# Vortex Fluidic assisted encapsulation of flaxseed oil using alginate and strawberry.

By

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# TABLE OF CONTENTS

# ABSTRACT

The present study investigates the process of encapsulating flaxseed oil through the utilisation of a Vortex Fluidic Device (VFD), alginate, and strawberry extract. The process of emulsion preparation consisted of the combination of 8ml of alginate, 1ml of flaxseed oil, and 1ml of Tween 80, utilising a VFD operation configured in a northward orientation and a clockwise motion. This procedure yielded emulsions characterised by remarkably diminutive nanoparticles, measuring approximately 10nm in diameter. In order to generate a novel emulsion, the process involved the utilisation of a systematic approach, as well as the manipulation of encapsulating materials and VFD parameters. The data were divided into two treatment groups: one group received treatment exclusively with the VFD, while the other group was exposed to both the VFD and a sonication bath. A statistical analysis, specifically an analysis of variance (ANOVA), was performed to compare the two treatment groups, indicating that there was no statistically significant difference observed between them. In conclusion, this study highlights the effectiveness of the VFD in producing nanoparticles of small size and maintaining their stability during the process of encapsulation. As a result, valuable insights into the operational mechanism of the VFD are gained.

# DECLARATION

I certify that this thesis:

1. does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university

2. and the research within will not be submitted for any other future degree or diploma without the permission of Flinders University; and

3. to the best of my knowledge and belief, does not contain any material previously published or written by another person except where due reference is made in the text.

Signed.....Mgbeodichinma Veronica Ogbodo.....

Date.....November, 2023.....

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This thesis signifies the completion of my scholarly endeavors, and I am enthusiastic about the prospects and obstacles that await me in the future.

# ABBREVIATIONS

| | |
|---|---|
| Sod. alginate | Sodium alginate |
| Min | Minute |
| Alg | Alginate |
| VFD | Vortex fluidic device |
| EE | Encapsulation efficiency |
| STDEV | Standard deviation |
| ESI | Encapsulation stability index |
| ml | Milliliter |
| mM | Millimolar |
| nm | Nanometer |
| rpm | Revolutions per minute |
| % | Percent |
| $CaCl_2$ | Calcium Chloride |

# LIST OF FIGURES



# LIST OF TABLES

# 1 LITERATURE REVIEW

## 1.1 Introduction

### 1.1.1 The current market for plant-based oils.

Reducing the cost, and improving the sustainability and access to essential fatty acids is a key target for researchers (Gutjar et al. 2015). The plant based market is poised to grow from 1.6 billion USD in 2019 to 3.5 billion USD by 2026 (Alcorta et al. 2021). The global vegetable oil crisis is steadily on the rise as it is a great plant-based alternative for those suffer from animal-based allergies, or who have ethical and religious considerations. Unfortunately, the cost and spoilage of vegetable oils is also increasing, and efforts needs to be made to improve the shelf life and retain bioactive capacity before they reach consumers. This has led to the shortage in supply resulting in unsustainable prices and becoming inaccessible/unaffordable for the global economy, particularly as the cost of living has also rises (Gutjar et al. 2015).

Gradually, populations are moving towards a more bioavailable and sustainable substitute due to the unreliability of animal-based oils and their consistently high prices (Bell et al. 2001; Caballero et al. 2002). Fish oil has also become un unreliable source of omega 3's as the global supply and demand is not sustainable (Piedecausa et al. 2007). Plant-based oil has now been sought after as a healthier and more dependable alternative. Due to the high demand for fatty acids derived from vegetable oils, these oils are economically exported to many parts of the world. In 2015, Indonesia exported 29.2 million tonnes of oil to the global market. Also, 19 tonnes of vegetable oils were exported by Malaysia. Unfortunately, these nations—along with other oil-producing nations—cannot keep up with the growing demand for vegetable oils (Mielke 2018). Thus, the need for researchers to develop products to help sustain and improve the availability of these vegetable oils.

### 1.1.2 Benefits of flaxseed oils.

Flaxseed oil is a type of vegetable oil that is derived from the seeds of the flax plant, scientifically known as *Linum usitatissimum* (Rubilar et al. 2010). It is a rich source of alpha-linolenic acid

(ALA), an essential omega-3 fatty acid, that is linked to various health benefits, such as reducing inflammation disorders, improving heart health, and aiding in brain function (Rubilar et al. 2010; Xu et al. 2012). It also contains a variety of other fatty acids, omega 6 and 9, an advantage over fish oil supplements that are very narrow in their nutritional value, only containing omega 3's.

### 1.1.3 Chemical composition and Nutritional Value of flaxseed oil

Flaxseed oil is a rich source of various bioactive compounds, including omega fatty acids, lignans, and phenolic acids (Martinchik et al. 2012) . The major vitamins of flaxseed are vitamins A, C, and E, whereas the major minerals are phosphorous, magnesium, potassium, sodium, iron, copper, manganese, and zinc (Akande et al. 2010). Flaxseed oil contains 98% triacylglycerol and phospholipid and small amounts of free fatty acids (0.1%) (Mueller et al. 2010). They are one of the richest sources of plant based omega 3's, AHA, DHA, and EPA (Gebauer et al. 2006). They are also low in saturated fatty acids (9%), monosaturated fatty acids (18%) and are high in polyunsaturated fatty acids (73% - omega 3, 6 and 9) (Goyal et al. 2014).

Recently, AHA (alphalinolenic acid) which is derived from oilseeds have been used to enhance the productive capabilities in n-3 PUFA profiles and its sensory properties for lamb as researchers believes that the inclusion of ALA rich diet can help to improve the overall gut health of the lamb without any negative effects(Don et al. 2018), hence the need to incorporate a plant based oil like flaxseed oil into our diets.

The nutritional value of flaxseed oil is significant and has captivated the attention of researcher's all-over the world (Rodriguez-Leyva et al. 2013). A 1 tablespoon (15 ml) serving of flaxseed oil provides approximately 120 calories, 13.6 grams of fat, 1.3 grams of saturated fat, 8.9 grams of monounsaturated fat, and 3 grams of polyunsaturated fat, including 2.5 grams of ALA (Rodriguez-Leyva et al. 2013). Researchers are raising awareness of this extremely nutrient-dense food. As there is a growing demand for fatty acids, researchers need to develop a way to preserve, and

increase its bioavailability. One of the ways to improve the stability of ALA (alpha linoleic acid), is to encapsulate the oil.

The process of encasing or trapping a substance inside a material that prevents oxidation, enhances its stability, and regulates its release is known as encapsulation (Choi & McClements 2020). Essential oils that have undergone oxidation can release disagreeable odours and tastes that consumers find intolerable (Choi & McClements 2020). To mitigate these effects, a range of materials, such as polymers, lipids, and proteins, which have numerous applications in fields like tissue engineering and bio-catalysis, can be used to encapsulate these oils (McClements 2018). By introducing encapsulation technique, flaxseed oil's oxidative impact will be reduced. Fish oil, for example, can be kept tasting and smelling fresh by encapsulating it; flaxseed oil can also be kept stable and of high quality by doing the same (McClements 2018). This encapsulation is especially important because it not only addresses taste and odour concerns, but also acts as a barrier against oxidation. Oxidation must be prevented in order to maintain the structural integrity and health benefits of flaxseed oil (David Julian 2018). Examples of foods that have been encapsulated to help improve the quality and the longevity of foods include encapsulated flavours in beverages. These flavours are often added to beverages to provide a burst of flavour upon consumption. Microencapsulation is a common technique for achieving this (Luiza Siede & Caciano Pelayo Zapata 2016). Another example is the fish oil capsules is encapsulated to prevent oxidation and mask its strong taste. Gelatin capsules are a common choice for encapsulating fish oil (Kolanowski et al. 2006). Other examples includes vitamin, mineral supplements and chocolate-filled confections. These are encapsulated frequently to help protect vitamins and minerals in dietary supplements, ensuring their stability and controlled release in the body (Norton et al. 2015) and to create a burst of flavour when eaten. These capsules are typically made using techniques like coacervation (Sousa et al. 2022).

### 1.1.4 Materials for encapsulation of flaxseed oil.

During encapsulation the most common materials used to form a protective layer are the walling agents (Selim et al. 2021). These agents protect the nutrients to help prevent the nutrient from spoilage (Selim et al. 2021). Walling agents include edible films and coating, gelatin, cellulose derivatives, lipids , waxes, resins , polymers, pectin and sodium alginate (Selim et al. 2021).

These walling agents can necessitate the formulation of capsules of various sizes and usage in the nutraceuticals and pharmaceuticals especially in food and dosage formulations (Delphine et al. 2008). To this aim, researchers like Choi and McClement (2020), delved into the bioavailability of encapsulated flaxseed oil, revealing significantly heightened absorption and bioavailability when compared to unencapsulated flaxseed oil. This work underscored the potential of nano emulsion-based delivery systems as a means to enhance the bioavailability of flaxseed oil (Choi & McClements 2020). Further research by Stephen et al. (2020) produced unique microcapsules through the formation of a double emulsion of water in oil (w/o). This finding highlights the importance of walling agents in an encapsulating process.

### 1.1.5 Encapsulation using Alginate.

Alginate, a biocompatible, non-toxic polysaccharide extracted from brown algae, is widely used in the food and pharmaceutical industries (Abasalizadeh et al. 2020). Alginate protects the core material from heat, humidity, and acidity by forming a gel matrix around it, thus permitting interactions with microcapsules. As a result of the divalent cations, particularly $Ca^{2+}$ a three-dimensional gel matrix is formed and this matrix cross-links alginate polymer chains (Wang et al. 2022). Research carried out by Helena et. al (2013) has shown that sodium alginate can aid encapsulation, and with the addition of a surfactants, encapsulation became better. Nutritional supplements such as, pharmaceuticals, probiotics, and enzymes are encapsulated in alginate-based microcapsules.

### 1.1.6 Encapsulation using antioxidants.

Natural polyphenols are secondary metabolites, which act as defence system against environmental stresses, such as UV radiation and oxidative damage, and can also play a role in plant-microbe interactions (Pandey & Rizvi 2009). These group of natural compounds are widely found in fruits and vegetables, including strawberries. They have been reported to possess various biological activities, including antioxidant, anti-inflammatory, and anti-cancer properties (Quiñones, Miguel & Aleixandre 2013). Sources of polyphenols include fruits, vegetables, nuts, and seeds (Pandey & Rizvi 2009) .Polyphenols from fruits (strawberry) in encapsulation systems can further enhance the stability and antioxidant properties of the encapsulated material (Pulicharla et al. 2016).

Polyphenols can also act as a cross-linker for alginate, improving the mechanical properties of the resulting beads (Paidari et al. 2021), also improving the release of the active ingredient from the beads by reducing the cross-linking density of the alginate matrix and improving the retention of the active ingredient within the beads, thus reducing its loss during processing and storage (Hanuka-Katz et al. 2022). Plants like strawberry have been reported to enhance the stability of encapsulated active ingredients such as oils, by forming a complex with the alginate matrix (Hanuka-Katz et al. 2022).

A research group tried to encapsulate curcumin with green tea polyphenols, which was used to co-precipitate curcumin (Yan et al. 2019). The nanoparticles were characterised using dynamic light scattering, transmission electron microscopy, and Fourier transform infrared spectroscopy. Encapsulation efficiency was 90%, and the nanoparticles released curcumin for 48 hours (Yan et al. 2019). These studies demonstrate polyphenols' potential to revolutionise encapsulation for ingredient protection and release.

### 1.1.7 Technologies that facilitate encapsulation.

Encapsulation in biotechnology can be achieved through a variety of methods, that involve emulsion-based techniques, electrostatic interactions, and physical entrapment within gels or microspheres. The choice of encapsulation method will depend on the specific biological material

being encapsulated, as well as the intended application and desired properties of the final product (Kandilogiannakis et al. 2021).

Various technologies have been used in the past to encapsulate oils. Emulsification involves the stable production of oil-in-water (o/w) or water-in-oil )w/o) emulsions with the aid of devices like the homogenizer (Garti & McClements 2012). Another technique is spray drying, which involves the effective conversion of o/w emulsions into dry, powdered forms with the aid of a spray dryer (Bhavesh et al. 2015). However, the use of high temperature processes in this type of technology can lead to thermal degradation for heat sensitive compounds (Bhavesh et al. 2015). Other technologies include coacervation, extrusion, and solvent evaporation,These also involve the careful and controlled release of encapsulated materials in small and large scales. However these technologies emit much carbon during processing, as the process requires high temperatures during encapsulation, which is not suitable for the materials that are temperature and pH sensitive and to the environment large (Garti & McClements 2012).

To this aim, a new device that generates high-speed vortex flow within a tubular chamber, speeds up mixing and mass transfer, accelerating chemical reactions and improving material processing and controlled nanostructure synthesis without disrupting the encapsulated material's pH and temperature, has been employed. This device is known as the vortex fluidic device (Yasmin et al. 2013).

### 1.1.8 Vortex fluidic Device.

The vortex fluidic device (VFD) is a type of high-shear mechanical mixer that has been used in various applications, such as chemical synthesis, nanoparticle production, and biomaterials processing (Britton, Smith, et al. 2017). The VFD consists of a cylindrical chamber with a narrow gap between two cones that create a vortex flow when rotated at high speeds (Das & Giri 2016). This vortex flow generates high shear forces that can be used to break down and disperse materials, such as polymers, proteins, and nanoparticles (Britton, Smith, et al. 2017).

The VFD has several advantages over other types of mixers, such as its ability to operate at high speeds and shear rates with low energy consumption (Das & Giri 2016). These advantages make the VFD a promising tool for various industrial applications, such as drug delivery, food processing, and energy production (Britton, Smith, et al. 2017).

In recent years, the VFD has been used for encapsulating bioactive compounds, such as oils and proteins, using natural polymers, such as alginate (Singha, Pandit & Maity 2021). The high shear forces generated by the VFD can help to disperse the bioactive compounds, which can then be encapsulated in the alginate matrix to form particles with improved stability and bioavailability (Singha, Pandit & Maity 2021).

Researchers have investigated the use of a vortex fluidic device to fabricate zein nanoparticles for encapsulation of bioactive compounds. The authors demonstrated that the VFD was able to produce zein nanoparticles with a narrow size distribution and high encapsulation efficiency. However, the study did not investigate the long-term stability of the nanoparticles, nor did it address the potential cytotoxicity and immunogenicity of zein-based nanoparticles (Wang et al.,2016).

Another study explored the use of a VFD to produce alginate nanoparticles for oral delivery of insulin. The authors demonstrated that the VFD was able to produce insulin-loaded alginate nanoparticles with high encapsulation efficiency and controlled release properties. The study highlighted the potential of the VFD for the production of oral drug delivery systems (Mohandoss et al. 2023).

Lai et al. (2020) developed a method for encapsulating *Lactobacillus reuteri* DSM 17938 using a vortex fluidic device with alginate and carrageenan to achieve gastro-resistance and controlled release. The study aimed to investigate the effectiveness of this encapsulation method in protecting the probiotic bacteria from the harsh conditions of the gastrointestinal tract and to assess its potential for controlled release in the small intestine by using a combination of experimental methods, including a vortex fluidic device to encapsulate the probiotic bacteria in alginate and

carrageenan, followed by in vitro testing of the encapsulated bacteria's resistance to acidic conditions and the effectiveness of the controlled release mechanism in simulated intestinal fluid. The results of the study demonstrated that the encapsulated *L. reuteri* DSM 17938 exhibited significantly higher survival rates in acidic conditions compared to free bacteria. Additionally, the encapsulation method provided controlled release of the bacteria in simulated intestinal fluid, indicating its potential use as a delivery system for probiotic bacteria.

Furthermore, another research aimed to explore the use of a VFD for chemical transformations (Britton, Stubbs, et al. 2017). The objective was to investigate the potential of the VFD to improve reaction rates, selectivity, and efficiency compared to conventional methods. The study used a combination of experimental and computational methods to evaluate the performance of the VFD for various chemical reactions, including peptide bond formation, esterification, and Diels-Alder reactions. The results of the study demonstrated that the VFD can significantly improve reaction rates and selectivity for various chemical reactions. Additionally, the study identified the key factors that influence the performance of the VFD, such as the operating conditions and the type of reactants used (Britton, Stubbs, et al. 2017).

In conclusion, the chronological analysis of these research papers demonstrates the potential of the vortex fluidic device as an efficient tool for the encapsulation of bioactive compounds by providing stability, efficiency, and better morphology to the encapsulated capsules. Therefore, this research seeks to explore the use of the vortex fluidic device in the encapsulation of flaxseed oil using alginate and strawberry polyphenols.

### 1.1.9 Significance of the study

This project aims to encapsulate flaxseed oil with strawberry polyphenols using a vortex fluidic device, enhancing its stability and bioavailability, making it a viable alternative for individuals allergic to animal-based products. The encapsulation process ensures the nutritional value and antioxidant properties of the final product, promoting overall well-being. This eco-friendly

approach not only addresses the needs of individuals with allergies but also contributes to sustainable and eco-friendly alternatives on the market. The vortex fluidic device can be used on both small and large scales, ensuring that plant-based products retain their nutritional value and taste. The device allows for the creation of novel formulations by manipulating materials and VFD parameters, leading to constant growth and development in various industries. For example, in the pharmaceutical industry, it can enable the creation of more effective drug delivery systems that enhance the bioavailability of medications. In the research and development sector, it can facilitate the production of novel compounds with improved stability, leading to breakthrough discoveries and advancements in biotechnology.

### 1.1.10 Aim and hypothesis

This research seeks to explore the use of the VFD in the encapsulation of flaxseed oil using alginate and strawberry polyphenol.

I hypothesize that the vortex fluidic device will facilitate the development of nanoparticles as evaluated by stability, efficiency, and particle size measurement.

In addition, the strawberry extract will further improve the stability of nano-capsules.

# 2 MATERIALS AND METHODS

## 2.1 Materials

Flaxseed oil containing omega-3, 6 and 9 fatty acids were purchased from Melrose Laboratories Pty Ltd. Sodium alginate powder from brown seaweed (cat# 9005-38-3), calcium chloride, Folin-coicalteu reagent, sodium carbonate, vitamin C, 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ), ferric chloride hexahydrate, ferrous sulfate heptahydrate, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and methanol were purchased from Sigma-Aldrich. Tween 80 surfactant was purchased from N-essentials Pty Ltd. Strawberries were purchased from a local fruit and vegetable supplier in Adelaide, South Australia.

## 2.2 Encapsulation of flaxseed oil

### 2.2.1 Preparation of reagents

Sodium alginate (2%) was dissolved in Milli-Q water using magnetic stirrer at 1000 rpm at 20 °C for 24 h. Calcium chloride (1.5%) was prepared in water. A systematic combination of how materials were used in the vortex fluidic device is outlined in appendix 1.

### 2.2.2 Sonication bath

Sonication bath (Elmasonic S70, In Vitro technologies) was used as the control process for creating nano-emulsions and adapted from He et al. (2019) Briefly, alginate (1 ml) and flaxseed oil (1 ml) were prepared in a 1:1 ratio in a borosilicate tube, and sonicated for 30 min at 30 °C. Samples were taken and nano particles diameter was measured using dynamic light scattering (DLS) and microscopy.

### 2.2.3 Vortex fluidic device (VFD) processing

The protocol used in this encapsulation process was adopted from He et al. 2019, with modifications. The selected combination of materials was added to a borosilicate tube in the VFD. A range of parameters were used to undergo an optimisation process. Tube rotation time was 5, 15

and 30 min at a constant angle of 45 °. Then different rotation speeds were applied, 3000, 5500 and 7500 rpm, followed by optimisation of the orientation of tube relative to the Earth's magnetic field at North and West. This experiment was carried out in a confined mode, at ~20 ℃. The processed product was then collected and left in -20℃ until further analysis. The choice of processing parameters of the VFD was based on consideration of optimal parameters previously established in the Raston research group (He et al. 2020) The issue in relation to the Earth's magnetic field is a new development in using VFD processing, albeit with no publications as such. Nevertheless, the effect is associated with water dissociated under high shear in the VFD and the moving charged components as the fluid flow are impacted by the magnetic field, which alters the fluid flow in a controllable way.

## 2.3 Extraction and validation of strawberry polyphenols

### 2.3.1 Extraction

Fresh strawberries were crushed with mortar and pestle for 30 min. Milli-Q water (45 ml) was added to the crushed strawberries and vortexed. The extract was centrifuged at 10,000 g for 15 min. The supernatant was collected and freeze dried for further analysis.

### 2.3.2 Total phenol assay

In a 96 well plate, 20 ul of strawberry sample or standard and 100 µl of Folin-coicalteu reagent (10%) was add and incubated at 23℃ for 5 min. After incubation, 80 ul of sodium carbonate (7.5%) was added, and then incubated for another 2 h. The absorbance was measured at 725 nm (OMEGA Fluostar plate reader). Results were calculated as gallic acid equivalents per g of extract.

### 2.3.3 Ferric reducing antioxidant power (FRAP)

FRAP reagent was prepared with 25 ml of acetic acid solution (300 mM), 2.5 ml of 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ, 10 mM) in hydrochloric acid (40 mM), and 2.5 ml of ferrous chloride hexahydrate (20 mM) in Milli-Q water. The solution was warmed to 37 ℃ until further use. Strawberry samples or standard (6 ul) were pipetted into a 96 well plate, with the addition of 18 ul

of milli-Q water. Warmed FRAP solution (180 ul) was added to start the reaction. The reaction was incubated for 20 min at 23°C, and absorbance as measured at 593 nm (OMEGA Fluostar plate reader). Results were calculated as mM ferric ion ($Fe^{2+}$) equivalents per gram of extract.

### 2.3.4 DPPH free radical assay

To assess the antioxidant capacity to scavenge free radicals, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was used. Sample or standard (100 ul) was added to each well, then DPPH in 100% methanol (10 mM) was added. The reaction was left to incubate for 20 min at 23°C. Absorbance was read at 520 nm (OMEGA Fluostar plate reader). Results were expressed as vitamin C equivalents per gram of extract.

## 2.4 Characterisation and validation of encapsulation methods

### 2.4.1 Microscopy

Samples were observed using a light microscope (p-cartridge) at magnification x 4. Images were taken through the lens.

### 2.4.2 Dynamic light scattering

Dynamic light scattering (DLS) was used to determine the average particle size and assess the stability of the emulsion (Malvern Zetasizer nano ZS). Prior to testing, the emulsion was diluted 1:3 with Milli-Q water.

### 2.4.3 Encapsulation efficiency

To assess the encapsulation efficiency (EE) of the VFD processed emulsion with strawberry extract, the method as follows (Anna et al. 2018) was used with minor modifications. After the emulsion process with added strawberry extract, phase separation was allowed to occur after 24 h. Calculations were based on the total phenolic content (TPC) to assist quantifying small amounts of processed emulsions. Thus, the difference between the initial concentration of strawberry extract used (W1) and non-encapsulated strawberry concentration (W2) was used to calculate the concentration of strawberry extract in the encapsulated oil (W3). The

percentage of encapsulated oil and strawberry extract with respect to its initial amount was expressed as encapsulation efficiency (EE%). (Huang et al., 2001; Soliman et al., 2013).

$$W3 = W1 - W2$$

$$EE\% = \frac{W3}{W1} \times 100\%$$

### 2.4.4 Encapsulation stability

Encapsulation stability index (ESI) was calculated to understand the stability of the emulsion with strawberry extract. The method from Iris and Strain (1996) was used. After phase separation, the water layer served as a proxy for the unencapsulated material to calculate an encapsulation stability index. This index was determined by deducting the initial total phenolic content ($TPCi$) of the processed samples from the final total phenolic content ($TPCf$) of the processed samples. The fraction was multiplied by 100%.

$$ESI = 100 - \left(\frac{TPCi - TPCf}{TPCi}\right) \times 100\%$$

## 2.5 Statistical analysis

Experiments were performed in triplicates. One-way ANOVA test was used to validate for significant differences of particle size between control process (sonication) and the VFD processed emulsions. It was also used to identify any significant differences in encapsulation efficiency of total phenols from strawberry extract.

# 3 RESULTS AND DISCUSSION

## 3.1 Optimisation of materials for encapsulation

### 3.1.1 Encapsulation of flaxseed oil in a matrix of sodium alginate.

Flaxseed oil is a very rich source of omega 3 including ALA, DHA and EPA, and omega 6 and 9. These oils contribute significantly to improving human cardiovascular and anti-inflammatory diseases. However, shelf life of fatty acids, particularly APA, is minimal as it is highly susceptible to oxidation (Abbasi et al. 2019). Finding suitable materials to encapsulate the oil helps to reduce oxidation. Seaweed alginate can act as a sustainable and natural plant based stabiliser, and has been successfully used as a gelation material and in encapsulation processing (Abbasi et al. 2019), but combining seaweed alginate with an oil requires additional processing, like mechanical energy, to improve the emulsion (ref). The vortex fluidic device is a green processing tool that can act as a facilitator in enhancing reactions and thus, encapsulate oil without the need of added chemicals and energy input.

Sodium alginate, flaxseed oil and water (1:1:1) were first selected in a preliminary step to assess the efficacy of the VFD for facilitating the encapsulation process compared to conventional mechanical shaking of the sample. Microscopy images showed that a stable emulsion did not form before and after the VFD. However, after VFD processing there is a much more uniform emulsion than the control, suggesting that the VFD has some influence over the final product (figure 3.1a and 3.1b).

Sodium alginate is a hydrophilic polysaccharide, while flaxseed oil contains a mixture of hydrophobic fatty acids, so almost immediately after processing phase separation occurred. Sodium alginate is an ionotropic material that can react with oppositely charged molecules and commonly with calcium ions ($Ca^{2+}$). This combination has been used for improving encapsulation of oils. Linseed oil was encapsulated with a calcium-alginate mix improved the nano-emulsion and oxidation, while also delaying gastrointestinal release of APA, DHA and EPA (Rahiminezhad et al. 2020).

Calcium chloride in the VFD has not yet been used in the VFD for encapsulation of flaxseed oil with alginate. The addition of cations would improve the gelation of sodium alginate and therefore increasing the stability of the emulsion post VFD processing. The VFD would also facilitate the particle size and uniformity.

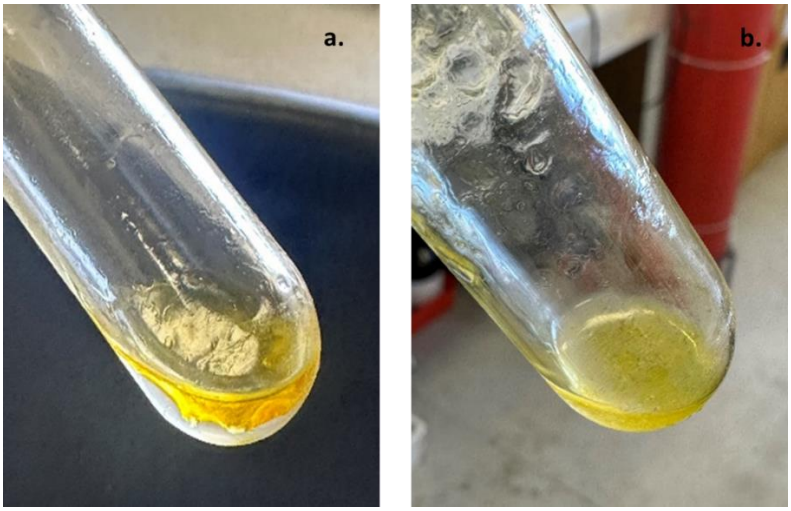



Figure 3.1 Before VFD processing (1a) and after VFD processing (1b) of sodium alginate, flaxseed oil and water (1:1:1) showing that the VFD does assist in the emulsion process. VFD parameters (Speed: 7500 rpm, time: 30 minutes, tilt angle: 45 degrees, direction of flow: clockwise, position of the VFD: North, mode of operation: confined mode, temperature: 23˚C)

### 3.1.2 Encapsulation of flaxseed oil using sodium alginate and calcium chloride.

Encapsulation of a crosslinking agent, like calcium chloride, has been reported to enhance the gelation of alginate and thus, the stability of the micro and nano-capsules. Calcium chloride (0.5%) was added to the emulsion using the VFD combined with a jet feed with syringe and needle that deposits droplets into the rotating mixture of sodium alginate, water, and flaxseed oil (1:1:1). The mixture was rotated at 7500 rpm for 30 min. Samples were compared between conventional mixing (control) and post-VFD processing using light microscopy and particle diameter (figure 3.2).

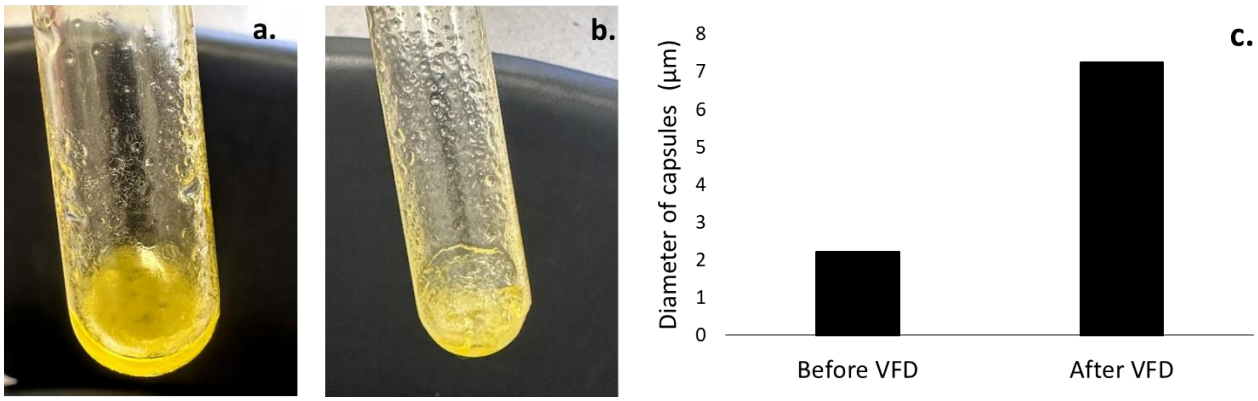



Figure 3.2 Images of control (a) and post-VFD processing (b). Dynamic light scattering of samples showed that calcium chloride increased the size of the crosslinked material, amplified by the VFD (c).

Calcium ions ($Ca^{2+}$) clearly act as a strong crosslinking agent. Homogenous material in the VFD under high speed (7500 rpm) undergoes intense micro-mixing due the figure-of-eight fluid dynamics. However, when there is an a significant in-balance of density in the materials, then the rotation can act as a centrifugal force, pulling the dense materials to the side and this is consistent for the thin film of alginate forming in the VFD after the addition of calcium chloride (Chuah et al. 2023) (figure 3.2b). The control did not show the same alginate film (figure 3.2a), but this would be expected as there is calcium chloride is being added to an uncontrolled environment (e.g. no rotation), therefore microcapsules form instantly. The DLS results support these observations clearly comparing microcapsules (2.2 µm) and a film (7 µm). Choi and McClements (2020) also address the encapsulation of flaxseed oil and the challenges faced using calcium chloride in hydrophilic components, therefore calcium chloride should not be added to the alginate and flaxseed oil mixture, during VFD processing, to improve the stability of the emulsion.

These findings collectively emphasize the importance of considering the specific properties of the core material and the encapsulation process parameters. While calcium chloride may have its uses in certain encapsulation contexts, alternative materials for nano-emulsion stability, like surfactants, should be investigated.

### 3.1.3 Addition of surfactant improves the emulsion size and stability.

Surfactants act as emulsifiers and exist as non-ionic or ionic. Non-ionic surfactants, like tween 80, are more suitable for food applications in that they are less of an irritant, less toxic and do no form the 'soap' effect like ionic surfactants. Like other non-ionic surfactants, tween 80 also has a longer fatty acid chain allowing them to find with oils more effectively (figure 3.3).


Figure 3.3. Tween 80 surfactant structure showing the long fatty acid chain and hydrophilic head (https://www.guidechem.com).

Therefore, tween 80 was mixed with the alginate, water, and flaxseed oil mixture to improve the stability and size of capsules in the emulsion. Optimisation of materials was then systematically performed to select the most stable nano-emulsion (appendix 1). The most stable emulsion was selected based on the minimum error displayed in the DLS result over a 15 min time period (table 1), therefore this combination of materials was used for subsequent experiments.

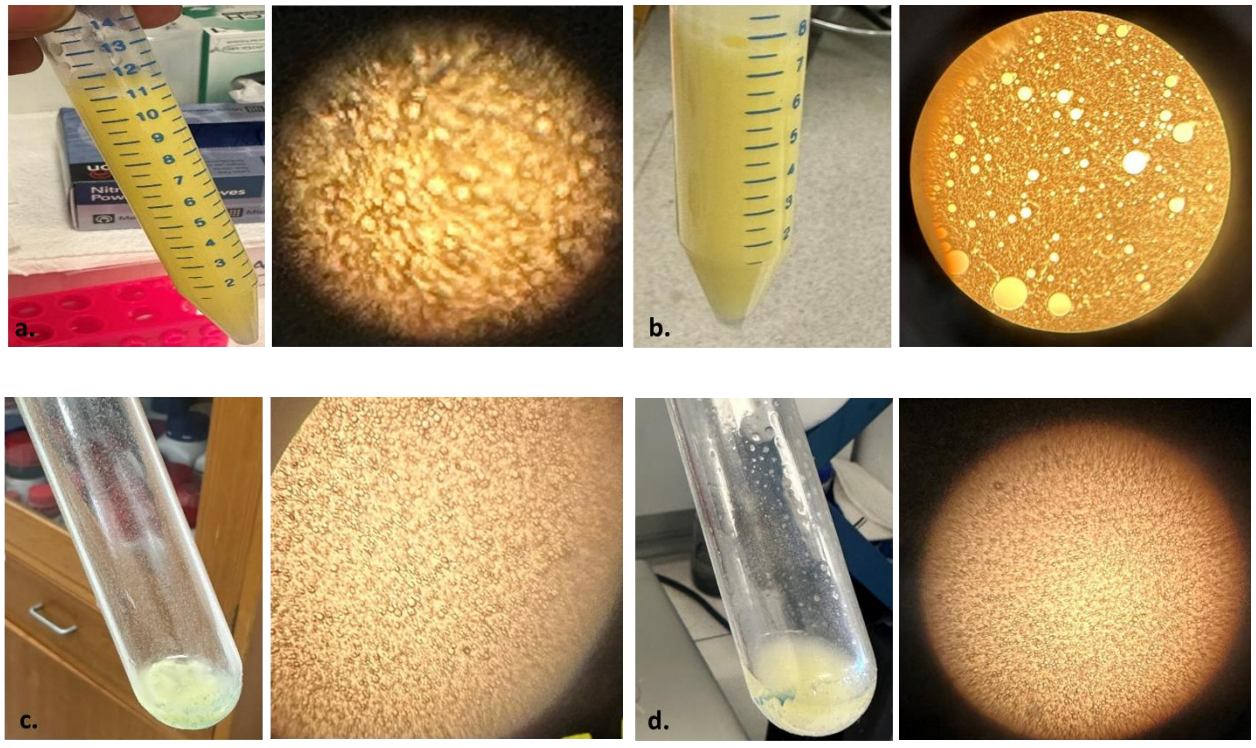
The new mixture of materials was then compared before and after VFD processing, shown in figure 3.4a and 3.4b, respectively. DLS of these results showed that before VFD the emulsion contained capsules that <5 µm and after VFD processing, ~ 20 µm. The elimination of water was then investigated, before and after VFD, and this resulted in a significant reduction in the size of capsules, <2 µm (figure 3.4c and 3.4d). The ANOVA test for the size of particles before VFD treatment (with and without water) should that the p-value between and within the group was 0.7, with an F-value of 0.4. While the result for the size of particle after the VFD treatment (with and without water) showed and F-value of 0.8 and a p-value of 0.4. Also, results for the three treatments

(VFD, sonication bath, and a combination of a sonication bath with the VFD). Result showed that the P-value is 0.4 and the f-value is 1.2.

These results show that tween 80, with the aid of the VFD, form capsules that perhaps absorb more oil due to the strong repellent force with the addition of water in the mix. Figure 4b shows larger oil droplets in the mixture, supporting this theory. Cao et al. (2021) also investigated the use of water in vortex fluidics and similarly found that eliminating water also reduced the particle size and improved stability of capsules. However, vortex fluidics in the VFD is unique and somewhat can be incomparable to the vortex fluidics in the literature, thus optimisation of VFD parameters needs to be investigated.

Table 3.1. Final material selection from a systematic approach to optimisation.

| Alginate (2%) | Flaxseed oil | Water | Tween 80 |
|---|---|---|---|
| 8 ml | 1 ml | 1ml | 1 ml |



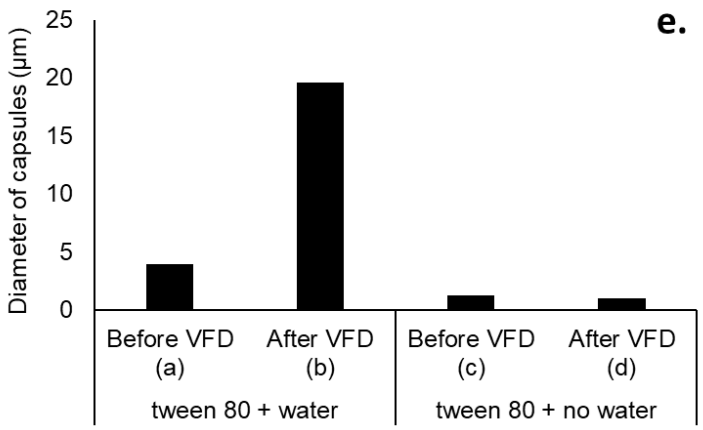



Figure 3.4 (a)Light microscopy images (x4) of the capsules formed in the emulsion before VFD processing (c) Light microscopy images (x4) of capsules formed with the addition of water and surfactant. (b) mixture without water in the mixture before VFD processing. (d)samples without water in the mixture after VFD processing. (e) DLS result showing capsule sizes of mixtures with water and mixtures without water.

## 3.2 Optimisation and evaluation of VFD parameters for encapsulation

The VFD has a number of operating parameters, notably the rotational speed, inclination angle, processing time, and the orientation of the tube relative to the Earth's magnetic field (Appendix 4). The latter discussed noting the effect of the field, is a recent discovery in the Raston research group, and it is a major ongoing project. For this research, the orientation of the tube was only briefly investigated at North and West, and warrants significant investigation in the future to determine the influence on the encapsulation process, but for now, is not primary the scope of this project (Appendix 4). As to the inclination angle, a 45° tilt angle is optimal for a large number of processes in the VFD, and this angle was therefore chosen for the present study. The rotational speed changes the mechanical energy induced into the thin film of liquid, which is in the form of high shear topological flows, namely a typhoon like flow which is a Coriolis force from the base of the tube, and double helical flow associated with induced Faraday waves. The dimensions of these flows, 1 µm in diameter alters with change in speed, and the choice of rotational speeds is representative of the major changes in these flows (Chen et al. 2022).

### 3.2.1 Evaluation of the VFD for facilitating encapsulation process

This section focuses on the study of material organisation and characteristics, with a specific emphasis on the examination of Vortex Fluidic Device (VFD) technology. The effectiveness of

VFD technology is then compared to that of standard homogenization procedures. The work utilised an experimental methodology that involved systematic adjustments of both the enclosing materials and the VFD device. The careful technique was employed in order to exert control on the organisation and qualities of materials, while also guaranteeing the elimination of any potential experimental bias. The utilisation of this particular methodology afforded me the chance to create a wide array of emulsions, hence facilitating a thorough examination of the effects of VFD technology on the stability and particle size of capsules.

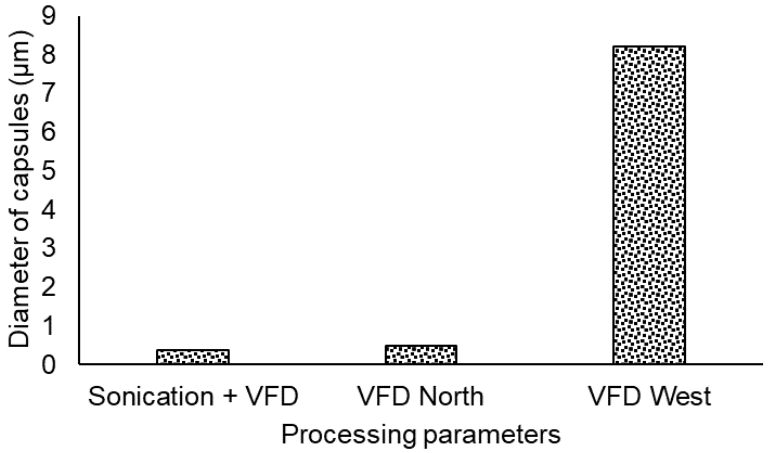



Figure 3.5. Exploratory analysis of the diameter of capsules based on the orientation of the tube relative to the Earth's magnetic field (North and West) compared to the control (sonication and VFD facing North).

The findings of this unique experiment revealed a precise combination of well controlled variables, including 8mls of sodium alginate, 1ml of flaxseed oil, and 1ml of tween 80. These variables were subjected to a clockwise motion direction, with the VFD (Vortex Fluidic Device) set to a northward orientation at a rotational speed of 7500 rpm for a duration of 30 minutes. The emulsions generated under the specified conditions indicated notable stability and exhibited a decreased range of particle sizes. Results show that the capsules in the emulsion had more stability and were more uniform in size averaging 8.2 µm facing west, and 0.49 µm VFD facing the north. Emulsions treated with sonication bath + VFD processing showed good stability and smaller size, averaging 0.36 µm (figure 3.5). These results are consistent with the conclusions drawn by Cao et al. (2021) whose

study focused on the manipulation of properties from tannic acid and gelatine as a means of encapsulating nutrients, using the VFD. The findings revealed that the entangled compounds exhibited improved stability, as seen by the confinement of particle sizes within the range of 1nm to 10nm. In contrast, the compounds created by the homogenization approach resulted in larger particle sizes, around 100nm. My result findings are inconsistent with these findings including the recent work done by Colin's research group investigating the influence of the Earth's magnetic field on particle size and stability during the utilisation of the VFD, where West generates smaller capsules in emulsions (from unpublished works). The results of this study also highlight the significant impact of modest changes in size, such as the inclusion or exclusion of water and the modification in the VFD's directional alignment from north to west.

For industry purposes, the repeatability and consistency of outcomes across many experimental settings are a concern, due to the need to upscale, thus further research is warranted to understand the modification and organisation of materials for encapsulation of flaxseed oil.

## 3.3 Strawberry extract as a functional material in the capsules

Encapsulated oils are often encased with synthetic ingredients to enhance flavouring and aroma and to improve stability of bioactive materials. These materials are usually synthetic, or animal based, therefore shifting to natural, plant-based sources to improve product shelf-life is critical. Flaxseed oil unfortunately oxidises over time reducing the functionality of the oil. Encapsulating oil with an antioxidant would therefore prolong the oxidation reaction. Plant polyphenols are compounds that are often associated with antioxidant activity (Stagos 2019). Currently, vitamin C is one antioxidant that is often combined with nutraceuticals to enhance absorption and retain shelf life of products, but when synthetically made, it does not have a suitable flavour or aroma. Strawberries could be a natural flavouring source for encapsulated material also being high in antioxidants (Balci-Torun & Ozdemir 2021)

In this study, strawberries were extracted, freeze dried and added into the optimised emulsion, containing alginate, flaxseed oil and tween 80. Total polyphenol content, antioxidant activity and scavenging capacity was analysed before and after VFD processing (figure3.6). As expected not all polyphenols were encapsulated in the process (figure 3.6). Interestingly, more strawberry polyphenols were not encapsulated in the process that warranted the most stable emulsions over time (sonication + VFD). Therefore, more strawberry polyphenols were encapsulated just using the VFD.

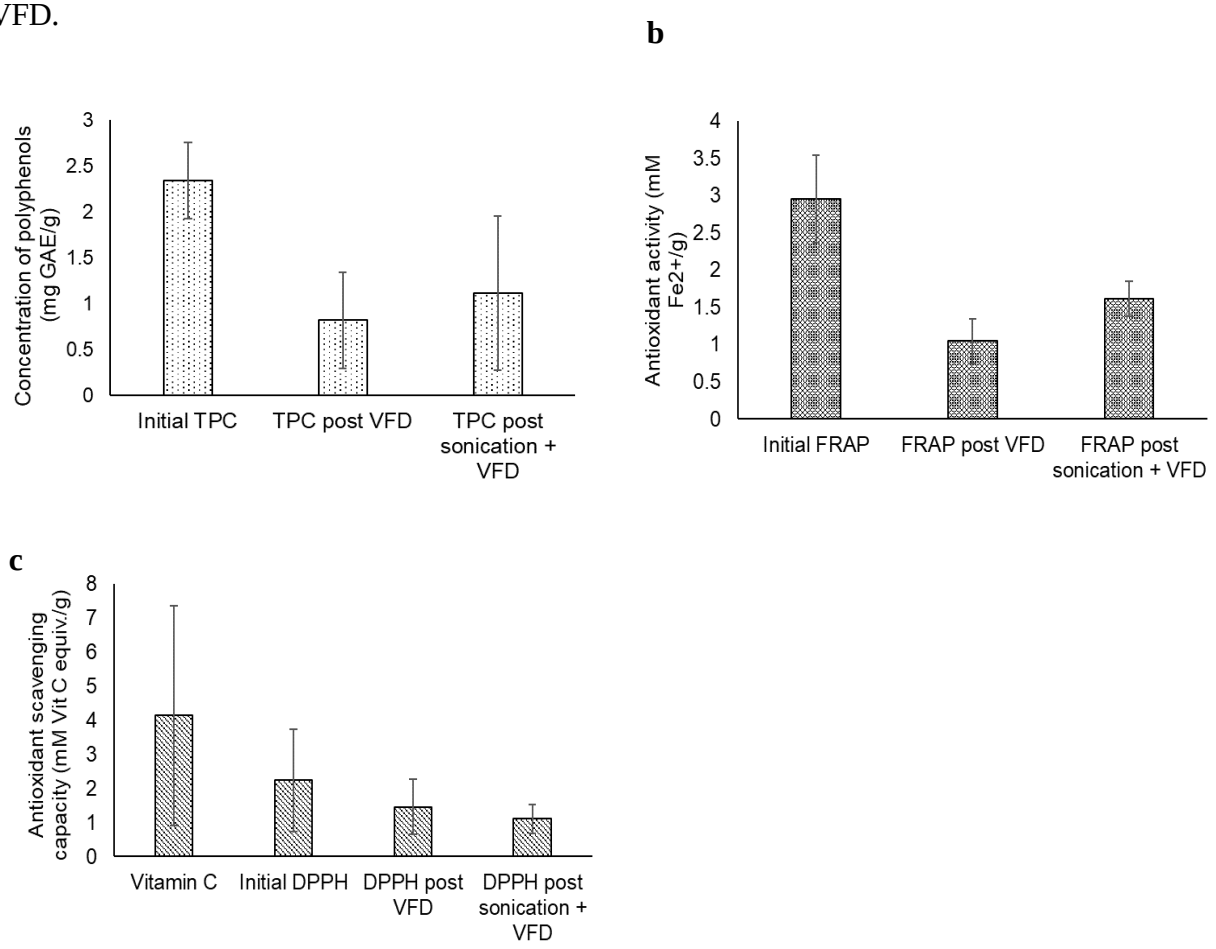



Figure 3.6. Showing the concentration of polyphenols in each treatment (a), the antioxidant activity of treatments (b) and the antioxidant scavenging capacity of treatments (c). Treatments: VFD and sonication + VFD)

Similar trends in results was shown for FRAP, supporting the hypothesis that polyphenols are responsible for antioxidant activity (figure 3.6b). Antioxidant scavenging capacity of the DPPH free radical was also compared. Strawberry polyphenols had less activity in the non-encapsulated emulsion. This does not align with the TPC and FRAP results, an ANOVA was performed to

compare between the types of processing (VFD vs sonication + VFD) and result showed that the P-value and F-statistic were greater than their significance level, 0.36 and 7.71, respectively, indicating no statistical difference resulted between the two groups. The strawberry extract showed moderate antioxidant activity with a mean percentage inhibition of 55.01%, at a mean concentration of 2.23 ±1.5 mM, compared to vitamin C with significant antioxidant activity, obtaining an average percentage inhibition of 81.67%, at an average concentration of 4.1 ±3.23 mM. ANOVA results for total phenolic content, FRAP assay, and DPPH assay were found across all three parameters analysed. The ANOVA test for total phenolic contents yielded a p-value of 0.357, and a f-value of 7.709. Similarly, the ANOVA test for the FRAP assay resulted in a p-value of 0.688, with an f-value of 7.709. the test for the DPPH assay produced a p-value of 0.364, and an f-value of 7.710. The ANOVA test results consistently indicated that there were no significant differences in the measured parameters between samples treated with the Vortex fluidic device only and samples treated with both sonication bath and the vortex fluidic device.

Comparing with the literatures, this results supports that strawberry extract has the capacity to eliminate a considerable amount of DPPH radicals, although to a lesser degree than vitamin C. these results is in agreement with (Karacam, Sahin & Oztop 2015) who also found a higher content of total phenolics in strawberry juice on multiple passes. However, while this result was gotten without the use of a homogeniser, the aforementioned author arrived at their result after the use of a homogenization treatment. These findings not only highlight the significant antioxidant power of vitamin C but also emphasize the potential of strawberry extract as a natural source of antioxidants.

The result of samples with VFD treatment, showed that the total phenolic content in the unencapsulated samples exhibited reduced concentrations. This suggests that most of the polyphenols are in the encapsulated emulsions and a fraction were not trapped in the matrix, leading to the reduced concentration of polyphenols present in my unencapsulated emulsion. On the other hand, emulsion samples treated with the combination method (sonication bath and VFD), showed

an increased concentration of polyphenols in its unencapsulated layer thus leaving a small concentration of polyphenols in its encapsulated sample. This result is consistent with a study by (Hu et al. 2023) who observed that the fresh blackcurrant juice resulted in a slight reduction in total phenolic content after VFD processing and a higher phenolic content with samples treated with ultrasonication was observed. Another researcher (Karacam, Sahin & Oztop 2015) also found a higher content of total phenolics in strawberry juice at 100 MPa and multiple passes after treatment using the homogenization method.

Although our findings are similar, it is worthy of note that our methods of sample preparation, volume of samples, direction, and mode of processing using the VFD and homogenisers/sonication alongside experimental procedures were slightly different. it is important to take these differences in methodology and experimental conditions into account. While interpreting the results and their practical implications,

The ANOVA results of the difference in the total phenolic content and antioxidant activity between the VFD samples and combined treated samples (sonication bath and VFD) was not statistically significant (Appendix 2). This aligns with the findings of (He et al. 2019; Hu et al. 2023) where the ANOVA test showed no significant difference in total phenolic content antioxidant activity and percentage inhibition between, VFD-treated samples, and sonicated samples. Another researcher (Koraqi et al. 2023) found that at an increased sonication time, the amount of polyphenols and antioxidant activity did not show a significant difference in antioxidant activity. These findings are in line with similar research studies that have examined the impact of different processing methods on the phenolic content and total antioxidant activity of fruit extracts (Britton, Stubbs, et al. 2017; Peng et al. 2018), Based on this result, Further research is needed to explore the specific technology, conditions and parameters that may help achieve better incorporation of the phenolic content in strawberry extracts into a matrix during processing.

### 3.3.1 Emulsion stability and efficiency of strawberry-enhanced microcapsules

In this section of this experiment, the expression of the encapsulation of bioactive compounds in the emulsion, containing strawberry extract was investigated, as the presence of this polyphenols will help protects these bioactive compounds from degradation and allows for their controlled release, enhancing their bioavailability and functionality (Peng et al. 2018). To this aim, the characterization of encapsulated flaxseed oil with strawberry polyphenols, with a particular emphasis on assessing encapsulation stability and efficiency was focused on. The encapsulation process involves the use of innovative techniques like VFD and sonication bath (Liu, X et al. 2022). Understanding the stability and efficiency of this encapsulation is vital for optimizing product quality and applications.

### 3.3.2 Encapsulation stability index of samples treated with the vortex fluidic device, and both the sonication bath and Vortex fluidic device.

To determine the concentration of polyphenols that was not encapsulated, the encapsulation stability index was calculated based on the total polyphenol content assay. This measures the concentration of polyphenols in the encapsulated layer comparing it with the total polyphenols in the original strawberry extract. The difference between the polyphenols in the unencapsulated layer and the strawberry extract will help estimate the concentration of polyphenols encapsulated into the oil and water mixture. Result showed that 37% of the phenolic content of the strawberry extract were incorporated into the emulsion after being treated with the VFD. The samples treated with homogenization had approx. 46% of the phenolic content not incorporated into the encapsulated matrix. This suggests that the VFD method is slightly more effective in preserving the phenolic content compared to the homogenization method (figure..) (Liu, G et al. 2019)

### 3.3.3 Encapsulation Efficiency of treated samples

The unencapsulated layer (water layer) was collected and analysed. Finally, the entire volume of each layer was also measured. Results showed that for samples treated with the sonication bath and the VFD had its unencapsulated layer of about 1.8 ml whereas the encapsulated layer measured 3.0

ml. The total volume measured 7.5 ml. while samples treated with the sonication bath only had its unencapsulated layer of about 2.5ml, its encapsulated layer at 1.5 and total volume of 7.5ml.with this result the encapsulated efficiency of the product was calculated using the formular from (Nico et al. 2023) with modifications. Result is summerized in fig 3.7

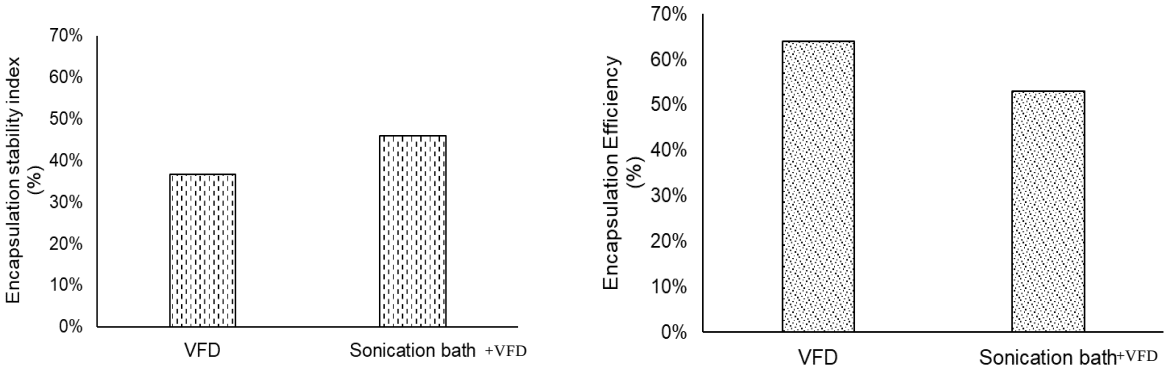



Figure 3.7. Encapsulation Stability (left) and encapsulation efficiency (right).

This means that about 65% of the strawberry polyphenols was successfully and efficiently encapsulated or trapped in the emulsion, compared to the samples treated with sonication bath and VFD, the concentration of polyphenols present in the sample was considerate at about 54%. This result is similar to the findings of (He et al. 2019) whose result showed that samples treated with the vortex fluidic device had the highest emulsion stability and encapsulation efficiency compared to samples treated with homogenization technology. This suggest that the use of the VFD technology could support encapsulation stability and efficiency without the additional sonication step before encapsulation.

## 3.4 Study limitations

While this research offered insightful information on the encapsulation of flaxseed oil using the Vortex fluidic device, this study faced numerous limitations including time. The vortex fluidic device (VFD) was mostly used in confined mode of operation throughout the study, as time did not allow for the alternative use of the continuous flow mode of operation to compare results. A full

analysis of tests designed to characterize emulsions was partially achieved due to time as some of these tests required multiple replications which can take weeks to achieve for each analysis and result. Not being able to test out different crosslinking agents and surfactants and see how they affected flaxseed oil encapsulation also constrained the study.

Additionally, an extended study is suggested to allow encapsulation of not only flaxseed oil but also alginate and strawberry polyphenols, utilizing the capabilities of the VFD to unlock potential applications in various fields of research and industry.

## 3.5 Future Research Directions

Subsequent investigations will involve the exploration of a broader spectrum of cross-linking agents and emulsifying agents, spanning varying concentrations to enhance emulsion stability, featuring diverse concentration levels, to enhance stability. Additionally, the study will extend its inquiry into diverse cardinal positions of the VFD examining eastward and southward positions, along with varying timeframes and operational speeds. This exploration will encompass both confined and continuous modes of VFD operation. Moreover, a rigorous assessment of capsule reliability featuring compression testing and sensory testing will be conducted, as these are vital facets for ensuring the quality, dosage precision, palatability, acceptance, and structural resilience of nutraceuticals and pharmaceutical products.

Furthermore, an expanded investigation is proposed for the encapsulation of flaxseed oil in conjunction with alginate and strawberry polyphenols, leveraging the capabilities of the VFD to unlock potential applications in diverse domains of research and industry. These research directions aim to contribute to the optimization of encapsulation techniques and further enhance the understanding of encapsulation processes within the context of pharmaceutical and nutraceutical industries.

## 3.6 Conclusions

This study aimed to examine the impact of the VFD on the encapsulation process of flaxseed oil fortified with strawberry polyphenols. The study's findings indicated that the controlled manipulation of the VFD resulted in enhanced stability and a reduction in particle size in comparison to conventional techniques. However, apart from minor variations, no significant distinction was observed between the conventional approach and the encapsulation method employing the VFD. It is crucial to acknowledge the constraints of this study. The constraints encompass the utilisation of a singular type of encapsulating material, a singular operational mode for the vortex fluidic device, and a limited duration for conducting experiments. Future research endeavours should aim to address these limitations by conducting experiments over extended time periods and exploring a broader range of encapsulating materials. The implications of this study extend beyond the confines of academia. The findings of this study possess the capacity to assist biotechnologists in the advancement of encapsulation techniques, particularly within the nutraceutical and pharmaceutical industries.

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# APPENDICES

## Appendix 1 – Systematic optimisation of encapsulation materials and VFD parameters

| **Sodium Alginate** | | **Flaxseed oil** | | **Water** | | **Surfactant Tween80** | | **Surfactant Tween20** | | **VFD Parameters** | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **ml** | % | ml | % | ml | % | ml | % | ml | % | Direction | CW motion | CCW motion | Angle | Rotation speed | Time |
| **8ml** | | | | | | | | | | N | CW | | 45 | 7500 | 30mins |
| **8ml** | | 1ml | | | | | | | | N | CW | | 45 | 7500 | 30mins |
| **8ml** | | 1ml | | 1ml | | | | | | N | CW | | 45 | 7500 | 30mins |
| **8ml** | | 1ml | | 1ml | | 1ml | | | | N | CW | | 45 | 7500 | 30mins |
| **8ml** | | | | | | | | | | N | | CCW | 45 | 7500 | 30mins |
| **8ml** | | 1ml | | | | | | | | N | | CCW | 45 | 7500 | 30mins |
| **8ml** | | 1ml | | 1ml | | | | | | N | | CCW | 45 | 7500 | 30mins |
| **8ml** | | 1ml | | 1ml | | 1ml | | | | N | | CCW | 45 | 7500 | 30mins |
| | | 8ml | | | | | | | | N | CW | | 45 | 7500 | 30mins |
| **1ml** | | 8ml | | | | | | | | N | CW | | 45 | 7500 | 30mins |
| **1ml** | | 8ml | | 1ml | | | | | | N | CW | | 45 | 7500 | 30mins |
| **1ml** | | 8ml | | 1ml | | 1ml | | | | N | CW | | 45 | 7500 | 30mins |
| | | 8ml | | | | | | | | N | | CCW | 45 | 7500 | 30mins |
| **1ml** | | 8ml | | | | | | | | N | | CCW | 45 | 7500 | 30mins |
| **1ml** | | 8ml | | 1ml | | | | | | N | | CCW | 45 | 7500 | 30mins |
| **1ml** | | 8ml | | 1ml | | 1ml | | | | N | | CCW | 45 | 7500 | 30mins |
| | | | | 8ml | | | | | | N | CW | | 45 | 7500 | 30mins |
| **1ml** | | | | 8ml | | | | | | N | CW | | 45 | 7500 | 30mins |
| **1ml** | | 1ml | | 8ml | | | | | | N | CW | | 45 | 7500 | 30mins |
| **1ml** | | 1ml | | 8ml | | 1ml | | | | N | CW | | 45 | 7500 | 30mins |
| | | | | 8ml | | | | | | N | | CCW | 45 | 7500 | 30mins |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1ml** | | | | 8ml | | | | | | N | | CCW | 45 | 7500 | 30mins |
| **1ml** | | 1ml | | 8ml | | | | | | N | | CCW | 45 | 7500 | 30mins |
| **1ml** | | 1ml | | 8ml | | 1ml | | | | N | | CCW | 45 | 7500 | 30mins |
| | | | | | | 8ml | | | | N | CW | | 45 | 7500 | 30mins |
| **1ml** | | | | | | 8ml | | | | N | CW | | 45 | 7500 | 30mins |
| **1ml** | | 1ml | | | | 8ml | | | | N | CW | | 45 | 7500 | 30mins |
| **1ml** | | 1ml | | 1ml | | 8ml | | | | N | CW | | 45 | 7500 | 30mins |
| | | | | | | 8ml | | | | N | | CCW | 45 | 7500 | 30mins |
| **1ml** | | | | | | 8ml | | | | N | | CCW | 45 | 7500 | 30mins |
| **1ml** | | 1ml | | | | 8ml | | | | N | | CCW | 45 | 7500 | 30mins |
| **1ml** | | 1ml | | 1ml | | 8ml | | | | N | | CCW | 45 | 7500 | 30mins |

# Appendix 2 – ANOVA data

ANOVA for samples with water and without water

BEFORE VFD

Anova: Single Factor

SUMMARY

| *Groups* | *Count* | *Sum* | *Average* | *Variance* |
|---|---|---|---|---|
| emulsion with water before VFD processing | 3 | 3639 | 1213 | 4414107 |
| emulsion without water before VFD processing. | 3 | 1257.9 | 419.3 | 387945.97 |

ANOVA

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Between Groups | 944939.535 | 1 | 944939.535 | 0.393556481 | 0.564466345 | 7.708647422 |
| Within Groups | 9604105.94 | 4 | 2401026.485 | | | |
| Total | 10549045.48 | 5 | | | | |

AFTER VFD

Anova: Single Factor

SUMMARY

| *Groups* | *Count* | *Sum* | *Average* | *Variance* |
|---|---|---|---|---|
| emulsion with water after VFD processing. | 3 | 436.8 | 145.6 | 42403.36 |
| emulsion without water after VFD processing. | 3 | 5560 | 1853.333333 | 10304533.33 |

ANOVA

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Between Groups | 4374529.707 | 1 | 4374529.707 | 0.845570015 | 0.409843637 | 7.708647422 |
| Within Groups | 20693873.39 | 4 | 5173468.347 | | | |
| Total | 25068403.09 | 5 | | | | |

Anova: Single Factor

TPC

SUMMARY

| *Groups* | *Count* | *Sum* | *Average* | *Variance* |
|---|---|---|---|---|
| Samples treated with the Vortex fluidic device only | 3 | 0.452 | 0.150666667 | 0.010209333 |
| samples treated with the sonication bath and the VFD | 3 | 1.258 | 0.419333333 | 0.189492333 |

ANOVA

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Between Groups | 0.108273 | 1 | 0.108272667 | 1.084344147 | 0.356526 | 7.708647 |
| Within Groups | 0.399403 | 4 | 0.099850833 | | | |
| Total | 0.507676 | 5 | | | | |

FRAP

Anova: Single Factor

SUMMARY

| *Groups* | *Count* | *Sum* | *Average* | *Variance* |
|---|---|---|---|---|
| Samples treated with the Vortex fluidic device only | 3 | 1.339 | 0.44633333 | 0.053394333 |
| samples treated with the sonication bath and the VFD | 3 | 1.1 | 0.36666667 | 0.048910333 |

ANOVA

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Between Groups | 0.00952 | 1 | 0.00952017 | 0.186114025 | 0.688404705 | 7.708647422 |
| Within Groups | 0.204609 | 4 | 0.05115233 | | | |
| Total | 0.21413 | 5 | | | | |

DPPH

Anova: Single Factor

SUMMARY

| *Groups* | *Count* | *Sum* | *Average* | *Variance* |
|---|---|---|---|---|
| Samples treated with the Vortex fluidic device only | 3 | 6.797 | 2.265666667 | 9.637066333 |
| samples treated with the sonication bath and the VFD | 3 | 1.275 | 0.425 | 0.063783 |

ANOVA

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Between Groups | 5.082080667 | 1 | 5.082080667 | 1.047759942 | 0.363887457 | 7.708647422 |
| Within Groups | 19.40169867 | 4 | 4.850424667 | | | |
| Total | 24.48377933 | 5 | | | | |

## Appendix 3 - The vortex fluidic device (VFD)

Removed due to copy right restriction.

## Appendix 4 - The rotation of the VFD in relation to the earth's magnetic field.

Removed due to copy right restriction.