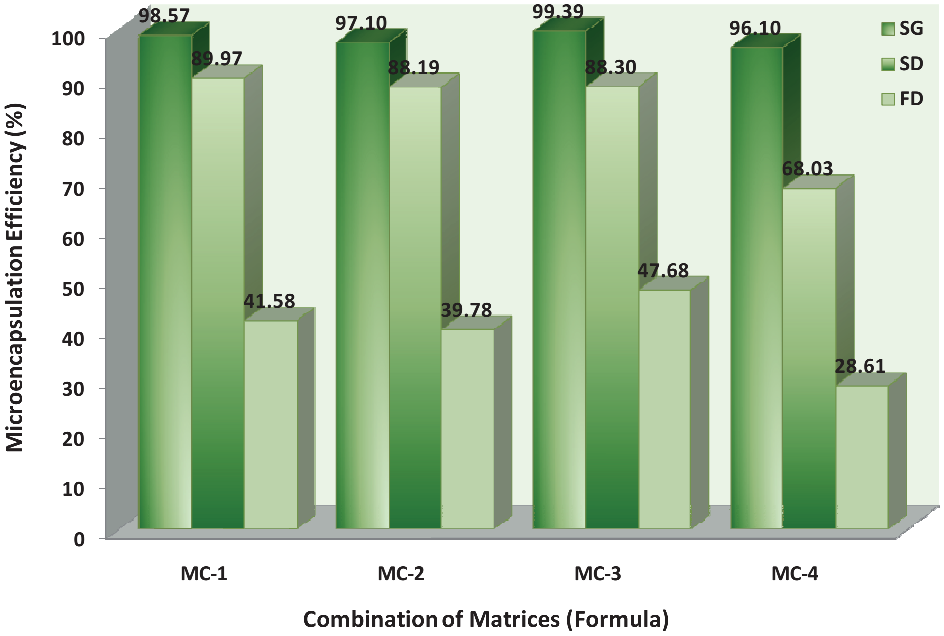


Fig. 6.10 Comparison of microencapsulation efficiency from 3 production methods; samples prepared from 4 different formulas

The statistical analysis verifies that within the same drying process, the ME of MC-4 formula is significantly different ($p < 0.05$) from the other 3 formulas (MC-1, MC-2, and MC-3). Microencapsulation efficiency (ME) obtained in each production method is statistically ($p < 0.05$) different from one to another.

6.2.4 The best outcomes and their comparison with other finding

The results in this study show that SSPS and OSA-*waxy* corn starch (MC-3 formula) are the best combination of coating materials to protect fish oil from oxidation during storage at ± 21 ºC. The protection is given starting from emulsification by stabilizing the fish oil droplets, minimizing formation of free radicals, and preventing droplets aggregation. SSPS is a negatively charged polysaccharide [246]. In emulsion, SSPS functions as an emulsifier by steric stabilizing effect, because its neutral sugar side chains are able to form thick layers (30 nm) on the droplet surface [201]. Similarly, OSA-starch is an anionic starch [258], stabilizing the emulsion by mechanism of steric hindrance, and is able to be adsorbed to form strong films at the O/W interface [278]. The peptidyl constituents of SSPS act as an anchor onto the surface of the oil droplets [180], and the octenyl parts of the OSA-starch bind to the oil droplets [200] to facilitate the carbohydrate or glucose moieties of both biopolymers to bind to the water phase.

SSPS and OSA-starch are working simultaneously to stabilize fish oil droplets via steric repulsion effects and protect them from being attacked by oxygen. McClements (2005) emphasized that steric interactions in food emulsion are the most common and important stabilizing mechanisms and occur due to layers of adsorbed emulsifier molecules [183]. This type of interaction is stronger than the electrostatic repulsion. Moreover, Hunter (1986) explained that when droplets are sufficiently close to each other, the electrostatic repulsion is usually weaker than the van der Waals attraction. Unlikely, the steric repulsion is stronger than the van der Waals attraction and keeps the droplets from being coalesced due to an extremely strong short-range repulsion [114].

Interestingly, though other 3 formulas (MC-1, MC-2 and MC-4) show remarkable differences in retarding oxidation in each of drying process, MC-3 formula demonstrates a comparable oxidation protection for microcapsules produced by any methods tested. Table 6.4 informs that propanal development detected in SG granules is 32.47 ± 6.60 μmol/kg oil, this value is lower than that in FD and both are lower than propanal in SD. Statistical analysis with 95% confidence interval confirms that in any production method, the propanal formation can be 13 μmol/kg oil more or less than the mean value.

Table 6.4 Comparison of oxidative stability based on propanal for the MC-3 formula

Production method	*Formula*	*Combination of matrices*	*Mean of propanal µmol/kg oil*	*95% Confidence interval*
SG	MC-3	SSPS + OSA-starch	32.47 ± 6.60	[19.37 ; 45.57]
SD	MC-3	SSPS + OSA-starch	36.22 ± 6.60	[23.12 ; 49.32]
FD	MC-3	SSPS + OSA-starch	35.32 ± 6.60	[22.22 ; 48.42]

In order to judge the results of this study, the MC-3 results were compared with the findings of a comparable study recently published by Serfert et al. (2009). The authors microencapsulated fish oil containing approx. 27% EPA and DHA with a combination of *n*-OSA-starch and glucose syrup to produce 18% fish oil powder. In preparing the emulsions, mixtures of antioxidants were tested and spray drying was used to dry the emulsion [253]. Similar parameters that can be compared with the current research are: drying temperature (180/70 ^{o}C *vs* 180/90 ^{o}C), the same storage period (56 days or 8 weeks), storage temperature (20 ^{o}C *vs* 21 ^{o}C), type of antioxidants (mixture of tocopherols); at least one of the matrices used is the same, i.e., OSA-starch. Some differences are: amount of PUFAs contained in fish oil (27% *vs* 55%), and amount of fish oil in emulsion (18% *vs* 12.5%). The comparison is based on the oxidative stability of samples as monitored by propanal development. The method applied for propanal analysis is similar.

The storage test showed that after 56 days, the sample in the above study which stabilized with 1000 mg/kg α-tocopherol and 100 mg/kg δ-tocopherol formed ± **140** µmol/kg oil of propanal and the sample with 100 mg/kg α-tocopherol and 1000 mg/kg δ-tocopherol exhibited ± **270** µmol/kg oil of propanal. The MC-3 in the present study showed that only **102.39 ± 0.06** µmol/kg oil propanal formed after 56 days. This was obtained from samples emulsified with MC-3 formula (SSPS and OSA-starch) and dried by spray drying. The antioxidant used in bulk fish oil is a mixture of tocopherols of 3 – 4.5 mg/g, which is higher than the amount the above-mentioned authors applied, but the amount of PUFAs in the present study is obviously much higher (55% EPA and DHA or 620 mg/g of total omega-3 fatty acids) compared to only 27% EPA and DHA.

Using the same production method, i.e., spray drying, but different combinations of matrix (MC-1, MC-2, and MC-4), the results showed less stability. The maximum propanal contents were higher than those reported by Serfert et al. (2009). One possible explanation could be the degree of unsaturation of fish oil in this research which is almost doubled, thus the risk of oxidation is actually multiplied. Only the MC-3 result shows that microcapsules stabilized by a combination of SSPS and OSA-starch are more stable than those prepared by a combination of OSA-starch and glucose syrup. These might be attributed to the amount of OSA-starch incorporated into the emulsion, and the role of SSPS to stabilize the oil droplets.

The results obtained by the SG process, however, demonstrate the superiority of SG compared to SD (Fig. 6.2 and 6.3). Samples that are microencapsulated by all combinations of matrix exhibited less propanal content than that reported by Serfert et al. (2009). The maximum propanal values that could be detected were 81.42 ± 0.63 µmol/kg oil, 99.45 ± 11.82 µmol/kg oil, 108.74 ± 4.88 µmol/kg oil, and 138.24 ± 1.67 µmol/kg oil for MC-1, MC-2, MC-3, and MC-4, respectively. Therefore, the multiple- protection system created by the SG process has successfully encapsulated fish oil and reduces the rate of oxidation.

VII. Conclusion

A systematical approach was used in the present study to investigate important factors affecting the oxidative stability of fish oil microcapsules. First, three types of fish oil were evaluated and microencapsulated by four combinations of coating material, and subsequently emulsions were dried using four drying methods. The results are compared based on microcapsule stability during storage at room temperature and at low temperature.

From the preliminary research, it can be concluded that the powder stability against oxidation depends on the total amount of PUFAs contained in the fish oil, and also on the type of fatty acids, i.e., EPA or DHA. Uncoated powder containing the lowest concentration of PUFA (18/12) was found to be stable during storage at room temperature with maximum PV of 3.98 ± 0.001 meq/kg oil. The PV increased sharply for uncoated powder with higher concentration of omega-3 (in 33/22 and 10/50 fish oils) after storage for 3 weeks. The PV values are in agreement with the concentration of propanal.

The powder with the higher content of DHA oxidized more rapidly and produced more hydroperoxides and propanal. Powder containing 620-670 mg/g omega-3 fatty acids can be stored up to 3 weeks at room temperature (± 21 ºC) if produced by the spray granulation (SG) process and only 2 weeks if produced by SG followed by film coating (SG-FC). However, powder containing less omega-3 fatty acids (360 mg/g) can be stored up to 6 weeks or even more at room temperature. Microcapsules stored at low temperature (3-4 °C) were found to be stable until the end of the storage test. At low temperature, the product shelf life can be estimated to be more than 6 months.

The role of SSPS to form membranes surrounding the oil droplets and to act as an emulsifier in combination with maltodextrin as a matrix less permeable matrix to oxygen is crucial for protection of fish oil. The SG process produces more stable microcapsules compared with SG-FC because of the unique structure of the granules developed in this process. Double exposure to the drying process, the quality of films formed by HPBCD, longer residence time and a possibility of particles overheating in the fluid bed might have induced further oxidation of the fish oil in the powder.

In the main research, the storage tests focus on monitoring oxidation at room temperature. The evaluation and analysis are particularly based on detection of propanal by headspace GC, as this measurement is proportionally related to the off-flavor formation or rancidity. The results show that the stability of powder containing a high amount of omega-3 (620 mg/g) is governed by the best combination of matrices and type of drying method. The mixture of soybean soluble polysaccharides (SSPS) and modified *waxy* corn starch (OSA-starch) is found superior with respect to stabilization of the microcapsules compared with the other three combinations. By using these matrices, the product stability is comparable when produced by any method (SG, SD, or FD).

The microcapsules produced by SD and FD are relatively stable during the first 5 weeks without apparent increase in propanal content. The most stable microcapsules are those produced by SG, where a very low propanal development is observed. In the first 4 weeks, no volatile propanal could be detected, and the concentration slightly increased in the following weeks. For 5 week's storage at ± 21 ^{o}C, the product stability encapsulated by SSPS and OSA-starch is in the order of SG > SD > FD.

Spray granulation is proved to be the best drying process to produce stable powder. Application of high drying temperature is found to be the most critical factor determining product stability. Processing or drying time is less crucial than the exposure to heat treatment. Though SG needs ± 60 min. to produce a desirable size of granules compared to only a few seconds in SD, the results confirm that spray granulation at ± 70 ^{o}C means a lower chances of lipid degradation by autoxidation than spray drying at ± 180 ^{o}C. High drying temperature induced initial development of primary oxidation products as well as hastened rapid degradation of them to become stable secondary oxidation products.

The results based on PVs and propanal verify that there is another cause of oxidation other than types of coating material and heat. This factor is the particle microstructure. Though freeze drying uses no heat or very low drying temperature in its process, oxygen diffusivity onto matrices becomes a rate-limiting factor toward lipid oxidation. The porous, irregular, and flake-like structure of the FD powder accelerates oxidation due to an easy oxygen access into matrices which thus reach the non-encapsulated oil.

The superiority of spray granulation (SG) to produce stable microcapsules is to a great extent affected by the particle microstructure obtained by this method. The term “multiple encapsulations” is appropriate and can be claimed for granules produced by SG. Agglomeration of seed particles containing oil droplets in the first stage of process, followed by the envelopment of the seed by the layers growth, and finally the granule surface is coated by very fine particles have create a multi-protection system for the lipids embedded inside the matrices. In addition, exposure to low-medium heat treatment has maintained minimum formation of free radicals and unstable peroxides. Lower accumulation of oxidation initiators may keep the granules from being affected by oxidation reactions.

This study suggests that unless one can assure the main protection can be given by the coating materials or by initial process such as double emulsifications, production of microcapsules containing sensitive ingredients, i.e. fish oil by spray drying, is not recommended. From the view point of economics and industrial application, further research should evaluate the feasibility of SG process for producing microcapsules with the longest shelf life at a reasonable selling price.

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