Master's thesis

NTNU
Norwegian University of Science and Technology
Faculty of Natural Sciences
Department of Biotechnology and Food Science

Parisa Izadinajafabadi

Spray drying microencapsulation of salmon oil with plant-based protein

Master's thesis in Ocean resources
Supervisor: Eva Falch
Co-supervisor: Ida-Johanne Jensen
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Abstract

Marine lipids and especially polyunsaturated omega-3 fatty acids play important roles in human nutrition and health (Hamed et al. 2022). Their unsaturated nature makes them highly susceptible to lipid oxidation that result in unpleasant taste and smell. Thus, there is a need to protect these oils from oxygen and conditions that catalyzes the lipid oxidation (Hamed et al. 2022). Microencapsulation is one way of physically protect the oil from lipid oxidation when used as food ingredient. Microencapsulation process, particularly the spray drying technique has been employed to minimize the lipid oxidation and simultaneously provide stable products (Islam et al. 2018). The aim of this study was to I) investigate whether a new plant-based wall material (rice protein and maltodextrin) exhibits good emulsifying properties or not and to II) optimize the spray drying variables (aspirator rate, inlet-drying air temperature, and spraying air mass flow rate) on encapsulation efficiency (EE), peroxide value (PV) and moisture content (MC) of microencapsulated salmon oil. The impacts of these variables were examined via Complete Randomized Designed. Results indicated that investigated variables had significant effects on studied microencapsulation properties and the optimal spray-drying variables giving the highest EE, lowest PV and MC were found. According to this research, plant-based wall materials (rice protein and maltodextrin) exhibited good emulsification properties and may be an alternative for animal-based wall materials to protect Omega-3 fatty acids in food industry.

Keywords: Microencapsulation properties, plant proteins, rice protein, salmon oil microencapsulation, spray dryer parameters.

Preface

This master thesis concludes two rewarding years studying ocean resources at NTNU. The thesis written at the Department of Biotechnology and Food Science. My supervisor is Eva Falch and my co-supervisor is Ida-Johanne Jensen.

SINTEF Ocean provided crude Salmon oil and the experiments were performed at biotechnology & food science department. Spray drying process has also done at Department of Energy and process engineering. I want to thank Wilhelm Glomm at Department of Biotechnology and Nanomedicine, SINTEF Industry, for giving me insights in all steps of experimental design and also thank Ignat Tolstorebrov at Department of Energy and process engineering for his valuable guidance through spray drying process. A special thanks my co-supervisor Ida-Johanne Jensen for all assistance during the experiments.

I want to thank my supervisor, Eva Falch, for all support through the work on this thesis. Despite the last years challenges due to the COVID-19 pandemic, you have enable collaboration and been available for questions and good fish oil oxidation related discussions.

Contents

1 Introduction

1.1 Salmon

1.1.1 Biology and cultivation

Salmon is the common name for several species of ray-finned fish in the family Salmonidae. Other fish in the same family include trout, char, grayling, and whitefish. Salmon are native to tributaries of the North Atlantic (genus Salmo) and Pacific Ocean (genus Oncorhynchus). Many species of salmon have been introduced into non-native environments such as the Great Lakes of North America and Patagonia in South America. Salmon are intensively farmed in many parts of the world. The term "salmon" comes from the Latin salmo, which in turn might have originated from salire, meaning "to leap". The nine commercially important species of salmon occur in two genera. The genus Salmo contains the Atlantic salmon (Figure 1-1), found in the North Atlantic, as well as many species commonly named trout. The genus Oncorhynchus contains eight species which occur naturally only in the North Pacific. As a group, these are known as Pacific salmon. Chinook salmon have been introduced in New Zealand and Patagonia. Coho, freshwater sockeye, and Atlantic salmon have been established in Patagonia, as well. Norway is the largest producer of farmed salmon in the world, with a yearly production of 1.2 million tonnes (Skjermo et al. 2014). The main species farmed in Norway is Atlantic salmon, representing 93% of total Norwegian aquaculture production (Diserud et al. 2022).

Figure 1-1: Atlantic salmon, Salmo salar

1.1.2 Chemical composition

The nutritional value of salmon can vary slightly among the varieties. For example, farmed salmon contains healthy lipids, whereas wild-caught salmon is a bit higher in protein. However, both types are great sources of many key nutrients, including selenium, phosphorus, and B vitamins (Opheim et al. 2015).

The nutritional value for a (100-gram) serving of cooked wild or farmed salmon is shown in Table 1-1 (Opheim et al. 2015).

Table 1-1: The nutritional value for a 3.5-ounce (100-gram) serving of cooked wild or farmed salmon

Nutritional value	Wild salmon	Farmed salmon
Calories	182	206
Protein	25 grams	22 grams
Fat	8 grams	12 grams
Vitamin B12	127% of the Daily Value (DV)	117% of the DV
Vitamin B6	56% of the DV	38% of the DV
Selenium	85% of the DV	75% of the DV
Niacin	63% of the DV	50% of the DV
Pantothenic acid	38% of the DV	30% of the DV
Thiamine	23% of the DV	28% of the DV
Phosphorus	21% of the DV	20% of the DV

1.1.2.1 OMEGA-3 fatty acids:

Our diet contains a complex mixture of fats and oils whose basic structural components are fatty acids. We generally consume at least 20 different types of fatty acids, which are classified as saturated, monounsaturated and polyunsaturated. Fatty acids have many fates in the body, including β-oxidation for energy, storage in depot fat or incorporation into phospholipids, which form the major structural components of all cellular membranes (Cholewski, Tomczykowa, and Tomczyk 2018).

Not all dietary fatty acids are created equally. Because humans do not have the enzymatic machinery required to synthesize omega-3 fatty acids, they must be obtained from the diet (termed "essential fatty acids"). Even among dietary polyunsaturated fatty acids, there are different families of compounds, and this is at the heart of the difference between omega-3 fatty acids and other dietary lipids (Cholewski et al. 2018).

Omega-3 fatty acids are found in fatty layers of cold-water fish and shellfish, plant and nut oils, English walnuts, flaxseed, algae oils, and fortified foods. You can also get omega-3s as supplements (Bradberry and Hilleman 2013). Fish such as tuna, trout and salmon are especially rich sources of these fatty acids. Fish-oil supplements are also a rich source, as they typically contain 30–50% omega-3 fatty acids by weight.

These are the two main types of omega-3 fatty acids (Bradberry and Hilleman 2013):

1-Long-chain omega-3 fatty acids are EPA (Eicosapentaenoic acid) and DHA (Docosahexaenoic acid). These are plentiful in fish and shellfish. Algae often provides only DHA (Bradberry and Hilleman 2013).

2-Short-chain omega-3 fatty acids are ALA (Alpha-linolenic acid). These are found in plants, such as flaxseed. Though beneficial, ALA omega-3 fatty acids have less potent health benefits than EPA and DHA. You'd have to eat a lot to gain the same benefits as you do from fish (Bradberry and Hilleman 2013).

Studies have shown that EPA and DHA are important for proper fetal development, including neuronal, retinal, and immune function. EPA and DHA may affect many aspects of cardiovascular function including inflammation, peripheral artery disease, major coronary events, and anticoagulation (Swanson, Block, and Mousa 2012). Some studies claimed that EPA and DHA have been linked to promising results in prevention, weight management, and cognitive function in those with very mild Alzheimer's disease (Swanson et al. 2012).

Different governments encourage their citizens to consume these fatty acids (Shabanpour et al. 2007). The latest proposal of the international community in the field of fatty acid studies is the consumption of EPA and DHA, 500 mg per day (Togarcheti and Padamati 2021). Most people don’t get the recommended daily levels of EPA and DHA in their diet, and this has led producers in many countries to produce useful and functional food products containing these compounds (Shabanpour et al. 2007).

1.1.3 Salmon oil:

Salmon oil, is a recognized source of n-3 long-chain Polyunsaturated fatty acids (PUFAs) (Alfaia et al. 2019). Most diets don’t provide the recommended levels of Omega-3 fatty acides for people (Foran et al. 2005). Deficiencies are associated with a number of health problems, like heart

disease, mood disorders, arthritis, and some cancers. Adding salmon oil to your diet can help you get enough of these health-boosting nutrients (Stoll 2001). It may offer benefits specified in the following chapters:

1.1.3.1 Heart Health

Studies show that the omega-3s in salmon oil help lower triglyceride levels. High levels of triglycerides, a type of fat in your blood, are a risk factor for heart disease and stroke (Director 2009).

Salmon oil also increases " good " (HDL) cholesterol levels. This helps clear your arteries of " bad " (LDL) cholesterol that can build up in your blood vessels over time. HDL cholesterol also promotes strong and flexible artery walls. This supports healthy heart function and lowers disease risk (Foran et al. 2005).

1.1.3.2 Lower Risk of Chronic Disease

Salmon oil's omega-3s have anti-inflammatory properties. While inflammation is your body's natural response to stress and infection, high levels over time raise your risk of chronic diseases, including cancer, diabetes, and arthritis.

By helping to reduce inflammation, research shows a diet high in omega-3s can help protect against these and other inflammation-related diseases (Lewis 2016).

1.1.3.3 Better Circulation

One study found that the omega-3s in salmon oil can improve your body's blood flow and oxygen circulation. The effect is stronger compared to other omega-3 sources (marine oils). Scientists think this is because the body converts omega-3s into nitric oxide, a substance that helps your blood vessels stay relaxed, flexible, and strong (Denis et al. 2013).

1.1.3.4 Cognitive Support

Salmon oil is one of the best available sources of DHA. While research is ongoing, studies show that this omega-3 helps repair and maintain brain cells. Getting enough of this fatty acid in your diet may reduce your risk of cognitive decline, including conditions like Alzheimer's and Parkinson's diseases (Denis et al. 2013).

1.1.3.5 Eye Health

The omega-3 content in salmon oil may support eye health as well. One study showed that omega-3 supplements lowered the risk of eye problems like glaucoma and age-related vision loss (Tang et al. 2021).

The fatty acid profile of the salmon oil is described as fllows (Kahveci et al. 2010):

The dominant FA in the substrate is oleic acid (OA, 18:1) accounting for 35.51% of total FAs, followed by palmitic acid (16:0) with a content of 12.1%. MUFA fraction has the highest portion (48.2%), which is believed to be resulted from the feed given in the salmon farm (the fatty acids

in the salmon is a reflection of the feed, so when they use more vegetable oils, it is easy to see it). Total omega 3 PUFA content, consist of EPA, Docosapentaenoic acid (DPA) and DHA, is 13.77% (Kahveci et al. 2010).

The biggest technical barrier for adding effective amounts of EPA and DHA to food is the oxidation and odor of fish due to lipid breakdown, which should be avoided (Cho, Shim, and Park 2003). In other word, these unsaturated fatty acids are susceptible to oxidative degradation, therefore there is a chance of generation of undesirable odor ("rancidity"), reducing consumer acceptance. Moreover, some of the reaction products of lipid oxidation exhibit toxicity, which may lead to chronic health problems if they are regularly consumed over long periods (Venugopalan et al. 2021). According to many studies, one of the best ways to protect fish oil against environmental factors and especially oxidation, is microencapsulation (Heinzelmann et al. 2000).

1.2 Microencapsulation

Microencapsulation can be defined as a process by which solids, liquids, and even gases are entrapped (core material) in microscopic particles through the formation of a thin coating (wall material) around an active substance (Figure 1-2) ((Aghbashlo et al. 2013a). The core materials can be food ingredients, enzymes, cells, or other materials and the wall materials are proteins, polysaccharides or a combination of these substances (Choudhury, Meghwal, and Das 2021). Microencapsulation is a technology that allows sensitive ingredients to be enveloped as a 'core' material within a polymer matrix or 'wall'. Encapsulated materials are, thus, protected against

adverse reactions such as lipid oxidation or nutritional deterioration (Hogan, O'Riordan, and O'Sullivan 2003). The main advantages of microencapsulation are masking the taste or odor of the core, isolating the core materials from deteriorating effects of oxygen, retard the evaporation of a volatile core and improve the handling properties of a sticky material (Choudhury et al. 2021).

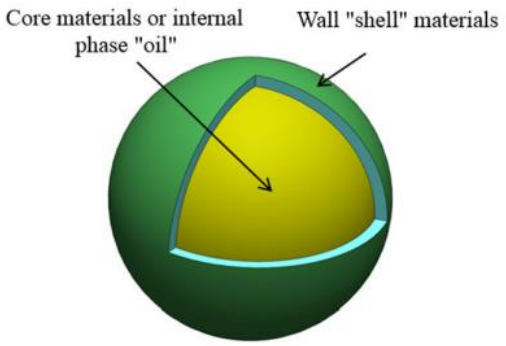


Figure 1-2: Composition of an oil microcapsule in simplified form.

Various techniques are used for encapsulation. These encapsulation techniques include spray-drying, spray cooling, freeze-drying, extrusion, fluidized bed coating, emulsification, and coacervation, (Choudhury et al. 2021).

1.2.1 Spray drying

Spray drying is the most commonly used microencapsulation method in the food industry. Spray drying is by definition the transformation of a feed from a fluid state (solution, dispersion or paste) into a dried particulate form by spraying the feed into a hot drying medium (Figure 1-3). The spray-drying technique has been widely used for drying heat-sensitive foods, pharmaceuticals, and other substances because of the rapid solvent evaporation from the droplets. Although most often

considered a dehydration process, spray drying can also be used as an encapsulation method when it entraps ‘‘active’’ materials within a protective matrix, which is essentially inert to the material being encapsulated. Compared to the other conventional microencapsulation techniques, it offers the attractive advantage of producing microcapsules in a relatively simple, continuous processing operation (I Ré 1998). The energy consumption of spray drying is 6 to10 times lower compared to freeze drying and it produces a high quality product (when it comes to microencapsulation efficiency).(Martín et al. 2015). This leads to evaporation of the solvent. It is important to underline that in this technique, the product feed, gas flow and temperature should be controlled. In this study, we also used spraying drying methods because it can be used for different encapsulating agents, it is economical, flexible, can be used for many different types of materials and can be scaled up easily (Choudhury et al. 2021).

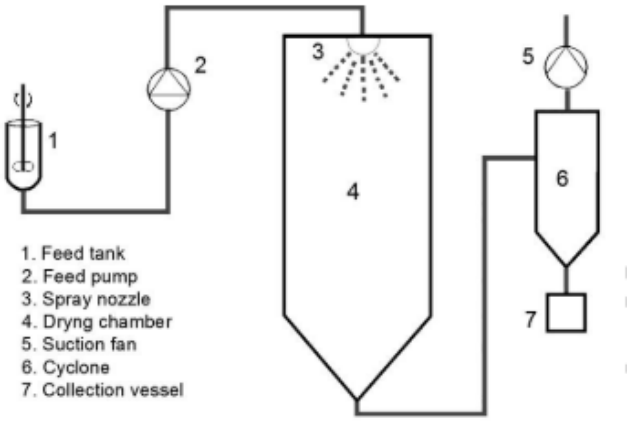


Figure 1-3: Schematic diagram for spray drying apparatus (Martín et al. 2015)

The disadvantage of this technic refers to the high temperature used in the process which may not be suitable for encapsulating of some material (Martín et al. 2015).

1.2.2 Spray cooling

Spray cooling is very similar to spray drying in operation and the major difference being the use of cold air in the chamber. Here, a mixture of core and wall materials atomized to form a mist inside the chamber, which cold air flows in. The low temperature within the chamber results in solidification of the micro droplets, leading to the formation of microencapsulated powder. This technique also has a huge potential in scaling up (Choudhury et al. 2021). Some successful implementations of this technique in encapsulation includes, microencapsulation of tocopherols within lipid matrix, with encapsulation efficiency as high as 90% (Gamboa, Gonçalves, and Grosso 2011), encapsulation of iron, iodine, and vitamin A within hydrogenated palm oil to fortify salt, where the microcapsules were highly stable (Wegmüller et al. 2006). However, this method also has some documented drawbacks. Some studies have shown that the microcapsules formed by spray cooling are not very stable and it leads to expulsion of the core material during storage (Jenning, Thünemann, and Gohla 2000).

1.2.3 Freeze-drying

Freeze-drying has been used to manufacture probiotic powders for decades but the combination of freeze- drying and encapsulation is relatively new concept. The process is based upon sublimation, occurring in three phases; freezing, primary, and secondary drying (Martín et al. 2015). Typically, cells are first frozen and then dried by sublimation under high vacuum (Santivarangkna, Kulozik, and Foerst 2007). As the processing conditions associated with freeze drying are milder than spray drying, higher probiotic survival rates are typically achieved (Wang, Yu, and Chou 2004). In this technique, the solvent is frozen and removed via sublimation (Solanki et al. 2013). However, freezing causes damage to the cell membrane because of crystal formation and also imparts stress

condition by high osmolarity. A variety of protectants have been added to the drying media before freeze drying to protect the viability of probiotics during dehydration, such as skim milk powder, whey protein, glucose, maltodextrin, trehalose among others (Martín et al. 2015).

1.2.4 Extrusion

Extrusion technology for microencapsulation can be used for producing highly dense microcapsules. To use this method, the core and the wall material should be immiscible. Here, the core and the wall materials are passed in such a way that the wall material surrounds the core and they are passed through concentric nozzles, thus, forming droplets containing the core surrounded by the wall material. Then solidification is done either by cooling or using an appropriate gelling bath wherein the droplets fall and solidify due to formation of complex. The capsules formed using this method are relatively larger in size than formed using any other method and also, this technology is useful with limited wall materials (Choudhury et al. 2021).

1.2.5 Fluidized bed coating

Fluidized bed coating is an encapsulation method in which coating material is sprayed onto the fluidized core material. Here, the core material is fluidized by application of air, onto which a coating material is sprayed. Different fluidized bed coating methods are: (a) Top spray (b) bottom spray, and (c) tangential spray. In this method of encapsulation, coating efficiency of the wall material is dependent on various parameters like feed rate of the wall material, atomization pressure of the nozzle, inlet air temperature, velocity, etc. (Choudhury et al. 2021).

1.2.6 Emulsification

Encapsulation using emulsification technique is done by dispersing the core in an organic solvent, containing the wall material. The dispersion is then emulsified in the oil or water, to which emulsion stabilizer is added. Encapsulation of the core occurs by formation of a compact polymer layer around it, by evaporation of the organic solvent. Emulsification on the other hand is a part of some other microencapsulation methods like spray and freeze drying. In other word, when the core material is oil, then the first stage of microencapsulation, regardless of the method, would be emulsification. This is one of the frequently used techniques of encapsulation as the procedures involved are very simple. This technique is widely used for encapsulating enzymes, oils and microorganisms (Choudhury et al. 2021).

1.3 Emulsion

Emulsions are dispersions made up of two immiscible liquid phases which are mixed using mechanical shear and surfactant. Particle size of this conventional emulsion grows continuously with time and hence finally separation occurs at gravitational force thus these emulsions are thermodynamically unstable (Kale and Deore 2017). Amphiphilic surface-active molecules are called as ‘surfactants’ which are responsible to reduce naturally existing attractive forces in the form of surface tension. Choice of surfactant on the basis of hydrophilic-lipophilic balance (HLB) value or critical packing parameter (CPP) helps to develop desired emulsion (Kale and Deore 2017). Surfactants with low HLB values are useful to form W/O (water in oil) emulsion and that of with high HLB values are used to form O/W (oil in water) emulsion. critical packing parameter (CPP) is ratio of hydrophilic and hydrophobic parts of surfactant molecule (Kale and Deore 2017). CPP also gives idea of nature of aggregates. recently two new concepts are emerged in emulsion

that is as follows: Micro-emulsion is clear, thermodynamically stable, isotropic liquid mixture (Kale and Deore 2017). It is prepared by using oil, water, surfactant and a co-surfactant. It incorporates very small size particles up to nano size as compared to conventional emulsion (Kale and Deore 2017). IUPAC defines micro-emulsion as dispersion made of water, oil, and surfactant(s) that is an isotropic and thermodynamically stable system with dispersed domain diameter varying approximately from 1 to 100 nm, usually 10 to 50 nm. Nano-emulsions are very similar to micro-emulsions that are dispersions of nano scale particles but obtained by mechanical force unlike to micro-emulsions which forms spontaneously (Kale and Deore 2017). Nano emulsions consist of oil droplets in the nano-ranged size, between 10 and 100 nm dispersed within an aqueous continuous phase, with each oil droplet surrounded by surfactant molecules. Nano emulsions can be produced using a variety of methods, which are classified as either high-energy or low- energy approaches. High-energy approaches use mechanical devices capable of generating intense disruptive forces that breakup the oil and water phases and lead to the formation of oil droplets, e.g., high-pressure valve homogenizers, micro- fluidizers, and sonication methods. Low-energy approaches rely on the spontaneous formation of oil droplets within mixed oil–water-emulsifier systems when the solution or environmental conditions are altered, e.g., phase inversion and solvent demixing methods.

High-energy Approaches

The formation of nano emulsions by high-energy methods is governed by the selected composition (i.e., surfactants and functional compounds) and by the quantity of energy applied. Therefore, nano emulsions produced through high- energy methods present a natural predisposition to preserve the nano emulsions formation against formulation modification e.g., addition of monomer, surfactant,

co-surfactant, etc (Anton, Benoit, and Saulnier 2008). The mechanical processes generating nano emulsions can be divided into three major groups based on the used devices that are presented below (Anton et al. 2008):

- High-pressure homogenization: in high-pressure homogenization, the mixture is subject to very high pressures and is pumped through a restrictive valve. The very high shear stress causes the formation of very fine emulsion droplets (Quintanilla-Carvajal et al. 2010);
- Ultrasound (Figure 1-4): when two immiscible liquids are submitted to high-frequency sound waves in the presence of a surfactant, emulsion droplets are formed by cavitation. This causes intense shock waves in the surrounding liquid and the formation of liquid jets at high speed is responsible for the formation of emulsion droplets. However, this technology has not yet been proved as efficient for industrial-scale applications (Quintanilla-Carvajal et al. 2010). In this study we used this technique because it was available and provided more stable emulsions.

Figure 1-4: Ultrasonic bath

- High-speed devices: rotor/stator devices (such as Ultra-Turrax (Figure 1-5) could form emulsions with mean droplet sizes in the lower micrometer region (Scholz and Keck 2015). It's also flexible and accessible. With the energy provided mostly being dissipated,

generating heat (Anton et al. 2008). In this study we used this technique to decrease the droplet size and improve the emulsion stability.

Figure 1-5: Ultra-Turrax

Low-energy Approaches

In low-energy approaches, nano emulsions are obtained as a result of phase transitions produced during the emulsification process which is carried out, generally, at constant temperature and changing the composition (Uson, Garcia, and Solans 2004) or at constant composition and changing the temperature (Izquierdo et al. 2002). The methods used more often are presented below:

- Membrane emulsification: it is a low-energy process that requires less surfactant (when compared with high- energy methods) and produces emulsions with a narrow size distribution range. This method involves formation of a dispersed phase (droplets) through a membrane into a continuous phase (Sanguansri and Augustin 2006);
- Spontaneous emulsification: this mechanism occurs when an organic phase and an aqueous phase are mixed, with the organic phase being a homogeneous solution of oil, lipophilic

surfactant and water-miscible solvent, and the aqueous phase consisting of water and hydrophilic surfactant (Sanguansri and Augustin 2006). The spontaneous features of this method result of the initial non-equilibrium states of two bulks liquids when they are brought into contact without stirring. It is only under specific conditions that spontaneous emulsification occurs. Spontaneous emulsification is produced by different mechanisms (e.g., diffusion of solutes between two phases, interfacial turbulence, surface tension gradient, dispersion mechanism, condensation mechanism) which seem to be affected by the systems' compositions and their physicochemical characteristics like the physical properties of the oily phase and nature of the surfactants (Sanguansri and Augustin 2006). This process itself increases entropy and thus decreases the Gibbs free energy of the system (Anton et al. 2008).

- Solvent displacement: this method consists of mixing a water-miscible organic solvent containing lipophilic functional compounds in an aqueous phase containing an emulsifier. The rapid diffusion of the organic solvent in the aqueous phase promotes the formation of nano emulsions enabling their preparation in one step at low-energy input with high yield of encapsulation. Finally, the organic solvent is removed from the nano dispersion under reduced pressure. Nevertheless, the use of this technique is limited to water-miscible solvents (Chu et al. 2007);
- Emulsion inversion point: this method consists in varying the composition of the system at a constant temperature. The structures are formed through a progressive dilution with water or oil in order to create kinetically stable nano emulsions (Anton et al. 2008);
- Phase inversion point: this method uses the specific ability of surfactants (non-ionic) to alter their affinities to water and oil in function of temperature at a fixed composition

(Shinoda and Saito 1968). It consists in suddenly breaking-up the micro emulsions maintained at the phase inversion point by a rapid cooling or by a dilution in water or oil. The nano emulsions immediately formed are kinetically stable and can be considered as irreversible. This process is relatively simple, prevents the encapsulated drug being degraded during processing, consumes low amounts of energy and allows an easy industrial scale-up (Silva, Cerqueira, and Vicente 2012).

1.4 Emulsion stability

Emulsion stability can be defined as the system's ability to resist changes in its physicochemical properties over time. Emulsion stability is important in many industrial applications, including coatings, food products, agriculture formulations, personal care and petroleum (Ravera et al. 2021). There are major experimental methodologies that are available to assess the physical stability of food emulsions such as gravitational separation, flocculation, coalescence, emulsifying capacity, and environmental stress tests (McClements 2007).

Normally, the density of the droplets in an emulsion is different from that of the liquid that surrounds them, and so a net gravitational force acts upon them. If the droplets have a lower density than the surrounding liquid then they have a tendency to move upwards, which is referred to as creaming (Figure 1-6). Conversely, if they have a higher density than the surrounding liquid then they tend to move downwards, which is referred to as sedimentation (Figure 1-6) (McClements 2007). The densities of most edible oils (in their liquid state) are lower than that of water, and

therefor there is a tendency for oil to accumulate at the top of an emulsion and water at the bottom. Thus, droplets in an O/W emulsion tend to cream, whereas those in a W/O emulsion tend to sediment. Having said this, it is sometimes possible to observe the opposite tendency, e.g., if the density of the oil phase is increased appreciably because it contains a weighting agent or fat crystals. Gravitational separation is one of the most common instability mechanisms encountered in the food industry and food scientists would like to establish the degree of creaming or sedimentation that has occurred in a product at a particular time, as well as the susceptibility of a product to this kind of instability (McClements 2007).

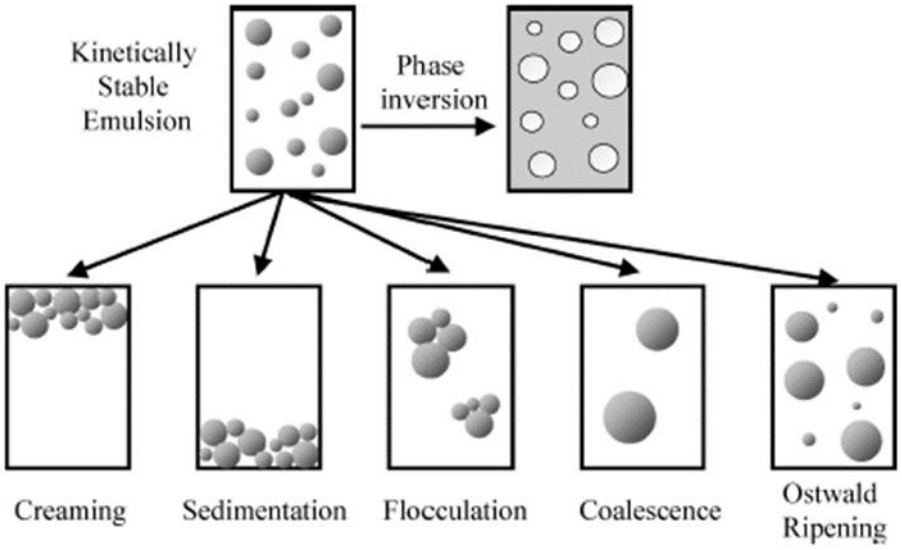


Figure 1-6: Schematic diagram of most common instability mechanisms that occur in food emulsions: creaming, sedimentation, flocculation, coalescence, Ostwald ripening and phase inversion (McClements 2007).

Creaming is one of the key manifestations of instability of emulsions. This is attributed to the tendency of oil droplets in the emulsion to move upwards and form a layer at the top (Najaf Najafi et al. 2016).

Most food emulsions are polydisperse systems, i.e., they contain a range of droplets of different sizes. The droplets in a polydisperse emulsion tend to cream at different velocities, with the

larger ones creaming more rapidly than the smaller ones (Figure 1-7) (McClements 2007). Consequently, the large droplets may move upwards rapidly and form a cream layer, whereas the smaller ones may remain dispersed in the lower part of the container (Figure 1-7 e & f). This can sometimes make it difficult to accurately characterize the amount of creaming that has occurred in an emulsion. For example, there may be an optically opaque emulsion layer in the middle or cream layer at the top, but a turbid serum layer at the bottom of the emulsion (containing the small droplets), rather than a clear serum layer. This kind of situation is commonly encountered in flocculated emulsions where only a fraction of the droplets is flocculated–the non-flocculated droplets form a turbid layer at the bottom, whereas the flocculated one's cream rapidly and form an opaque layer (McClements 2007).

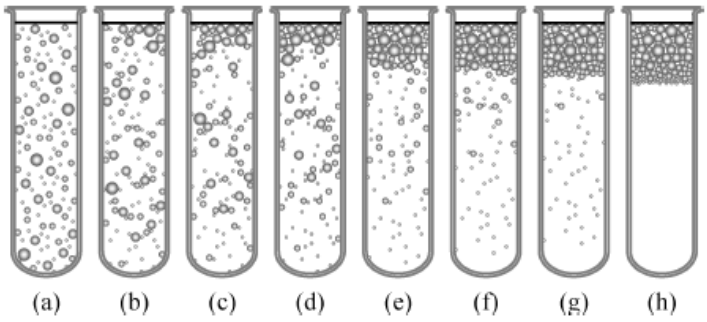


Figure 1-7: Schematic representation of creaming in a polydisperse oil-in-water emulsion (McClements 2007).

Obviously, the value of the creaming index will depend on the time that the measurement is made. Normally, the creaming index should start at zero and increase over time as the emulsion droplets move upwards until a fairly constant value (CI_{final}) is reached when all the droplets are packed tightly into the cream layer. The creaming stability of an emulsion can therefore be characterized by measuring the height of the serum layer over time (Figure 1-8) (McClements 2007).

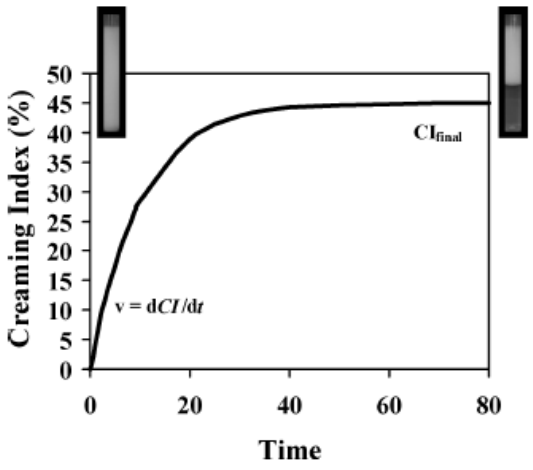


Figure 1-8: Schematic representation of evolution of creaming index versus time for an oil-in-water emulsion (McClements 2007).

If the size of the particles in the emulsion does not change during storage, then the creaming stability can be adequately characterized by the creaming velocity calculated from the initial slope of CI versus time. On the other hand, if the size of the particles changes during storage (e.g., due to droplet aggregation), then one should measure the full dependence of creaming index on time (McClements 2007).

1.5 The choice of wall materials

One of the most important challenges in the field of microencapsulation is choosing the right wall material with maximum stability and the ability to protect the core material against external factors and over time (I Ré 1998). An ideal coating should have properties such as favorable rheological properties at high concentrations, easy efficiency during microencapsulation, ability to disperse and stabilize active compounds, non-reaction with core materials during the micro encapsulation process and long-term storage, ability to retain active materials within the its structure during

processing and storage, the maximum ability to protect the active ingredients against environmental conditions (e.g., oxygen, heat, light and moisture) and soluble in solvents used in the food industry (Jun-xia, Hai-yan, and Jian 2011). Carbohydrates such as gum Arabic, maize starch and maltodextrin as well as proteins including gelatin, milk protein, whey protein concentrate, and soy protein isolate, has good functional properties for use as a wall material (Sharif, Khoshnoudi-Nia, and Jafari 2020). Maltodextrin, either alone or in combination with other biopolymers, was chiefly used as a wall material in several studies. Maltodextrin has been revealed to be an effective drying agent, helping in protection of microencapsulated fish oil. Maltodextrin shows economic advantage coming from the price and availability of this polymer; so, its combination with more expensive carrier agents might be a financial advantage (Sharif et al. 2020). Preparation of nano/microcapsules by means of biopolymer mixtures is of great interest for various food applications with hydrophilic or lipophilic media. Some biopolymers such as modified starches have the potential to form blends with a low consistency, thus hindering agglomeration and film formation prior to the spray drying process (Sharif et al. 2020). In addition, maltodextrin offers a greater flexibility in industrial food production systems where the cost and ease of acquisition of inputs are important factors to be considered in technical and economic feasibility studies. Several studies have also shown that biopolymers combined with each other result in a better efficiency and stability in the encapsulation process compared with their individual use (Sharif et al. 2020). On the other hand, Rocha et al. (2019) showed that combination of two or three biopolymers can decrease the encapsulation efficiency. Emulsifying properties, rigidity, porosity, activation energy, and surface-active properties of different (bio) polymers can affect the encapsulation efficiency of various micro/nanocarriers. Therefore, it seems that proper selection of biopolymers and blending of them can play an important role in encapsulation performance

(Sharif et al. 2020). The wall material for encapsulation should have good emulsification properties and perform as an excellent protective agent during the storage of microcapsules. Often, these requirements cannot be met with a single wall material. Thus, combination of polysaccharides and proteins, or a combination of a few wall materials with similar origins but different properties, are often used (Kurek and Pratap-Singh 2020). Maltodextrin is one of the most common polysaccharides used during spray-drying microencapsulation because of its low viscosity at high concentration, good solubility, adequate protection against oxidation, relatively low cost, and neutral aroma and taste (Kurek and Pratap-Singh 2020). However, maltodextrin has a low emulsifying capacity and emulsion stability (Kurek and Pratap-Singh 2020). Therefore, a combination of maltodextrin and other compounds could be employed for the purpose of microencapsulation. Proteins are generally perceived as suitable wall materials and emulsifiers due to their amphiphilic nature. On the other hand, because of the growing interest of non-GMO (Genetically Modified Organisms) products and vegan diets, there is a need to search for new sources of plant-based proteins that are not negatively associated with consumer perception (Kurek and Pratap-Singh 2020). Rice protein is also an essential source of protein, as it is a by-product derived from rice bran (Kurek and Pratap-Singh 2020). The nutritional profile of rice protein is generally perceived as positive, but the emulsifying properties still need better understanding, especially in terms of its' role in emulsifying hempseed oil emulsions (Kurek and Pratap-Singh 2020). Kurek and Pratap-Singh (2020a) studied the properties of emulsions and spray-dried microcapsules prepared from hempseed oil by employing a combination of maltodextrin with hemp, pea, and rice protein as carrier materials. The highest encapsulation efficiency was observed in samples with rice protein, while the lowest was with hemp protein. Combination of maltodextrin

and proteins had a preventive effect on the oxidative stability of hempseed oil (Kurek and Pratap-Singh 2020).

So based on all the aforementioned remarks, we decided to use the combination of Maltodextrin and rice protein as our wall materials for protection of salmon oil as the core material of microcapsules.

1.6 Application Fields of Microencapsulation

Microencapsulation technology is widely used in several industries, especially food and pharmaceutical industries, since it can increase solubility, enhance stability, and improve the controlled release properties of compounds such as essential oils, antioxidants, enzymes, drugs, etc. Therefore, this section focuses on the applications of microencapsulation in these industries (Choudhury et al. 2021).

1.6.1 Applications in the food industry

The food industry utilizes functional ingredients to improve flavor, color, and texture properties and to extend the shelf-life of products. Moreover, ingredients that have functional health benefits, such as antioxidants and probiotics, are of great interest. However, most of these ingredients have low-stability and are easily decomposed by environmental factors. Thus, the preparation of high stability bioactive compounds is important. Microencapsulation is one way to address these issues.

In recent years, there has been a great deal of research on the production of high efficiency microcapsules and their applications in the food industry (Peanparkdee, Iwamoto, and Yamauchi 2016). Microencapsulation has many applications in the food industry. To stabilize the material inside the dunk, control the reaction start, control the time and speed of release of the material, to cover the flavors, colors and odors, to detect pressure, increase shelf life, protect food ingredients and maintain nutritional value. This method is used to convert a liquid into a solid, to reduce volatility and to keep the active ingredients separate (Prakash and Jones 2005). The shelf life of raw milk is increased by the addition of calcium alginate bonds containing Lactic acid bacteria including *Lactococcus lactis* and *Lactobacillus helveticus*. In fact, these bacteria reduce the activity of natural cold-blooded bacteria by up to 50% (Siuta-Cruce and Goulet 2001). It is also possible to immobilize the cells used in the fermentation of dairy products and enter the process in large quantities, thus reducing the fermentation time. Obviously, such cells will be recoverable and reusable at the end of fermentation (Teunou and Poncelet 2005). Culture fluid bacteria must survive in order to be useful to their host. Therefore, such bacteria must not only reach the intestines safely, but also survive during food storage. Microencapsulation technique can be used to protect food ingredients against factors such as processing temperature, humidity and other food ingredients (Allan-Wojtas, Truelstrup Hansen, and Paulson 2008). One of the reasons food ingredients are finely coated is to delay the release of these compounds during the process. For example, they put artificial sweeteners inside the oven and protect them against the high temperature of the cooking process. In this case, the artificial sweetener does not decompose due to high temperatures, but at the end of the process, it is released out of the dunk, and as a result, the final product will have a sweet taste (Keogh 2005). The important thing is that under a certain stimulus, the material inside the dunk is released at a controlled rate. In food, the controlled release

of probiotics into the dunk means that these bacteria are not exposed to food and gastrointestinal conditions at the same time. Rather, it is gradually released from the dunk, and over a long period of time, the population of probiotics in the intestine is constantly renewed (Belitz, Grosch, and Schieberle 2004).

1.6.2 Application in pharmaceutical industries

The microencapsulation technique has been widely used in the pharmaceutical industry for controlled release of drugs, enhancing stability, and flavor-masking (Peanparkdee et al. 2016).

Arimoto et al. (2004) explored the use of microcapsule formulations for the colon-specific delivery of a water-soluble peptide drug. In general, peptides are heat-sensitive and have low permeability through polymeric membranes. Thus, this study aimed to preserve the stability of heat-sensitive drugs and the desired permeability allowing for a delayed-release profile of macromolecular drugs. The results showed that poly (EA/ MMA/HEMA) with a molar ratio of 95:85:40 exhibited good film-formability at 40°C. These conditions could be proposed as an appropriate way to prepare delayed-release microcapsules containing water-soluble drugs for colon-specific delivery (Arimoto, Ichikawa, and Fukumori 2004).

1.7 Three important microcapsule properties:

1.7.1 Moisture content

Microcapsules when exposed to an environment with relatively high humidity, tend to absorb moisture from the environment and this property is called hygroscopicity. It decides the stability of the core material. Hygroscopicity of a microcapsule largely depends on the type of wall material which is used to hold the core material. For example, for microencapsulation of oils and certain flavors, a wall material with less hygroscopic such as WPI will be used. This property of the microcapsules during storage can be determined by using the sorption isotherms and gravimetric static method (Choudhury et al. 2021).

1.7.2 Encapsulation efficiency

Encapsulation efficiency is defined as the amount of core material that is encapsulated within a wall material, against the concentration of the core that was used for encapsulation, using a particular technique. It depends on the concentration of core material that is used for encapsulation. With the increasing levels of the core material, encapsulation efficiency tends to decrease (Choudhury et al. 2021).

1.7.3 Peroxide value

Microencapsulation is considered to be effective only if the core material is protected until its release is desired. Lipid oxidation of fish oil after encapsulation and during storage is monitor by measuring lipid hydroperoxides value. Peroxide value is an indicator of the oxidative stability and initial oxidation of the edible oils. Peroxide value of the encapsulated oil can be influenced by

several factors such as temperature, homogenization rate and wall material composition (Cihat Icyer et al. 2017). Although hydroperoxides decompose to a mixture of volatile and non-volatile products and they also react further to endoperoxides and other products, the PV measurement is a useful method of monitoring oxidative deterioration of oils, although it should normally be combined with a method of monitoring secondary oxidation products to provide a fuller picture of the progress of oxidation. A high PV value may reflect either increased formation of hydroperoxides or reduced decomposition (Gordon 2004).

Lipid oxidation

Lipid oxidation is a process that results in rancidity and deterioration of fats and progresses via free radical propagated chain reactions. Free radicals are produced by the reaction of unsaturated fatty acids with molecular oxygen and traces of other oxidants, as metal ions (Fe^{3+} and Cu^{2+} are particularly effective) and H_2O_2, and by UV light (Ramis-Ramos 2003).

Mechanism of autoxidation

Deterioration of foods by lipid oxidation generally displays an induction period during which very little oxidation occurs (Figure 1-9) (Gordon 2004).

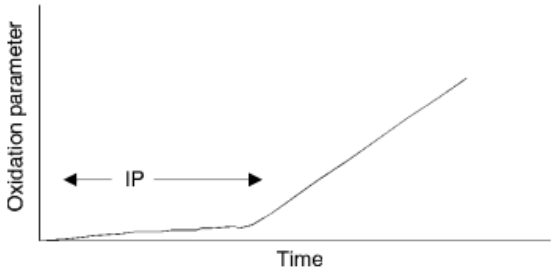


Figure 1-9: Typical change in a fat during oxidation showing the presence of an induction period (Gordon 2004).

This is followed by a stage when deterioration proceeds rapidly. The length of the induction period is shortened dramatically by low concentrations of metals such as iron or copper, and these are described as pro-oxidants, but it can be extended considerably by low concentrations of antioxidants. The rate of deterioration increases markedly with an increase in temperature. These characteristics have led researchers to conclude that the reaction is a free-radical chain reaction. As a free-radical reaction, autoxidation proceeds in three distinct steps (Figure 1-10) (Gordon 2004).

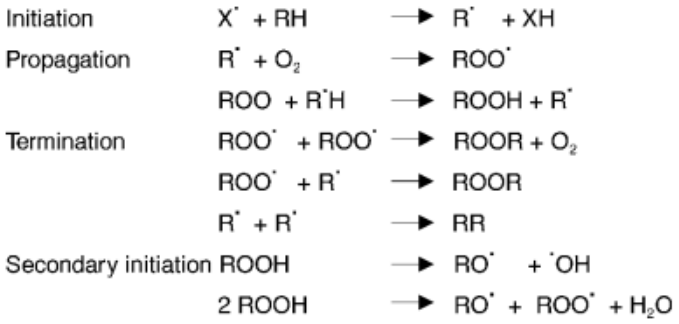


Figure 1-10: Mechanism of autoxidation (Gordon 2004).

The first step is initiation in which lipid radicals are formed from lipid molecules. Abstraction of a hydrogen atom by a reactive species such as a hydroxyl radical may lead to initiation of lipid oxidation. However, in oils there is often a trace of hydroperoxides, which may have been formed by lipoxygenase action in the plant prior to, and during, extraction of the oil. Secondary initiation by homolytic cleavage of hydroperoxides is a relatively low energy reaction, and is normally the main initiation reaction in edible oils (Gordon 2004). This reaction is commonly catalysed by metal ions. After initiation, propagation reactions occur in which one lipid radical is converted into a different lipid radical. These reactions commonly involve abstraction of a hydrogen atom from a

lipid molecule or addition of oxygen to an alkyl radical. The enthalpy of reaction is relatively low compared with that of the initiation reactions, so propagation reactions occur rapidly compared with initiation reactions. At normal atmospheric pressure of oxygen, the reaction of alkyl radicals with oxygen is very rapid, and the peroxyl radicals are present at much higher concentrations than the alkyl radicals. Alkoxy radicals formed by hydroperoxide decomposition can decompose to release volatile hydrocarbons, alcohols or aldehydes, that are no longer bound to the glycerol backbone when the fatty acid is present as a glyceride (Gordon 2004).

1.8 Aim of thesis

I. Produce a stable emulsion of Salmon oil with Rice protein and Maltodextrin as wall materials.

II. Optimize spray drying parameters on encapsulation efficiency, peroxide value and moisture content, by adjusting feed mass flow rate (2.26, 7.04 and 11.2 L/min), inlet drying air temperatures (100, 120, 140, 160 and 180℃) and spraying air mass flow rates (200, 250, 300 and 350 L/min) in spray dryer.

A factorial type of experiment was used in association with a completely randomized design (CRD). The results were processed by IBM SPSS statistics 19.0. Each experiment was performed in triplicate. The mean values pertaining to the data were compared using Duncan's test. The differences were considered significant at p value < 0.05.

1.9 Complete Randomized Design

An experiment is a procedure carried out to support or refute a hypothesis or determine the efficacy or likelihood of something previously untried. Experiments provide insight into cause-and-effect by demonstrating what outcome occurs when a particular factor is manipulated (Tang et al. 2017). A completely randomized design (CRD) is one where the treatments are assigned completely at random so that each experimental unit has the same chance of receiving any one treatment. For the CRD, any difference among experimental units receiving the same treatment is considered as experimental error. CRD is used when the experimental material is homogeneous. CRD is often inefficient. CRD is more useful when the experiments are conducted inside the lab. CRD is well suited for the small number of treatments and for the homogeneous experimental material. ANOVA is a table that tells you if the dependent variable changes according to the level of the independent variable. For example: your independent variable is social media use, and you assign groups to low, medium, and high levels of social media use to find out if there is a difference in hours of sleep per night (St and Wold 1989).

1.10 Hypotheses

- ✓ With increasing the feed mass flow rate and decreasing the inlet-drying air temperature and spraying air mass flow rate the moisture content of samples will increase.
- ✓ With increasing the inlet-drying air temperature and spraying air mass flow rate and decreasing the feed mass flow rate the extraction yield of sample will increase.
- ✓ High feed mass flow rate and inlet-drying air temperature will lead to high peroxide value.

2 Materials and methods

2.1 The fish oil

The fish oil utilized in this master thesis were obtained from SINTEF Ocean that had received samples from a commercial salmon oil producer. The oils were flushed with nitrogen and were stored at -45°C in containers protected from light. After each sampling, the oils were flushed with nitrogen and returned to the freezer to limit undesired lipid oxidation.

2.2 Reagents and solvents

The following reagents and solvents were used in the experiments performed in the work with this thesis (listed in 2-1).

Table 2-1 The reagents and solvents used in this study

Reagents & solvent	Producer company	Producer country	CAS number
Chloroform 99%	VWR	USA	67-66-3
N-hexane 97%	VWR	USA	110-54-3
Methanol 99.9%	VWR	USA	67-56-1
Sodium polyphosphate	Merck	Germany	7758-29-4
Rice protein 80 %	My vegan	UK	-
Maltodextrin	Sigma	USA	9050-36-6
Triton x-100	Merck	Germany	9036-19-5
Sodium hexametaphosphate	Merck	Germany	68915-31-1
Acetic acid	VWR	USA	64-19-7
Sodium thiosulfate	Alfa Aesar	USA	10102-17-7
Sodium carbonate	Prolab	Canada	497-19-8
Potassium iodate	Prolab	Canada	7758-05-6
Starch	Merck	Germany	232-679-6

2.3 Emulsion formulation stages:

The emulsion formulation stage was the most challenging process in this master thesis.at first we had 2 plant protein candidates which expected to be used in combination with Maltodextrin as our polysaccharide. The diagram below, briefly shows the challenges that we had in this process and how we finally reached the final emulsion formulation.

Please note that the emulsification method was selected based on a paper which used the same proteins with different core material.

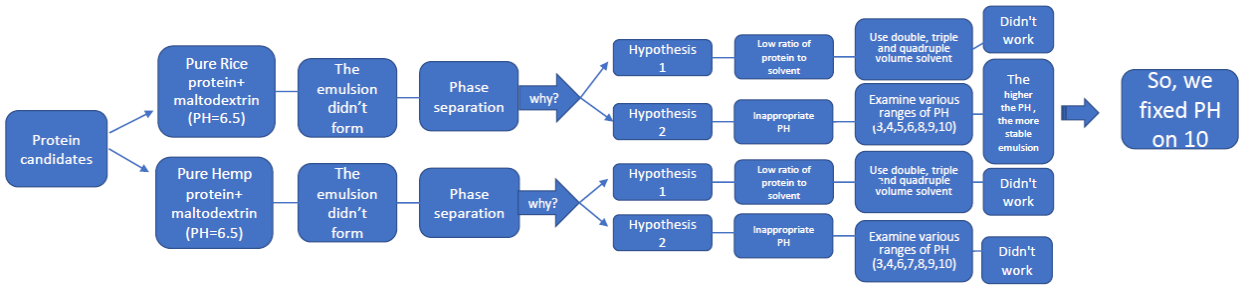


Figure 2-1: The emulsion formulation stage used in this master thesis

2.4 Emulsion Preparation and Spray-Drying Micro-Encapsulation

First dissolving 9g of rice protein in 40 ml sodium phosphate buffer with a pH of 10 that was then solubilized by constant stirring for 2 h. Then, the mixture combined with solubilized maltodextrin (9g/40 ml deionized water) and mixed at 20,000 rpm for 10 min using an Ultra-turax (Model T50 digital, IKA, Japan) (Figure 2-1A). The ratio of protein to maltodextrin was kept constant at 1:1 through all the experiments. The content of solid compounds in all the emulsions was kept at 20%. 2g fish oil was then added to the wall material suspension and homogenized at 20,000 rpm using an ultra-turrax for 10 min at 25 ˚C. Next, an ultrasound bath (Model RK100, BANDELIN) (Figure 2-1B) with ultrasonication power of 50W was conducted at room temperature for 30 min. Sodium

azide (0.02 wt%) was added to the emulsions as an antimicrobial agent (Kurek and Pratap-Singh 2020) .

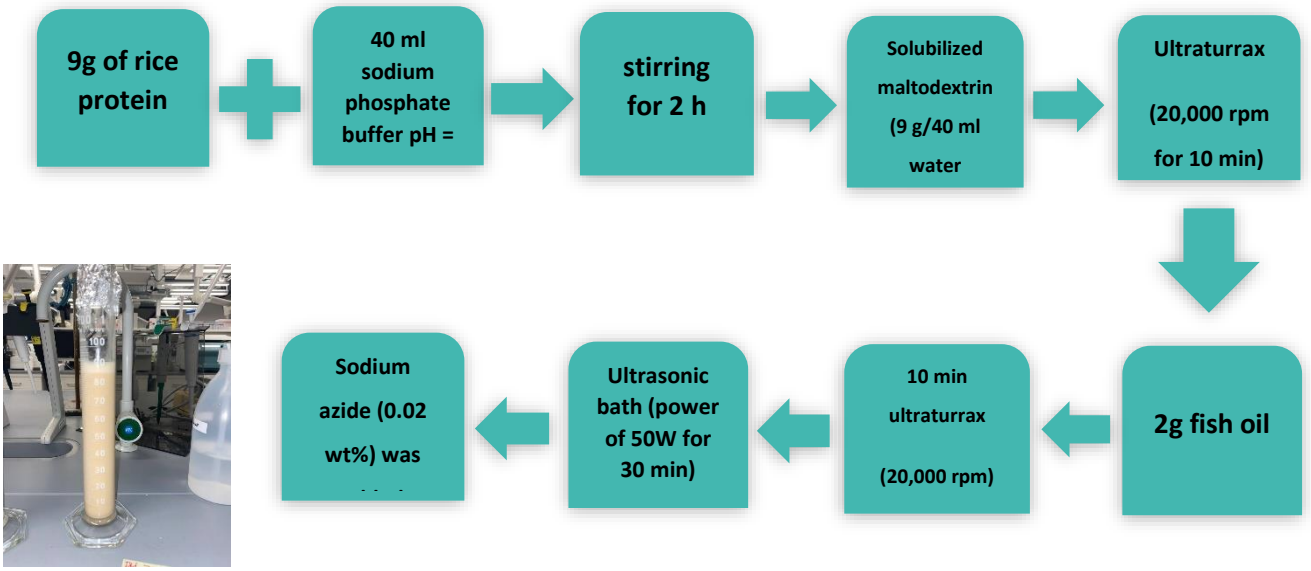


Figure 2-2: Stages of emulsion production.

The emulsions were dried using a spray-drier (Model Pulvis GB22, Yamato Scientific Co., Ltd) (Figure 2-1C). Dried powders (Figure 2-1D) were collected, closed hermetically, then the capsules were flushed with nitrogen and stored at −45℃ before further examination.

2.5 Analytical methods

2.5.1 Measurement of stability

As explained in section 2.3, this test was performed at emulsion formulation step. The aim of this test was to obtain a suitable pH with the highest emulsion stability. So, various rang of pH (3, 4, 5,6, 8, 9, and 10) was used for preparation of emulsion.

The creaming stability of the prepared emulsions was studied according to the Kargar et al. (2012). The samples were poured in glass test tubes (2.8 cm diameter 7.5 cm high) and were sealed to prevent evaporation. Samples were monitored for 3 days. The emulsions separate into a top ‘‘cream layer’’ and a bottom ‘‘serum layer’’ (Hs). The creaming index (CI) was calculated as:

CI (%)=(Hs/He) ×100

where Hs is the serum layer high and He is the total emulsion height.

2.5.2 Moisture Content

The moisture content of the powders was determined by the gravimetrically method. Briefly, 1 g powder were accurately placed into the aluminum pan and heated in an oven (TS8056, Termax, Norway) at 105 °C. The moisture content (%) was recorded for each sample after stable weight was obtained (~ 24 h). Measurements were carried out in duplicates after powder production. Finally, the moisture content was calculated using Equation 1:

$$\text{Moisture content (\%)} = \frac{W_1 - W_2}{W_0} \times 100 \quad (1)$$

Where, W_1 is the weight of container and sample before drying; W_2 is the weight of container and sampler after drying; and W_0 is the weight of initial sample before drying (Kurek and Pratap-Singh 2020).

2.5.3 Encapsulation Efficiency

Encapsulation efficiency was determined using a method based on the difference between the

total oil and surface oil.

Surface oil (S.O) content measurement:

The presence of S.O was determined after extracting the oil using hexane. Briefly, 0.5 g of beads were dispersed in 10 ml of hexane, followed by slow shaking for 30 s using a shaker (Model Ks 260, IKA, Japan). Then the mixture was filtered, diluted and the palm oil content was determined spectrophotometrically (model G10S UV-Vis, Thermo Fisher Scientifiic) at 228 nm.

Figure 2-3: The Spectrophotometer that used in this study

Total oil (T.O) content measurement:

T.O content was determined by modifying a method reported in a previous publication. Five ml of hot water (60 °C) was added to 0.5 g of beads and stirred for three h to disintegrate the beads. Then 20 ml of hexane was added and stirred for 60 min, followed by ultrasonication for 2 min to ensure complete extraction of the oil by hexane. The mixture was subsequently centrifuged for 15 min at 8,000 rpm, 5 ml of supernatant fluid was taken, diluted and the oil content was measured at 228 nm. The encapsulation efficiency of the beads was calculated using the following equation (Sathasivam et al. 2018):

$$\text{Encapsulation efficiency } (\%) = \left[1 - \frac{SO}{TO}\right] \times 100 \qquad (2)$$

2.5.4 Peroxide Value

The evaluation of the lipid oxidation in microencapsulated oils is important because it results in loss of nutritional value and development of undesirable reactions. The oxidative stability of the encapsulated soybean oil was evaluated by measuring the peroxide value.

immediately after drying. For determined the peroxide value of encapsulated fish oil first fish oil was extracted from spray-dried powders using a method described by (Hogan, O'Riordan, and O'Sullivan 2003).For this test, 1.5 g powder was placed in a 7 mL graduated Pyrex test tube and 4 mL de-emulsification reagent (30 g Triton X-100 and 70 g sodium hexametaphosphate/L) added. The test tubes were stoppered, mixed using a vortex shaker and allowed to stand for 3 h with occasional mixing. The test tubes were then placed in a boiling water bath for 5 min and subsequently centrifuged at 500 g (Model ROTINA420R, Hettich, German). Following centrifugation, the supernatant containing the fish oil was carefully removed using a Pasteur pipette and analyzed for peroxide value.

Peroxide value of encapsulated fish oil, as extracted above, was measured according to 0.3–5 g fish oil was weighed into a 250 mL Erlenmeyer flask, 30 mL acetic acid/chloroform mixture (3: 2 v/v) was added and the contents were swirled to dissolve the fish oil. Saturated KI solution (0.5 mL) was added, the flask was left to stand for 1 min and then gently mixed. Distilled H_2O (30 mL) was added and the contents were titrated against 0.01 N sodium thiosulphate ($Na_2S_2O_3$) (using 0.5 mL 1% starch indicator). Blank analyses were performed in the same way, but with the fish oil sample omitted. The peroxide value was calculated as milli-equivalents of active oxygen per kg of oil (meq O_2/kg oil) as follows:

$$\text{Peroxide value} = \frac{N \times (S - B) \times 1000}{\text{W}} \quad (3)$$

Where, S and B are the titration volumes of $Na_2S_2O_3$ for the oil sample and for the blank (in ml), respectively. Furthermore, N is the normality of $Na_2S_2O_3$ solution (0.01 N) (Hogan et al. 2003).

2.5.5 Scanning Electron Microscopy

Particle size and morphology were evaluated by scanning electron microscopy (SEM, Apreo) (Figure 2-3A). Powders were mounted on aluminum stubs and were coated with a 20 nm gold (Au) (Figure 2-3B) using a sputter coater (Figure 2-3C). SEM images were acquired with a scanning electron microscope under a low vacuum with a 3 kV acceleration voltage (Kurek and Pratap-Singh 2020).

A B C

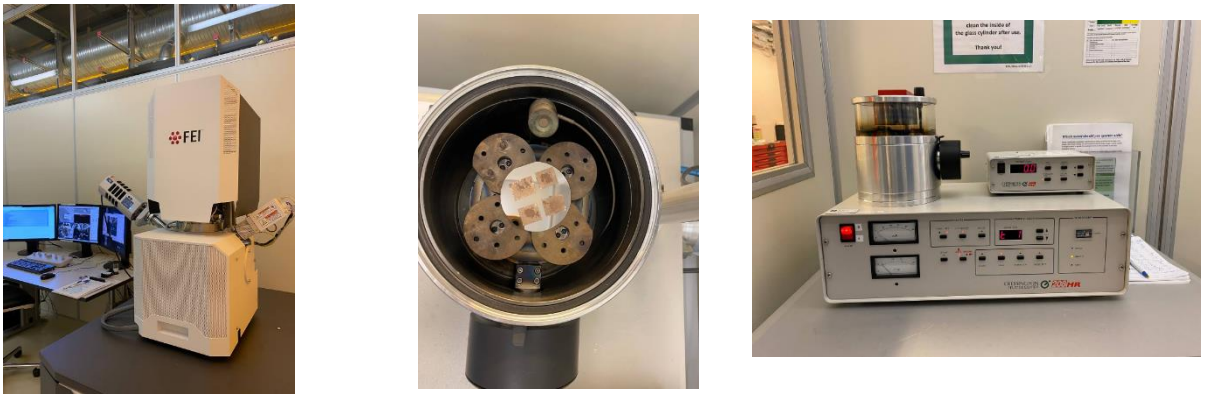


Figure 2-4: Scanning electron microscopy system.

2.6 Statistics

The data obtained were subjected to statistical analysis using SPSS 19 software and results data was imported to Microsoft Excel (2016) software to plotted charts. SPSS calculates the t-statistic and its p-value under the assumption that the sample comes from an approximately normal

distribution. If the p-value associated with the t-test is low (0.05 is often used as the threshold), there is evidence that the mean is different from the hypothesized value. A somewhat arbitrary convention is to reject the null hypothesis if $p < 0.05$. The mean differences for all treatments were tested with one-way ANOVA and statistical significance differences between the mean values were established at $p < 0.05$ and Duncan's new multiple range test. The results were expressed as mean ± SE.

3 Result and discussion

3.1 Creaming index

As it is shown in figure 2-1 previously, the creaming index was measured during the pre-tests to determine the final emulsion formulation. The creaming index should be as low as possible and therefore this was important to find before setting up the experimental design. According to Table 3-1 the creaming index of emulsion decreased from 12 % to 2 % with increasing pH from 3 to 10. It means, the stability of emulsion increased with increasing the pH.

*please note that the isoelectric point of rice protein is 7 and according to several studies, when the droplets in the emulsions are in close proximity (PH close to the isoelectric point or high ionic strength) the proteins can re-arrange themselves, which can promote droplet flocculation through increased hydrophobic attraction and disulfide bond formation between proteins adsorbed onto different droplets and that's the reason why we didn't measured creaming index in PH=7 (Östbring et al. 2021).

Table 3-1: Experimental values of response of creaming index

pH	Creaming Index (%)
3	12
4	11.2
5	9.2
6	9.2
8	6.4
9	3.2
10	2

3.2 Effect of feed mass flow rate

As shown in Table 3-5, feed flow rate has a significant effect on moisture content, encapsulation efficiency, and peroxide value of fish oil microcapsules ($P \leq 0.001$).

As shown in Figure. 3-1A and Table 3-2, by increasing the feed mass flow rate from 2.26 L/min to 11.2 L/min, the moisture content of microcapsules has a significant increase ($P \leq 0.001$) from 4.07 ± 0.01 % to 5.41 ± 0.02 %. This is due to the larger size of generated droplets and higher amount of entering water (in the emulsion) to the drying chamber (Aghbashlo et al. 2013a). A similar trend of increasing moisture content was shown by Aghbashlo et al. (2013) when the peristaltic pump rate (feed mass flow rate) increased from 5 to 15 %.

According to Figure. 3-1B and Table 3-2, with an increase in the feed mass flow rate from 2.26 L/min to 11.2 L/min, the encapsulation efficiency of microcapsules has a significant decrease ($P \leq 0.001$) from 69.18 ± 0.11 % to 56.41 ± 0.02 %. It is argued that the larger particle size obtained by increasing feed mass flow rate was responsible for the lower encapsulation efficiency. A larger droplet size causes the retarded crust formation and makes easier the diffusion of oil towards to droplets' surface (Aghbashlo et al. 2013a). A similar trend was observed by Aghbashlo et al. (2013a) for encapsulated fish oil and Hee et al. (2017) for encapsulated virgin coconut oil.

Fig. 3-1C and Table 3-2 verifies that, the peroxide value of microcapsules is significantly increased from 6.38 ± 0.03 meq O_2/kg oil 6.80 ± 0.02 meq O_2/kg oil with increasing the feed mass flow rate from 2.26 L/min to 11.2 L/min ($P \leq 0.001$). It is argued that the lower encapsulation efficiency could probably play a significant role in producing microcapsules with higher peroxide value by increasing the feed mass flow a rate (Aghbashlo et al. 2013a). The present result was in acceptable agreement with that reported by (Aghbashlo et al. 2013a). It should note that, our purpose wasn't to optimized the conditions for keeping the oxidation as low as possible (during emulsification and spray drying process) and there was oxygen involved in the encapsulation process. Our goal was to optimize the spray drying conditions and parameters.

Table 3-2: Moisture content of fish oil microcapsules produced at different spray-drying variables.

Drying variables			Microcapsules attribute
Feed mass flow rate (L/min)	Inlet-drying air temperature (°C)	Air mass flow rate (L/min)	Moisture content (%)
2.26	160	300	4.07 ± 0.01
7.04	160	300	4.59 ± 0.01
11.2	160	300	5.41 ± 0.02
2.26	100	300	5.09 ± 0.01
2.26	120	300	4.57 ± 0.01
2.26	140	300	4.14 ± 0.01
2.26	160	300	4.04 ± 0.01
2.26	180	300	4.03 ± 0.01
2.26	180	200	4.37 ± 0.01
2.26	180	250	4.21 ± 0.01
2.26	180	300	4.07 ± 0.01
2.26	180	350	4.06 ± 0.01

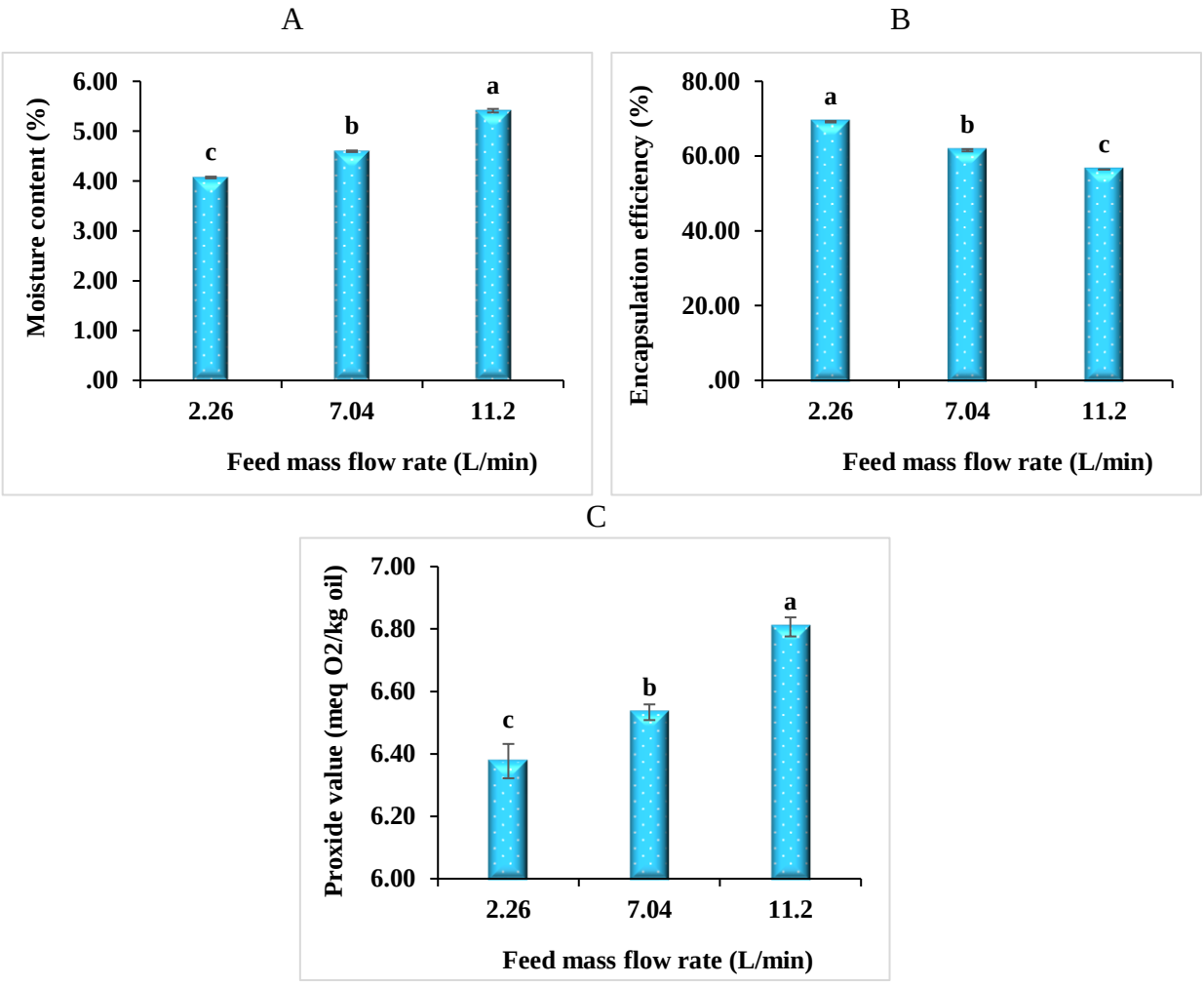


Figure 3-1: The effect of feed mass flow rate on A) moisture content, B) encapsulation efficiency, and C) peroxide value of salmon fish oil microcapsules.

3.3 Effect of inlet-drying temperature

As shown in on Table 3-5, the effects of inlet-drying air temperature were statistically significant on moisture content, encapsulation efficiency, and peroxide value of fish oil microcapsules ($P \leq 0.001$).

As Figure. 3-2A shows and Table 3-3, with increase in inlet-drying air temperature from 100-160℃, the amount of moisture content of microcapsules has a significant decrease ($P \leq 0.001$) from 5.09 ± 0.01 % to 4.04 ± 0.01 %. Increasing drying air temperature provided more energy and

thus led to higher moisture evaporation from droplet/particle because of higher heat and mass transfer values (Aghbashlo et al. 2013a). Similar findings have been reported by Aghbashlo et al. (2013), by Aghbashlo et al. (2013b) in the spray-dried of fish oil microcapsules, by Goula, Adamopoulos, and Kazakis (2004) for spray-dried fruit and vegetables powder. However, the amount of moisture content for a 160℃ inlet-drying air temperature treatment (5.41 ± 0.02 %) had no significant difference with an it's amount at 180℃ inlet-drying air temperature treatment (4.03 ± 0.15 %) ($P > 0.05$).

In Figure. 3-2B and Table 3-3, it can be seen that, the highest encapsulation efficiency (64.09 ± 0.01 %) was obtained at 180℃ inlet-drying air temperature. Higher drying air temperatures accelerated the drying rate of droplets, promoting the fast formation of particle crust. The crust, as soon as formed, provided a firm membrane around the particles, preventing further leaching of oil from the droplet (Aghbashlo et al. 2013b). Aghbashlo et al. (2013b) verified that the use of a higher inlet drying air temperature increased the encapsulation efficiency of fish oil microcapsules.

As can be seen in Figure. 3-2C and Table 3-3, the peroxide value of fish oil microcapsules increased from 6.77 ± 0.01 meq O_2/kg oil to 7.11 ± 0.01 meq O_2/kg oil at the early stage of inlet-drying air temperature (100℃ to 140℃), thus indicating the formation of peroxide and hydro-peroxide compounds. When inlet-drying air temperature continued much longer (140℃ to 180℃), peroxide value drastically dropped (7.11 ± 0.01 meq O_2/kg oil to 6.09 ± 0.01 meq O_2/kg oil) due to the degradation of peroxide and hydro-peroxide compounds (Samaram et al. 2015).

Tonon, Grosso, and Hubinger (2011) reported that peroxide value of flaxseed oil microcapsules increased with increasing drying temperatures from 150℃ to 170℃. Aghbashlo et al. (2013a) reported that the peroxide value of produced fish oil capsules varied between 5.6 and 6.5 meq O2/ kg oil, due to higher inlet- air temperature. Andrea et al. (2018) found that, lipid oxidation of palm

fiber oil carotenoids microcapsules was decreased at inlet- air temperature varying between 130 184 °C and then increased with more increase in inlet air temperatures more than 184 °C. Inlet air temperatures lower than 148 °C may not be sufficient for droplet drying, leading to an inefficient encapsulation, while temperatures higher than 184 °C accelerate the lipid oxidation reaction.

Aghbashlo et al. (2013b) observed that the peroxide value of pure fish oil was 5.00 ± 0.20 meq/kg and increased during spray drying with increasing drying air temperature. The higher the process temperature, the higher was the amount of generated peroxides, owing to the rapid oxidation of lipids which occurred due to the intensive energy provided at higher drying temperatures. Serfert et al. (2009) found that the hydro-peroxide content of microencapsulated fish oil at inlet/outlet temperatures of 210/90 °C was three times higher than that produced at 160/ 70 °C. Tonon et al. (2011) also verified that the use of a higher inlet drying air temperature increased the peroxide value.

Table 3-3: Encapsulation efficiency of fish oil microcapsules produced at different spray-drying variables.

Drying variables			Microcapsules attribute
Feed mass flow rate (L/min)	Inlet-drying air temperature (°C)	Air mass flow rate (L/min)	Encapsulation efficiency (%)
2.26	160	300	69.18 ± 0.11
7.04	160	300	61.55 ± 0.18
11.2	160	300	56.41 ± 0.02
2.26	100	300	64.09 ± 0.01
2.26	120	300	61.44 ± 0.02
2.26	140	300	60.20 ± 0.02
2.26	160	300	69.12 ± 0.02
2.26	180	300	73.22 ± 0.02
2.26	180	200	72.73 ± 0.01
2.26	180	250	73.07 ± 0.01
2.26	180	300	73.23 ± 0.02
2.26	180	350	73.27 ± 0.02

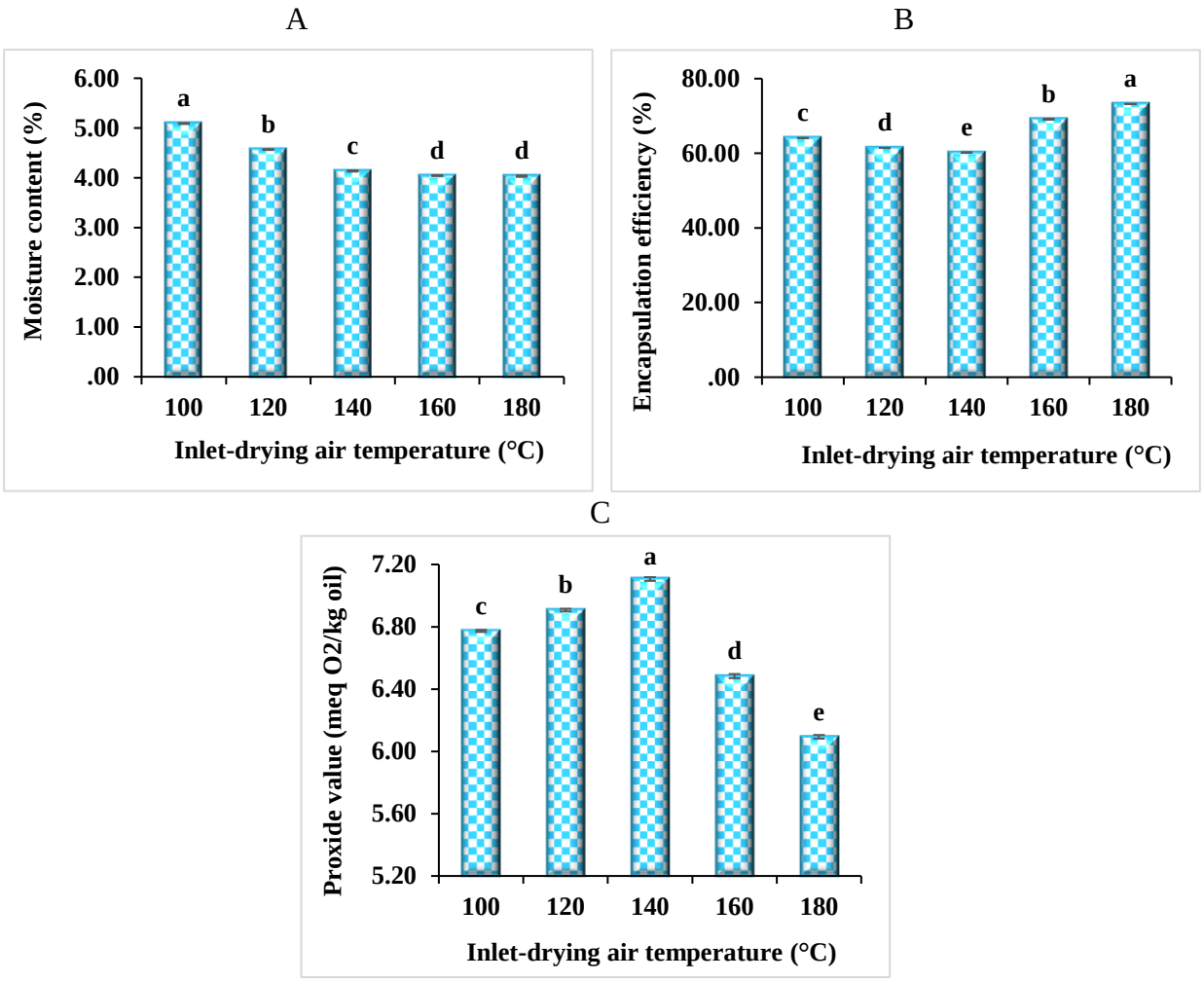


Figure 3-2: The effect of inlet-drying air temperature on A) moisture content, B) encapsulation efficiency, and C) peroxide value of salmon fish oil microcapsules.

3.4 Effect of spraying air mass flow rate

As illustrated in Table 3-5, the spraying air mass flow rate has a statistically significant on the moisture content, encapsulation efficiency, and peroxide value of fish oil microcapsules ($P \leq 0.001$).

As shown in Figure. 3-3A and Table 3-4, an increase in the spraying air mass flow rate from 200 L/min to 350 L/min led to the significant decrease in the moisture content from 4.37 ± 0.02 % to

4.06 ± 0.01 % ($P \leq 0.001$). It should be noted that, there were no significant difference between two treatments of 300 L/min air mass flow rate treatment (equivalent to 4.07 ± 0.02 %) and 350 L/min air mass flow rate treatment (equivalent to 4.06 ± 0.01 %) in term of the moisture content ($P > 0.05$). Increase of spraying air mass flow rate decreased the moisture content due to a decrease in the size of atomized droplets and a significant increase in contact surface for heat and mass transfer. The severe heat and mass transfer accelerated the water vaporization from droplet/particle and led to particles with lower moisture content (Aghbashlo et al. 2013a). Similar results were reported by Aghbashlo et al. (2013a) for fish microcapsules. They found that the moisture content of samples was decreased by increasing the spraying air mass flow rate (Aghbashlo et al. 2013a).

As Figure. 3-3B and Table 3-4 reveals, as spraying air mass flow rate increased from 200 L/min to 350 L/min the average of encapsulation efficiency of fish oil microcapsules was significantly increased from 72.73 ± 0.01 % to 73.27 ± 0.02 % ($P \leq 0.001$). Increasing spraying air mass flow rate increased encapsulation efficiency because of faster crust formation. A hardened crust did not allow the oil to diffuse out to the surface of particle and consequently the encapsulation efficiency increased (Aghbashlo et al. 2013a). It was reported by Aghbashlo et al. (2013a) that encapsulation efficiency of Fish oil microcapsules significantly increased ($P < 0.05$) with increasing spraying air mass flow rate from 600 to 800 (L/h) (Aghbashlo et al. 2013a), which is in good agreement with the present results. It should be noted that, two treatments of 300 L/min and 350 L/min air mass flow rate (equivalent to 73.23 ± 0.02 % and 73.27 ± 0.02 %, respectively) did not show any statically significant difference in terms of encapsulation efficiency ($P > 0.05$).

Results of Figure. 3-3C and Table 3-4 indicate that, by increasing the spraying air mass flow rate from 200 L/min to 350 L/min, the average of peroxide value of fish oil microcapsules significantly

decreased from 6.73 ± 0.01 meq O_2/kg oil to 6.14 ± 0.01 meq O_2/kg oil, respectively ($P \leq 0.001$). It should be noted that, two treatments of 300 L/min and 350 L/min air mass flow rate (equivalent to 6.16 ± 0.02 meq O_2/kg oil and 6.14 ± 0.01 meq O_2/kg oil, respectively) did not show any significant difference in terms of peroxide value ($P > 0.05$). Aghbashlo et al. (2013a) stated that the increase in spraying air flow rate reduced ($P < 0.05$) the peroxide value possibly due to higher encapsulation efficiency of finished microcapsule, which lowered the direct contact of deteriorating oxygen with encapsulated oil.

Table 3-4: Peroxide value of fish oil microcapsules produced at different spray-drying variables.

Drying variables			Microcapsules attribute
Feed mass flow rate (L/min)	Inlet-drying air temperature (°C)	Air mass flow rate (L/min)	Proxide value (meq O_2/kg oil)
2.26	160	300	6.38 ± 0.03
7.04	160	300	6.53 ± 0.01
11.2	160	300	6.81 ± 0.02
2.26	100	300	6.77 ± 0.01
2.26	120	300	6.91 ± 0.01
2.26	140	300	7.11 ± 0.01
2.26	160	300	6.48 ± 0.01
2.26	180	300	6.09 ± 0.01
2.26	180	200	6.73 ± 0.01
2.26	180	250	6.38 ± 0.01
2.26	180	300	6.16 ± 0.02
2.26	180	350	6.14 ± 0.01

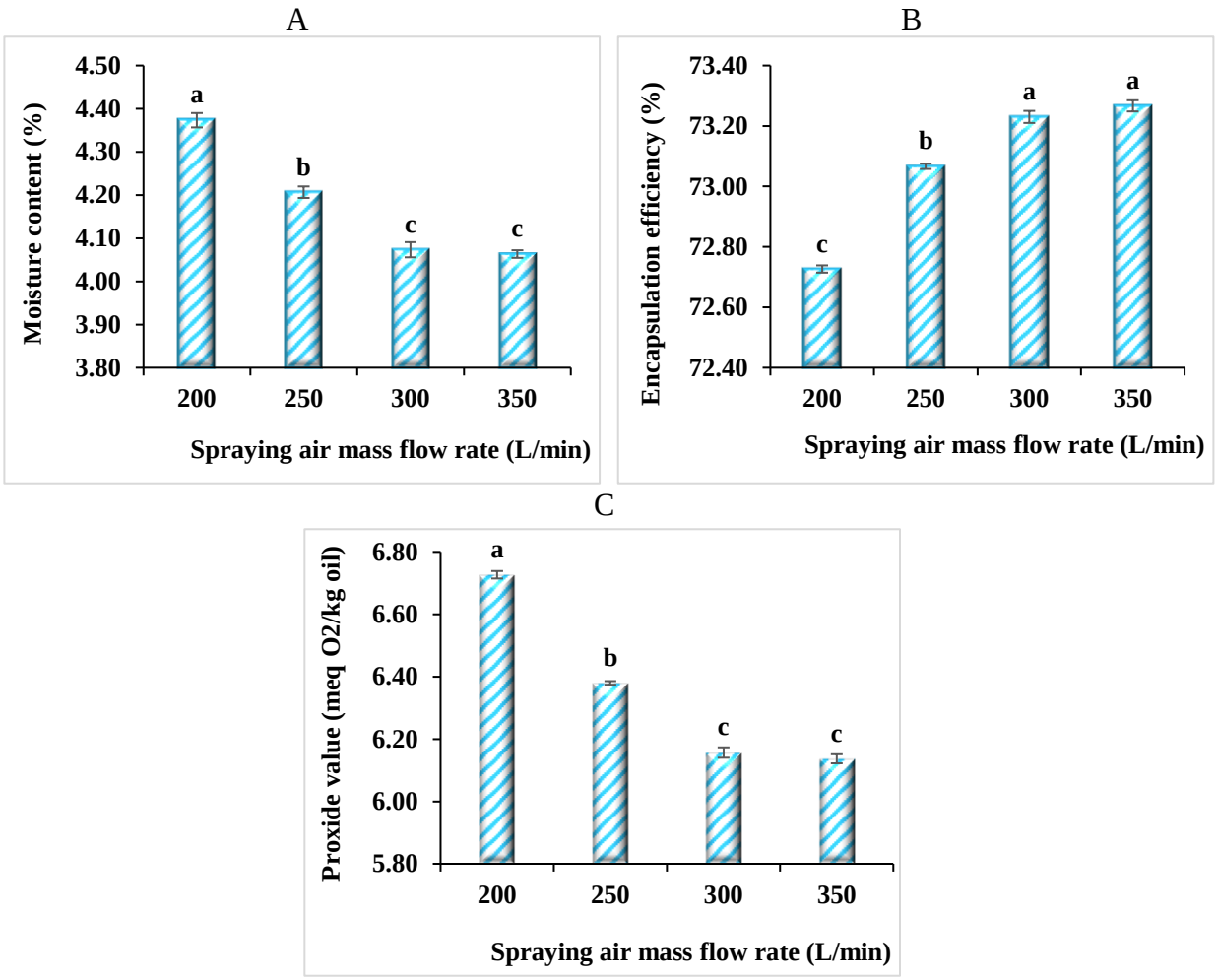


Figure 3-3: The effect of spraying air mass flow rate on A) moisture content, B) encapsulation efficiency, and C) peroxide value of salmon fish oil microcapsules.

Table 3-5: ANOVA results for feed mass flow rate, inlet-drying air temperature, and air mass flow rate (L/min) effect on the measured dependent responses.

Change sources	Degrees of freedom	Dependent responses		
		Moisture content (%)	Encapsulation efficiency (%)	Peroxide value (meq o_2/kg oil)
Feed mass flow rate (L/min)	2	9.427***	11.032***	12.286***
Inlet-drying air temperature (°C)	2	5.668***	9.091***	24.500***
Air mass flow rate (L/min)	2	0.365***	0.795 ***	3.929***

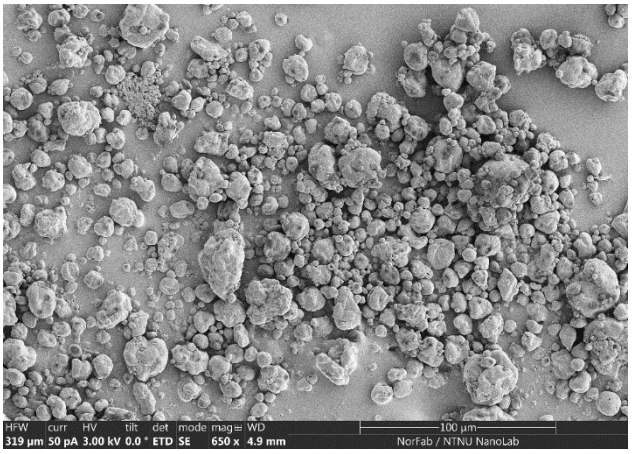
*** $P \leq 0.001$, extremely significant correlation

3.5 Microscopic observation

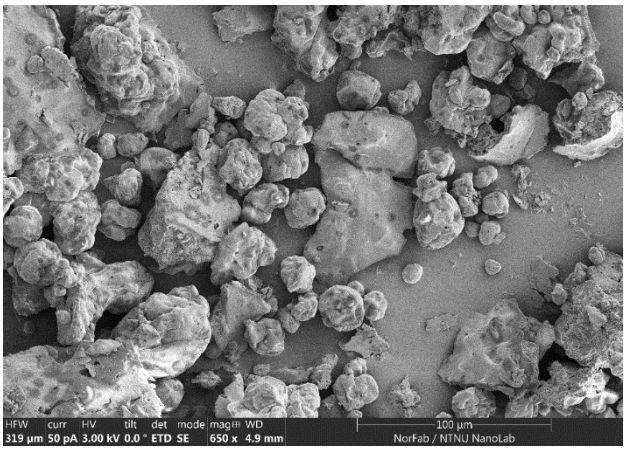
Figure 3-4 A-C displays SEM images of the microcapsules, which were prepared from different treatments (A) the treatment with the highest encapsulation efficiency (feed mass flow rate of 2.26 L/min, inlet drying air temperatures of 180℃, spraying air mass flow rates 350 L/min), B) the treatment with the lowest encapsulation efficiency (feed mass flow rate of 11.2 L/min, inlet drying air temperatures of 160℃, spraying air mass flow rates 300 L/min), and C) the treatment with the highest peroxide value (feed mass flow rate of 2.26 L/min, inlet drying air temperatures of 140℃, spraying air mass flow rates 300 L/min)). As can be seen in Figure 3-4 A and B the treatment with the highest encapsulation efficiency (Figure 3-4 A) had the larger number of particles with rigid smooth surface and lower shrinkage compared to the treatment with the lowest encapsulation efficiency (Figure 3-4 B) which had discontinuous and broken walls. Kurek et al. (2020) found that the application of proteins as wall material generally could lead to more extensive variations and non-uniformity in the morphology of particles (Kurek and Pratap-Singh 2020). Figure 3-4 C illustrated that the particles of the treatment with the highest peroxide value were very agglomerated.

Agglomeration has been reported to be partly related to the presence of a high level of surface free fat (Bae and Lee 2008) which is shows that the capsules can't retention the core.

A



B



C

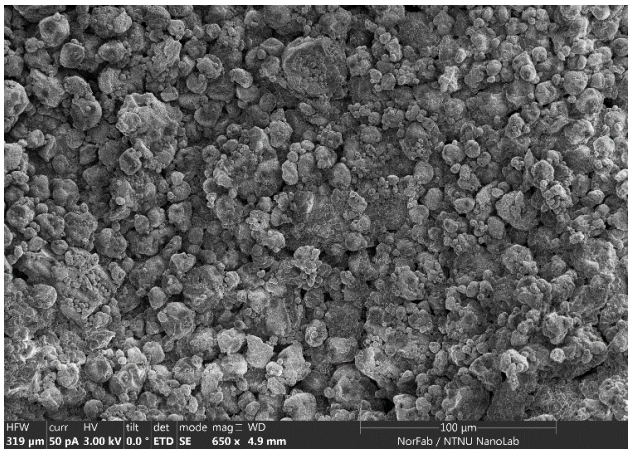


Figure 3-4: Scanning electron microscopy picture of A) the treatment with the highest encapsulation efficiency (feed mass flow rate of 2.26 L/min, inlet drying air temperatures of 180℃, spraying air mass flow rates 350 L/min), B) the treatment with the lowest encapsulation efficiency (feed mass flow rate of 11.2 L/min, inlet drying air temperatures of 160℃, spraying air mass flow rates 300 L/min), and C) the treatment with the highest peroxide value (feed mass flow rate of 2.26 L/min, inlet drying air temperatures of 140℃, spraying air mass flow rates 300 L/min).

4 Conclusion

In this study, the combination of the maltodextrin and rice protein were applied as the wall for microencapsulation of salmon oil via spray drying. The effects of feed mass flow rate (peristaltic pump rate) (in three levels of 2.26, 7.04 and 11.2 L/min), inlet drying air temperatures (in five levels of 100, 120, 140, 160 and 180℃), and spraying air mass flow rates (in four levels of 200, 250, 300 and 350 L/min) on microencapsulation properties of fish oil including moisture content, particle size, bulk density, encapsulation efficiency and peroxide were investigated. The findings demonstrated that The moisture content of microencapsules varied from 4.03 ± 0.01 % to 4.57 ± 0.01 %. This parameter was significantly increased by increasing the feed mass flow rate ($P \leq 0.001$). However, it was decreased significantly ($P \leq 0.001$) with by increasing the inlet drying air temperatures and spraying air mass flow rate.

The encapsulation efficiency of microencapsules was varied from 56.41 ± 0.02 to 73.22 ± 0.02 %. This parameter was significantly increased by increasing the inlet drying air temperatures and spraying air mass flow rate ($P \leq 0.001$). However, it was decreased significantly ($P \leq 0.001$) with by increasing the feed mass flow rate.

The peroxide value of microencapsules varied from 6.09 ± 0.01 meq O_2/kg oil to 7.11 ± 0.01 meq O_2/kg oil. This parameter was significantly increased by increasing the feed mass flow rate and. However, it was decreased significantly ($P \leq 0.001$) with by increasing spraying air mass flow rate the. It should be noted that, the highest peroxide value of microencapsules was achived at the inlet drying air temperatures of 140 °C.

The findings suggest a potential application of combination of maltodextrin with rice protein proteins to generate microparticles containing fish oil using spray drying method.

5 Future work

A lot of different aspects revealed in this study would be interesting to investigate further. According to different spray dryer condition it would be interesting to also investigate the effect of spray dryer operational condition on encapsulation properties of fish oil, additional studies are awaited to assess the influence of different types and diameters of atomising nozzle, drying media, chamber designs, air-droplet contact systems, powder and air discharge systems, and control systems on the encapsulation properties of fish oil. Also, some other properties of microcapsules such as particle size and morphology of microcapsules, porosity, surface hydrophobicity, flow properties, micromechanical properties, thermal properties, solubility, heat and light stability surface tension, controlled release of the core material can be studied. Also, some parameters such as higher concentrations of omega-3 to be used in food or nutraceuticals, examine the lipid oxidation in the whole process, and how a “rizecoated” oil will act in different food systems can be investigated.

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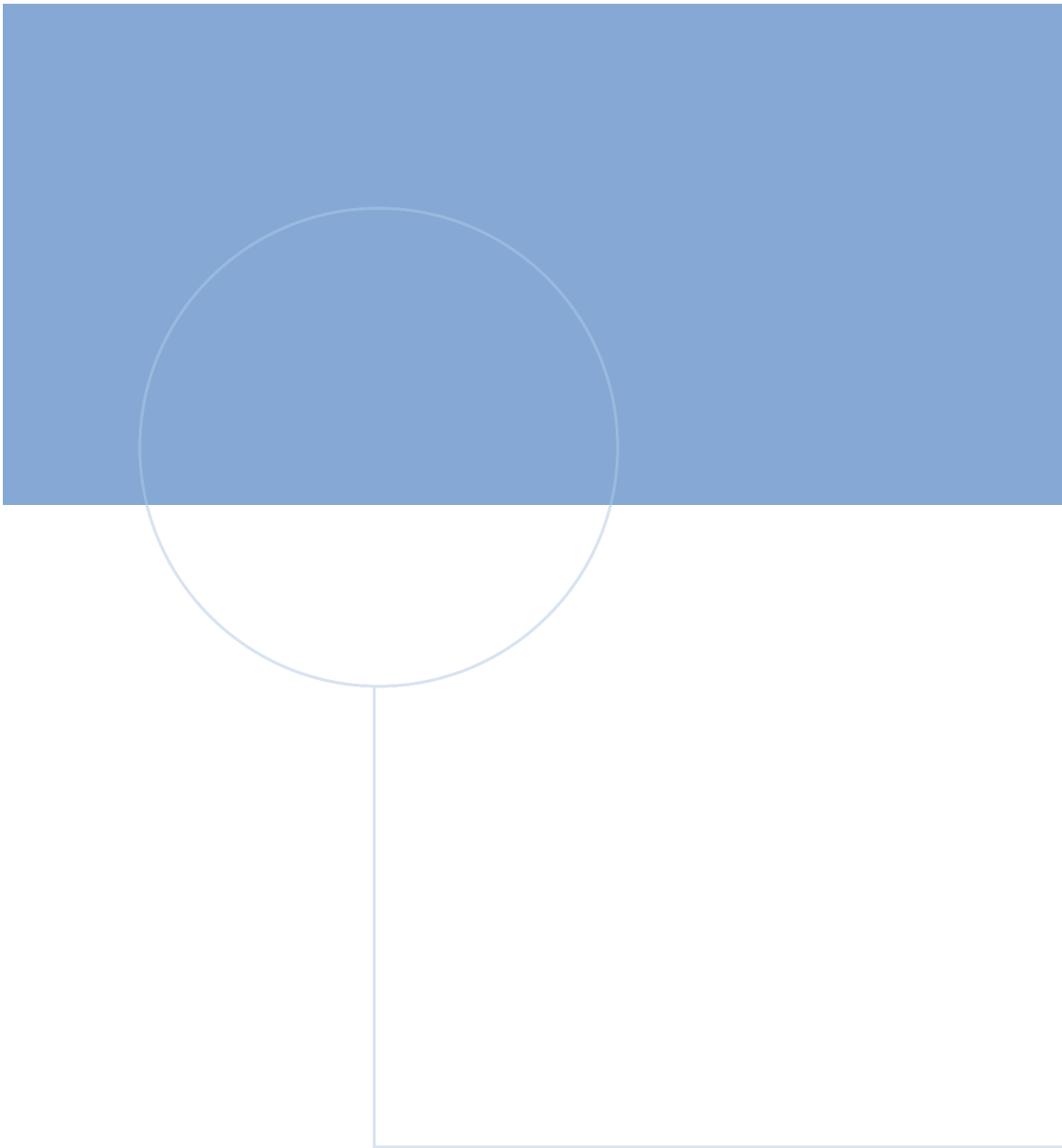

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