Master's thesis

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

Magnus Sebastian Øysteinsson Sølvesen-Myhre

Enrichment of instant foods using microencapsulated fish oil; chemical, rheological, sensory, and textural properties

Master's thesis in Food Science, Technology and Sustainability
Supervisor: Eva Falch
Co-supervisor: Sewuese Okubanjo
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Magnus Sebastian Øysteinsson Sølvesen-Myhre

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Summary

Fish oil, derived from fatty fish such as salmon, mackerel, and sardines, is renowned for its high content of omega-3 fatty acids, specifically eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These bioactive compounds have been linked to numerous health benefits, including cardiovascular health, cognitive function, and inflammation reduction. As a result, there has been growing interest in incorporating fish oil into various food products to increase their nutritional value. However, the addition of fish oil to food formulations presents challenges due to its susceptibility to oxidation, resulting in undesirable flavors and potential loss of the nutritional benefits. To overcome these limitations, microencapsulation techniques have gained attention as an effective means to protect sensitive bioactive compounds, such as fish oil, from oxidation and improve their stability during food processing and storage.

The objective of this master's thesis is to investigate the effect of adding microencapsulated fish oil (MFO) on the quality of bread, fish soup, and porridge, and to evaluate the sensory properties of these food products through consumer testing. Texture and viscosity were measured. scanning electronic microscopy (SEM) was performed to study the stability of microcapsules (used) in bread. Additionally, consumer testing was performed to evaluate the sensory attributes of the fortified and control samples. A panel of ordinary consumers assessed the taste, aroma, texture, overall liking, and purchase intentions of the products using check all that apply (CATA) and hedonic scaling. By assessing these attributes, this study aims to provide valuable insights into the physical- and sensory-impact of microencapsulated fish oil on bread, fish soup, and porridge.

Results showed significant changes in texture, viscosity, Liking and Purchase intentions. Adding powder positively affected color of bread and fish soup. Adding powder also improved texture-development of bread, slowing down the rate of retrogradation. Consumers were able to distinguish reference sample from fortified sample. Samples also proved to be significantly different in liking and purchase intentions. One can therefore conclude that the powder can negatively or positively affect foods when implemented. Therefore, one further should look at how to optimize process conditions so that the negative aspects can be minimized.

Sammendrag

Fiskeolje, fra fet fisk slik som laks, makrell og sardiner, er kjent for sitt høye innhold av omega-3 fettsyrer, spesielt eikosapentaensyre (EPA) og dokosaheksaensyre (DHA). Disse bioaktive forbindelsene har vært knyttet til en rekke helsemessige fordeler, inkludert kardiovaskulær helse, kognitiv funksjon og betennelsesreduksjon. Som et resultat har det vært økende interesse for å inkorporere fiskeolje i ulike matprodukter for å øke deres ernæringsmessige verdi. Tilsetning av fiskeolje til matformuleringer byr imidlertid på utfordringer på grunn av deres mottakelighet for oksidasjon, noe som resulterer i uønskede smaker og potensielt tap av de ernæringsmessige fordelene. For å overkomme disse begrensningene har mikroinnkapslingsteknikker fått oppmerksomhet som en effektiv måte å beskytte sensitive bioaktive forbindelser., som fiskeolje, mot oksidasjon og forbedre deres stabilitet under matforedling og lagring.

Målet med denne masteroppgaven er å undersøke effekten av å tilsette mikroinnkapslet fiskeolje (MFO) på kvaliteten på brød, fiskesuppe og grøt, og evaluere de sensoriske egenskapene til disse matvarene gjennom forbrukertesting. Tekstur, viskositet ble målt. SEM ble utført for å se på stabiliteten til mikroinnkapsler i brød. I tillegg ble forbrukertester utført for å evaluere de sensoriske egenskapene til de berikede- samt kontrollprøvene. Et panel av vanlige forbrukere vurderte smaken, aromaen, teksturen, hvor mye de likte produktet og kjøpsintensjon ved å benytte CATA og hedonisk skalering. Ved å vurdere disse egenskapene har denne studien som mål å gi verdifull innsikt i den fysiske og sensoriske påvirkningen av mikroinnkapslet fiskeolje på brød fiskesuppe og grøt.

Resultatene viste signifikante endringer i tekstur, viskositet, liking og kjøpsintensjoner. Pulveret påvirket fargen på brød og fiskesuppe positivt. Pulver forbedret også teksturutviklingen av brød og bremset ned graden av retrogradering. Forbrukerne var i stand til å skille referanseprøve fra beriket prøve. Det viste seg også å være betydelige forskjeller i hvor mye de likte produktene og kjøpsintensjoner. Man kan derfor konkludere med at pulveret har evnen til å påvirke matvarer negativt eller positivt når det implementeres. Derfor bør man se nærmere på hvordan man kan optimalisere prosessforholdene slik at de negative aspektene kan minimeres.

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1. Introduction

Norwegian aquaculture is massive and produced over 1,64 million tons of fish in 2020. Of this Salmon constituted 1,6 million tons, demonstrating how big a portion of the marked Norwegian Salmon-production constitutes. (Fiskeridirektoratet, 2022) This faction of the industry created over 531 300 tons of rest raw materials (RRM). Head, viscera, bones, and residual meat within the bones are examples of RRM. We as consumers may not think much of these by-products, but they do carry valuable components for human nutrition and health. (Myhre M., 2022) Content of protein and lipids within RRM vary greatly depending of the parts of the animal but numerous studies show that these byproducts contain anywhere from 13,3-38,9% crude protein and 0,6-25% lipids (Chuaychan et al., 2016, Gajanan et al., 2016, Kittiphattanabawon et al., 2005, Nithin et al., 2013, Taheri et al., 2009). The omega-3 oils within the tissue is what's most important to this current project.

The benefits of consuming omega-3 oils are well documented. Examples of prominent omeg-3's are alpha-linoleic acid (ALA), docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). The first are commonly found in plants such as nuts and seeds while the other two can be found in fish. (Griffin, 2021) The latter two are especially important as they have been linked to aiding cardiovascular health, cognitive function, lowering complications related to rheumatoid arthritis and potentially aid in preventing age related muscle degeneration and cancer (Nih, 2023).

The European Food Safety Authority (EFSA) recommends a daily intake of at least 250 mg EPA and DHA to maintain normal heart function (Efsa, 2012). Nevertheless, many adults do not have enough intake to attain these benefits. Annual seafood consumption is declining for various reasons. In Norway it has been reported to have fallen consistently over the years. (Eurofish, 2020) In addition, fish lipids are prone to oxidation which causes off flavors and can deter consumers from buying products containing them (Secci and Parisi, 2016). A good way to tackle both these problems would be to extract the oil and feed it to the consumer via products they are familiar with. We can encapsulate the oil, thereby protecting the oil against air which has been shown to increase oxidative stability. (Menin et al., 2018)

One way of encapsulating the fish lipids is to use spray drying. In this process the lipids are dissolved and mixed with an encapsulating agent and fed into a spray dryer that atomizes the solution into small droplets using a high-pressure nozzle. The droplets are then passed through a heated chamber leaving behind a dry powder that contains the encapsulated lipids. It is during the spray drying process, the encapsulating agents form a protective barrier around the lipid droplets, which helps to prevent oxidation and other forms of spoilage. (Karrar et al., 2021)

This master's thesis is a part of the OMEGA project led by SINTEF Industry, with the national partners SINTEF Ocean, NTNU and Høgskolen på Vestlandet. Their aim is to help people increase their intake of omega-3 oil and utilize more rest raw materials from fish production for human consumption. By utilizing rest raw materials, marine process waste can be reduced at the same time as it is a sustainable way to increase omega-3 intake through the enrichment of common instant-food products found in Norwegian grocery stores. (Johnsen H., 2021) For this project self-produced MFO was to be used, but as there were problems with producing enough poweder for all analyses, commercial MFO was also used.

The main objective of this project is to find the effect of fortification on the quality of selected foods and to evaluate sensory properties of the foods through consumer testing.
The sub-goals of this study are to:

- Find suitability of omega-3 microcapsules as functional ingredients in three selected food models.
- Test the chemical and rheological qualities of the food products before and after enrichment with omega-3 powders.
- Create a food product that can cover a percentage of the recommended daily intake of EPA & DHA and have high consumer acceptance.

2. Theory

When implementing MFO in food, it is important to know of the different food matrixes one is working with. In the context of food science, a food matrix refers the complex and organized structure formed by the combination of different components within a food product. These components can include macronutrients such as proteins, carbohydrates, and fats, or water, fibers, and other bioactive compounds. (Aguilera, 2019) The food matrix is not just a simple mixture of individual components. It is more of a system where components interact and influence each other's properties. Arrangement and interactions between the components within the matrix play a crucial role in determining the overall characteristics of the food product. It can influence its texture, flavor, aroma, nutritional availability. (Turgeon and Rioux, 2011)

The properties and behavior of said components can be significantly altered when they are part of a food matrix. (Turgeon and Rioux, 2011) The matrix can also influence the release and perception of flavors and aromas, as well as the physical attributes such as texture and mouthfeel. This will be discussed later in this chapter. Additionally, the food matrix can provide structural support and stability to the product. (Thomas et al., 2018) It can influence the water-holding capacity, viscosity, and rheological properties of the food (Fischer and Windhab, 2011). The food matrix can also affect the rate of heat and mass transfer during cooking or processing, impacting the final texture and sensory attributes of the food (Turgeon and Rioux, 2011). Understanding and studying the food matrix is therefore important for food scientists and technologists, as it helps them in developing and improving food products. By manipulating the composition, structure, and interactions within the matrix, it is possible to optimize the sensory, nutritional, and functional properties of foods.

2.1 Fish lipids

Fish is a rich source of several nutrients, including lipids, which are an essential part of the human diet. The lipids found in fish play important roles in providing energy, absorption of fat-soluble vitamins, and contributing to the sensory attributes of fish products. (Raatz and Bibus, 2016) Fish lipids can be broadly classified into two categories: structural lipids and storage lipids. Structural lipids, which include phospholipids, sphingolipids, and cholesterol, are primarily found in cell membranes and are responsible for maintaining membrane fluidity and permeability. Storage lipids, which include triacylglycerols (TAG's), are stored in adipose tissue and serve as a source of energy for the fish. (Johnson, 2009)

But one of the key characteristics of fish lipids is their high content of long-chain polyunsaturated fatty acids (LC-PUFA's), and two important ones being eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Fish, especially fatty fish such as salmon, mackerel, and herring, are rich sources of EPA and DHA, thereby making them an important dietary source of these compouinds. In addition to LC-PUFAs, fish lipids also contain other types of fatty acids, such as saturated fatty acids (SFA's) and monounsaturated fatty acids. The proportion of these fatty acids, however, vary depending on the species of fish and the environmental conditions in which they are raised. For example, farmed salmon may have higher levels of SFA's and lower levels of LC-PUFA's compared to wild salmon due to the composition of their feed. The type of lipid and fatty acid composition of fish can also affect the quality and stability of fish products. For example, the presence of unsaturated fatty acids, such as EPA and DHA, can make fish more prone to oxidation, leading to off-flavors and odors in the product. (Kris-Etherton et al., 2002)

2.2 Eicosapentaenoic acid and docosahexaenoic acid

The chemical compositions of eicosapenteanoic acid (EPA; 20:5) and docosahexaenoic acid (DHA; 22:6) are shown in figure 1.

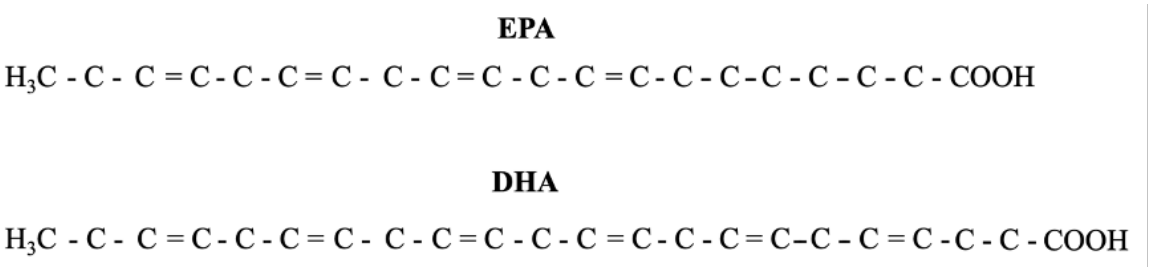


***Figure 1**: Showcasing the chemical composition of EPA (above) and DHA (below) (adapted from Kamal-Eldin, 2003 p.39).*

Both consists of a long carbon chain, with a carboxyl-group and a methyl-group at the terminal end. (Coultate, 2016)

One of the main health benefits of EPA and DHA is their ability to support heart health. Studies have shown that consuming EPA and DHA can help reduce the risk of heart attack and stroke and improve cholesterol levels. These fatty acids can also help to reduce inflammation, which is a risk factor for many chronic diseases, including heart disease and certain types of cancer (Mason et al., 2020) Additionally, EPA and DHA also play important roles in the development of brain health. DHA is a key component of brain cell membranes, and studies have shown that adequate intake of DHA can improve cognitive function, memory, and learning ability (Devarshi et al., 2019) EPA and DHA have also been shown to be beneficial for mental health, and some studies have found that they may be helpful in the treatment of depression and other mental health conditions. EPA and DHA have also been shown to have anti-inflammatory effects in the body, which again can be beneficial for a number of conditions, including joint pain, rheumatoid arthritis, and inflammatory bowel disease. These fatty acids have also been shown to be beneficial for skin health and may help to reduce the appearance of wrinkles and other signs of aging. (Kris-Etherton et al., 2002)

2.3 Lipid oxidation

Because of the high degree of unsaturation of LC-PUFAs, this makes them susceptible to oxidation. This is therefore an important parameter that greatly affects shelf life.
There are 3 stages of lipid oxidation which is seen in figure 2.

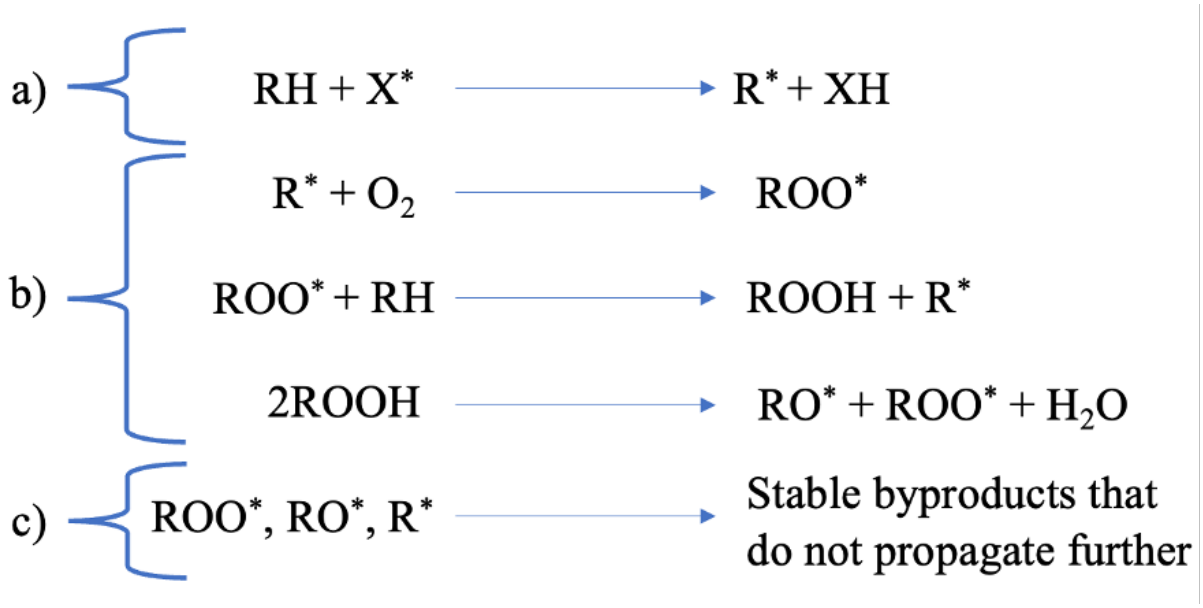


Figure 2: Showing the stages of lipid oxidation, whereas a) being initiation, b) being propagation and c) being termination (adapted from Coultate, 2016 p.127). The star (*) represents an unpaired electron.

The first stage (a) is called initiation. In this stage, free radicals, such as hydroxyl radicals (X*) or peroxyl radicals (R*), are formed and react with the double bonds of unsaturated fatty acids, leading to the formation of lipid radicals. This process can be initiated by numerous factors i.e. light, heat, metal ions, and enzymes within the raw material. Many of these radicals are highly reactive and therefore short lived as they will quickly pair up their electrons. (Frankel, 2005)

The second stage (b) is called propagation. In this stage, the lipid radicals react with oxygen molecules to form peroxy radicals ($ROO^{\cdot}$), which then attack neighboring fatty acids to form more lipid radicals, and thus creates a chain reaction. The end products from this stage are also highly reactive and can react with other PUFAs making hydroperoxides (ROOH) and regenerate free radicals ($R^{\cdot}$). These radicals can then repeat the process by forming a chain reaction which results in accumulating amounts of free radicals. These free radicals will continue the reaction by reacting with oxygen. (Coultate, 2009, Frankel, 2005)

At some point the concentration of free radicals will come to a limit where they start reacting with each other and form stable by/end products. This stage (c) is called termination. In this stage, the chain reaction is terminated by the removal of free radicals and occurs when the reactive intermediates generated during lipid oxidation, such as lipid peroxyl radicals, are either deactivated or consumed by various termination reactions. Termination reactions help to neutralize the reactive species thereby preventing further oxidation. (Frankel, 2005)

Volatiles from the propagation-stage are especially important for sensory as the secondary oxidation products formed here have low flavor-threshold ranging from 0.00003-1,6 µg/g depending on the oxidation product. EPA and DHA have 4 and 5 bis allylic methylene groups respectively that initiate hydrogen abstractions (from the methylene groups). They also contain 4 and 5 pentadienyl groups respectively that can produce positional hydroperoxide isomers (8 from EPA and 10 from DHA). Since there are many geometrical and positional isomers that can be formed then this gives rise to a complex mix of volatile secondary oxidation products. An example of major aldehydes that are formed from EPA is shown in figure 3 below. (Kamal-Eldin, 2003)

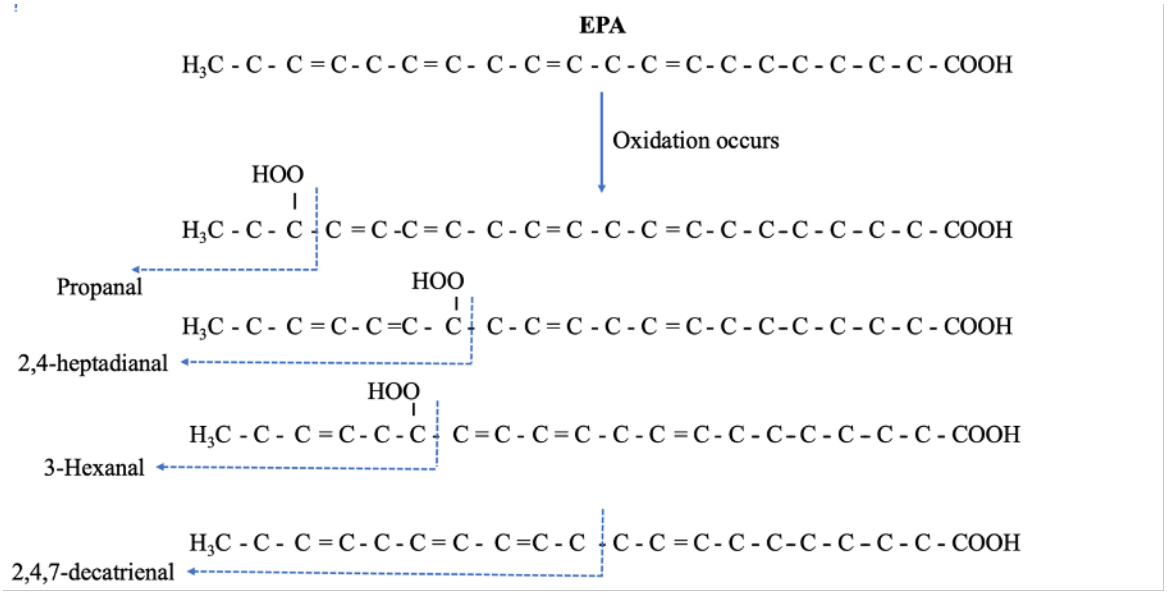


Figure 3: The pathway for three of the major aldehydes that can be formed from EPA. The dotted lines represent the oxidation sites in the carbon-link (adapted from Kamal-Eldin, 2003 p.41).

2.4 Microencapsulation to prevent oxidation

Microencapsulation is a versatile technique in which you can enclose active compounds. By creating a function barrier between the core and wall material one can avoid both chemical and physical reactions and protect said compounds from degradation and improve stability. There are various ways one can employ to encapsulate a compound. The most common one when microencapsulating oils is arguably emulsion-blends combined with spray drying. (Bakry et al., 2016) The process of creating a stabilized emulsion can be done by combining water and oil together with a suitable solvent in a homogenizer. One method of creating an emulsion is by using ultrasound. Here the mixture is circulated around a ultrasound probe until the emulsion is formed. (Cabrera-Trujillo et al., 2018)

Spray drying is a continuous process that usually involves several steps. Phase 1 is the concentration phase where a feedstock (with the active compound and wall material both dissolved in solvents) is concentrated and introduced to the spray dryer. Phase 2 called atomization stage is the stage that creates an optimal environment for evaporation of the desired product. This happens within the drying chamber of the spray dryer and is the most critical stage. The feed material must be dispersed into small enough droplets and be well distributed so that the compound mixes thoroughly with the hot gas. In phase 3 the droplets come into contact with hot air that evaporates the water contained in them leading up to phase 4 where the lack of moisture causes a “dried shell” to form on the surface of the droplets. This causes dried granules to form, creating a powder. (Patel et al., 2009) The physical characteristics of the materials are important to ensure an even operation and a sufficient production rate. To have low viscosity even at high solids-content, being able to disperse in uniform droplet size and being able to completely release from the solvent when drying are among the most important. This will produce a dense film around the granules with good physical strength. (Desai and Jin Park, 2005) It is in the spray drying process one introduces the materials for encapsulation. The type of wall materials differ, each possessing their own unique trait. It can be proteins such as casein or gelatin, carbohydrates such as starch or hydrophilic gums such as gum acacia. (Lu et al., 2021)

2.5 Starch

Starch is a complex carbohydrate and is characterized by its unique molecular structure, which gives it distinct physical and chemical properties. Starch is composed of two types of

polymers: amylose and amylopectin. Amylose constitutes approximately 20-30% of starch and is a linear chain of glucose units connected by α-1,4-glycosidic bonds. The presence of these linkages gives amylose its helical structure. In contrast, amylopectin accounts for the remaining 70-80% of starch and is a highly branched polymer. It consists of α-1,4-glycosidic bonds like amylose, but also contains α-1,6-glycosidic bonds at branch points, resulting in a tree-like structure. (Bertoft, 2017)

Starch granules exhibit a distinct morphology. They are generally oval or spherical in shape and vary in size across different plant species. Within each granule, amylose and amylopectin form distinct regions. The granules are further divided into concentric layers known as growth rings, reflecting the stages of granule development.
Amylose forms a tightly coiled helix that occupies the inner part of the granule, while amylopectin resides predominantly in the outer regions. This arrangement creates a matrix-like structure, with amylose acting as the core and amylopectin as the branches. The crystalline regions of amylopectin are responsible for the granules' semi-crystalline nature. (Buleon et al., 1998)

The presence of helical amylose chains allows starch to form complexes. Starch also exhibits different gelatinization properties depending on the source and processing. During gelatinization, starch granules absorb water, swell, and eventually rupture, resulting in the release of amylose and amylopectin. This process is crucial for cooking, as it contributes to the texture, viscosity, and stability of starch-containing foods. (Ratnayake and Jackson, 2008)

2.6 Casein

Casein is a group of proteins found in milk and is a crucial component of the mammalian diet. Casein is a mixture of several proteins, namely αS1-casein, αS2-casein, β-casein, and κ-casein. These proteins account for approximately 80% of the total protein content in bovine milk, with varying proportions across different species. Each type of casein has distinct amino acid sequences, resulting in different properties and functionalities. (Horne, 2002)

The primary structure of casein proteins consists of chains of amino acids linked together by peptide bonds. These chains fold and interact to form a complex three-dimensional structure.

The structure of casein is predominantly disordered, lacking well-defined secondary structures such as alpha-helices or beta-sheets. This disordered conformation contributes to its unique functional properties. (Kumosinski et al., 1991)
The casein proteins further assemble into micelles, which are the basic structural units of casein. Micelles are spherical aggregates consisting of thousands of casein molecules held together by hydrophobic interactions and stabilized by calcium and phosphate ions. The micelles exhibit a core-shell arrangement, with the hydrophobic regions of casein proteins forming the core and the hydrophilic regions facing the surrounding fluid. (Horne, 2006)
The outer shell of the micelles is formed by κ-casein molecules. These molecules play a crucial role in stabilizing the micellar structure and preventing casein aggregation. The κ-casein molecules bind to the surface of the micelles through hydrophobic interactions, shielding them from aggregation and helping maintain the stability of the casein system. (Creamer et al., 1998)

The unique structure of casein imparts various physical properties that contribute to its functionality. Casein micelles have a colloidal nature, displaying remarkable stability and resistance to changes in pH, temperature, and other environmental factors. (Zhang et al., 2021) Casein is also known for its ability to bind and transport calcium and other minerals. The micellar structure provides a protected environment for these minerals, allowing their efficient absorption in the digestive system.

The unique properties of casein make it valuable in a wide range of applications. In the food industry, casein is utilized as a source of protein, an emulsifying agent, and a texture modifier. It is used in the production of cheese, yogurts, and other dairy-based products. Casein-based materials are also employed in the pharmaceutical and cosmetic industries, owing to their biocompatibility and functionality. (Nascimento et al., 2020)

2.7 Gelatin

Gelatin is a widely used food ingredient and is also used in a variety of other applications such as pharmaceuticals, cosmetics, and photography. It is derived from collagen, which is a fibrous protein found in animal skin, bone, and connective tissue. The chemical properties of gelatin are determined by the amino acid composition of the collagen, but in general there are 18 amino acids that are connected across a triple matrix. The amino acid composition varies but the

general structure of gelatin is shown in figure 4. Gelatin is a hydrocolloid, meaning that it can form a gel when it is hydrated in water. This property is due to the presence of amino acids that have hydrophilic properties, such as glycine and proline, which are present in high concentrations in collagen. When gelatin is mixed with water the hydrophilic amino acids attract water molecules, causing the gelatin molecules to unfold and form a three-dimensional network that traps water within its structure. This network of gelatin molecules gives the gel its unique texture and ability to hold its shape. (Ross-Murphy, 1992, Ullah et al., 2017)

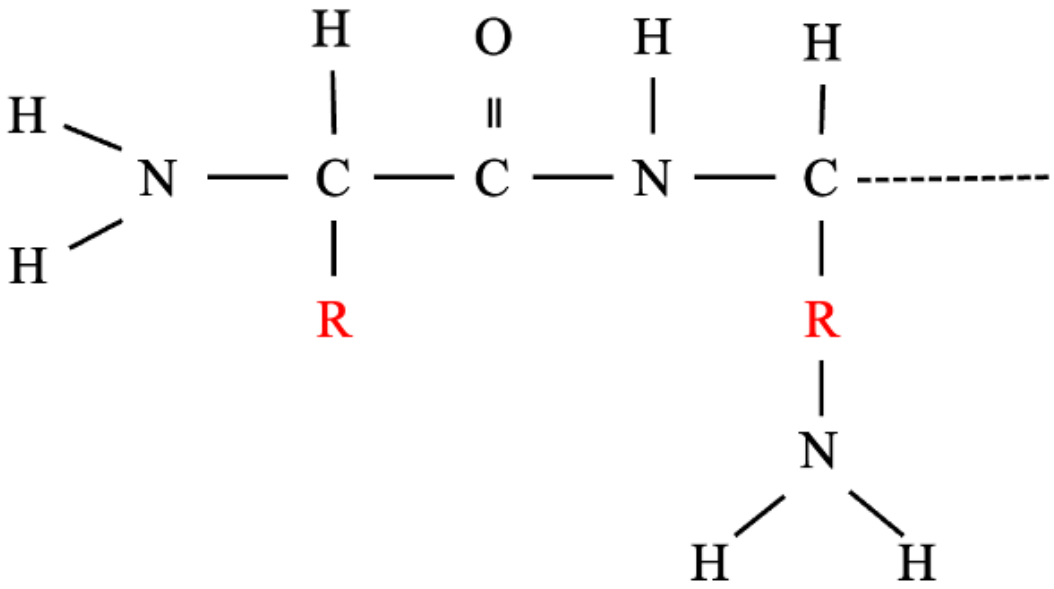


Figure 4: General structure of gelatin. R represents an amino acid group connected to the matrix (adapted from (Ullah et al., 2017) p.60).

The ability of gelatin to form a gel is dependent on several factors, including the concentration of gelatin in the solution, the temperature of the solution, and the pH of the solution. At low temperatures and high concentrations, gelatin molecules are more likely to aggregate and form a gel. At higher temperatures, the gelatin molecules become more soluble and are less likely to form a gel. The pH of the solution can also affect the ability of gelatin to form a gel, with the optimal pH range for gel formation typically being between 4.0 and 7.0.

Another important property of gelatin is its ability to form foams. When gelatin is whipped, air is incorporated into the mixture, creating a foam. The ability of gelatin to form a foam is due to the same three-dimensional network of gelatin molecules that allows it to form a gel. The air bubbles become trapped within this network, giving the foam its stability and structure. (Coultate, 2016)

The amino acid composition of gelatin is unique and distinguishes it from other proteins. The primary amino acids found in gelatin are glycine, proline, and hydroxyproline, which account for over 50% of the total amino acids. These amino acids are important for the functional properties of gelatin, including its ability to form gels and its water-holding capacity.
Glycine is the most abundant amino acid in gelatin, accounting for around one-third of the total amino acids. Glycine has a simple chemical structure and lacks a side chain, which allows it to be packed tightly together with other amino acids in the protein chain. This tight packing results in the formation of a helical structure in the protein chain, which contributes to the gelling properties of gelatin. Proline and hydroxyproline are also abundant in gelatin, accounting for around 20% of the total amino acids. These amino acids have unique chemical structures that result in the formation of kinks/twirls in the protein chain. These kinks disrupt the helical structure of the protein chain, which contributes to the flexibility and water-holding capacity of gelatin. (Huang et al., 2019)

Gelatin is an amphoteric substance, which essentially means that the molecule can act either as an acid (in the presence of a base) or a base (in the presence of an acid). Therefore, when the molecule is at its isoionic point (pI) the molecule has no net charge at the R-groups. This makes it great for microencapsulation in combination with gum acacia since gum acacia can react with the basic R-groups in gelatin and form a gelatin-acacia complex. (Baziwane and He, 2003) Research done on marine gelatine derived from various types of fish and shellfish possesses good emulsifying qualities that would make it applicable in food industry. Since gelatin has both hydrophilic (amino and carboxyl groups) and hydrophobic (aliphatic chains and aryl groups) regions this gives it the ability to absorb at the oil-water interface thus preventing coalescence of oil droplets and promote emulsion. (Aewsiri et al., 2009, Chen et al., 2018)

The chemical structure of gelatin can be modified (if needed) by chemical and enzymatic treatments, which can alter its functional properties and its suitability for different applications. For example, crosslinking of gelatin molecules can increase the strength of the gel and its resistance to heat and mechanical stress. Enzymatic treatments can modify the amino acid composition of gelatin, resulting in changes in its gelling and water-holding properties. (Huang et al., 2019)

2.8 Gum acacia

Gum acacia, which is also known as gum arabic, is a natural polysaccharide derived from the sap of the Acacia tree. It is widely used as a food and beverage ingredient, as well as in pharmaceutical, cosmetic, and industrial applications. In the EU it goes under the EC number of E414. (Coultate, 2016) The chemical properties of gum acacia are determined by its complex structure, which consists of a branched chain of sugars with various chemical functional groups attached.

Gum acacia is composed primarily of a complex mixture of carbohydrates, including arabinogalactans, rhamnogalacturonans, and glucuronic acid. The carbohydrate chain of gum acacia is highly branched and contains various chemical functional groups, including carboxyl, hydroxyl, and amino groups. The presence of these functional groups gives gum acacia unique chemical properties that make it useful in a wide range of applications. One of the most important properties of gum acacia is its ability to form stable solutions and suspensions in water. When gum acacia is added to water, the carbohydrate chains become hydrated, forming a network that traps water molecules within its structure. This network of hydrated carbohydrate chains gives gum acacia its characteristic viscosity and ability to form stable solutions and suspensions. (Mahendran et al., 2008)

At higher concentrations and lower pH values, gum acacia solutions exhibit higher viscosities due to the increased number of hydrated carbohydrate chains in the solution. This property makes gum acacia useful as a thickener and stabilizer in a wide range of food and beverage applications, including soft drinks, confectionery, and baked goods. When gum acacia is added to a mixture of two immiscible liquids, such as oil and water, the carbohydrate chains become adsorbed at the interface between the two liquids, forming a stable emulsion. The emulsifying properties of gum acacia are due to the presence of both hydrophobic and hydrophilic functional groups on the carbohydrate chain, which allow it to adsorb at the interface between the two liquids in the same way as gelatin (Kim et al., 1996) Studies show that gum acacia works better in combination with other wall materials. In a study done by Felix et al. (2017) they combined gum acacia with whey protein and maltodextrin. The result of this produced microcapsules with a higher rate of encapsulation efficiency when spray drying and better retention of the core material. It also produced walls with higher thermal stability, meaning the capsules would be able to tolerate a certain amount of heat exposure.

The main component of gum acacia is a polysaccharide called arabinogalactan, which accounts for over 85% of the total gum. Arabinogalactan is composed of two sugar units, arabinose and galactose, which are linked together in a branched structure. The branches of arabinogalactan are composed of different sugar units, such as rhamnose, glucuronic acid, and xylose. The protein and glycoprotein components of gum acacia are minor, accounting for less than 15% of the total gum. The proteins in gum acacia are composed of a mixture of globular and fibrous proteins, which contribute to the viscosity and stability of the gum. The glycoproteins in gum acacia are composed of a protein backbone that is modified with sugar units, such as arabinose and galactose. (Mahendran et al., 2008)

2.9 Sensory perception of foods fortified with MFO

When fortifying food products with MFO, sensory attributes are a major variable. In order to sell a product fortified with MFO one does not only rely on the nutritional value but also the sensory perception and acceptability of consumers. When adding MFO to the products attributes such as appearance, odor, taste, texture, and overall flavor can be affected. The extend of how these sensory parameters are affected, greatly depend on factors such as the type of product that is fortified, surroundings during production of both the powder (i.e. materials used for encapsulation as talked about in chapter 2.4) and the type food model (with regards to temperature during production and storage). (Jiménez-Martín et al., 2016, Serfert et al., 2010)

When the consumer is presented with a product, appearance is among the first sensory parameters that can impact acceptability. Our visual system is highly sensitive to the visual presentation of food. When we see a dish, our brain automatically processes the visual cues and forms expectations about its taste, texture, and quality. These expectations are based on our prior experiences, cultural influences, and personal preferences. The appearance of food creates expectations in terms of taste. For example, a visually appealing dish with vibrant colors and an attractive presentation can lead us to anticipate a delicious taste. On the other hand, if a dish appears unappetizing or poorly presented, we might have lower expectations of its taste. (Piqueras-Fiszman and Spence, 2015)

The visuals also influence our perception of texture. Presentation of food can actually enhance the overall enjoyment of a meal. As observed by Spence et al. (2016), when a dish is visually

appealing, it stimulates our appetite and increases our anticipation of the flavors to come. A visually appealing plate can elevate the dining experience, making it more satisfying and pleasurable. Conversely, unappealing visuals can negatively impact our perception and enjoyment of food. If a dish looks unattractive, it may dampen our enthusiasm and make it less appetizing, even if the taste and texture are perfectly fine. Speaking of texture, this is also something that needs to be taken into account.

As discussed above, the components of microencapsulation has the ability to bind water and change the properties of the products. In a study by Spence et al. (2016), they looked at how oral sensory cues affect the sensory liking and perception of cheeses where affected by differences in concentration (of added bell peppers), hardness and size. They concluded that the consumers hedonic responses are to a large extend determined by textural properties (with regards to the concentration, hardness and size of the particles) and the expectations of the consumer. When fortifying foods with MFO, one therefore has to take into account the degree to which the powder affects ether the viscosity or texture of the product.

Lastly is flavor. As discussed earlier, fish oil is prone to oxidation. The off-odor and off-flavor can have a dramatic effect on the sensory properties. As observed by Damerau et al. (2022), products fortified with MFO were detected by the sensory panel regardless of formulation, and higher content of EPA + DHA had a negative impact on the sensory perception. When describing attributes, the panelist mainly made distinction in fishy flavor and odor. In this project they fortified cookies which are heat treated and is an important factor. Depending on the thermal stability of the wall material, heat treatment can potentially break down the wall material (Fan et al., 2005). This could lead to the to the leakage of oil that is exposed to air, creating the undesirable compounds.

2.10 Analytical methods

2.10.1 Rheology

Rheology is a branch of physics that studies the mechanical properties of non-Newtonian and Newtonian fluids and their response to applied forces. Rheology can provide valuable information about the mechanical properties of materials and this information can be used to improve product quality, increase production efficiency, and optimize processing conditions.

Additionally it can also help in the development of new materials and products, as it provides a means of characterizing the flow properties of these materials and understanding how they will behave. (Rao, 2014)

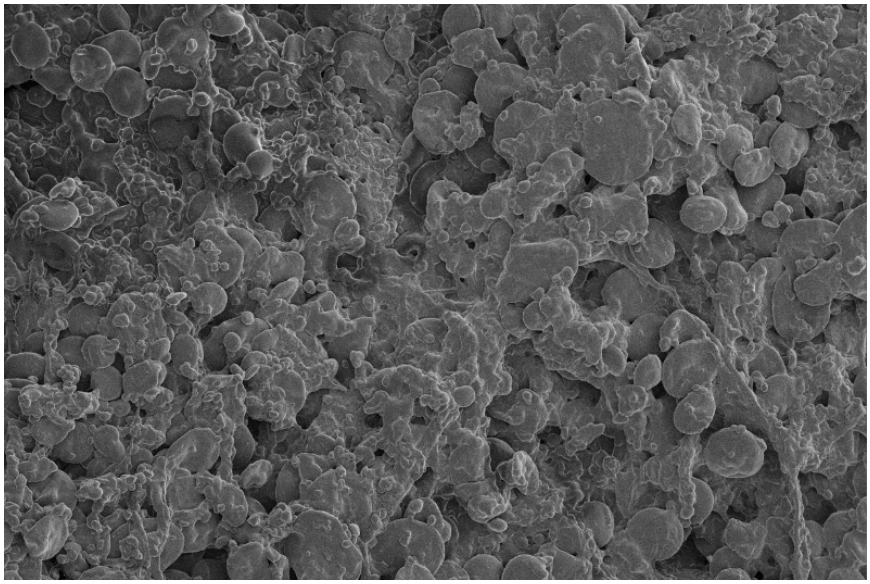
Rheology can be measured in a variety of ways, a good way to do this is with "pipe flow rheometry" that measures the pressure drop across a section of the pipe when the fluid is forced through it. The data contained from this can be used to calculate the viscosity and other rheological flow conditions (i.e., shear rate). (Chhabra and Richardson, 2008)

Newtonian fluids exhibit a linear relationship between shear stress (force that is applied to the liquid parallel to the surface) and the shear strain (deformation of the material in response to the shear stress). What this means is that the Newtonian viscosity of the fluid is constant regardless of the applied shear strain or stress. An example of this would be water. A non-Newtonian liquid on the other hand has a viscosity that changes depending on the applied shear stress or rate and can have quite the complex flow properties that vary with time, temperature and pressure. An example of this would be porridge. The rheological qualities of porridge changes depending on the content. This was demonstrated by Yadav et al. (2014) where they tested pearl millet porridge. They observed that grit size of the millet greatly affected the firmness and viscosity of the porridge, where higher grit size increased the parameters. When applying MFO one has to take into account the swelling capacity of mentioned wall-materials and water content of the product. In another study done by Tamjidi et al. (2014) they tested the rheological characteristics of yogurt enriched with MFO-powder. The wall material used in the MFO-powder was gelatin and gum acacia. The study concluded that the yogurt fortified with MFO coacervates, meaning that charged macromolecules in the homogenous mixture spontaneously separates and creates two unmixable liquids. This clearly showed in the flow behavior. Naturally with increased shear rate the viscosity of the non-Newtonian fluids decreased. However, when increasing the shear rate from 0,262-0,786 s^{-1} the viscosity of the enriched yogurt had a greater decrease.

Shear rate is the rate at which a fluid is sheared or deformed per unit of time. It is defined as the ratio of the velocity difference between two adject layers of the fluid to the distance between these layers and can be mathematically expressed as: $\gamma m = \frac{4Q}{\mu r3}$, Q being the volumetric flow rate through the pipe (distance/time) and being pipe radius. (Morrison, 2001)

2.10.2 Scanning electron microscopy

Scanning electron microscopy (SEM) is a analytical tool that provides high-resolution images that give information about the surface structure, composition, and morphology of materials. An example of this can be seen in picture 1. The technique has been widely used in a variety of fields. SEM works by directing a beam of electrons onto the sample surface and analyzing the interactions between the electrons and the sample atoms. The electrons in the beam lose energy as they travel through the sample, producing secondary electrons and other types of signals that are collected and analyzed to form an image of the sample surface. (Vernon-Parry, 2000)



Picture 1: Image taken of a piece of regular bread used in this project.

One of the key features of SEM is that it provides images with a much higher resolution than light microscopes, allowing researchers to see fine details of the sample surface that would be invisible to the naked eye. For example, SEM can resolve features as small as a few nanometers in size, which is much smaller than the wavelength of light. This makes SEM ideal for imaging materials that are too small or too transparent for light microscopy. (Nixon, 1971)

Several studies have been done to examine the behavior of microcapsules both in foods and their stability during storage. One study done by Krishnan et al. (2005) they studied the size and shape of microcapsules that were stored for 6 weeks at 25 °C. Core material for the microcapsules were oleoresin that is found in cardamom. Gum acacia, maltodextrin and modified starch were used as wall materials. The results showed that capsules made with gum acacia had a spherical shape with dents on the surface area and that they were less broken and

incomplete than the other capsules. In Gökmen et al. (2011) flaxseed oil was microencapsulated using high amylose corn starch and used to fortify bread. The results from SEM showed that the granules were still intact after baking. However, it differed between the crumb where the granules were intact and the crust where they appeared partially destroyed. Similar results were reported by Takeungwongtrakul et al. (2015b). In their study shrimp oil was microencapsulated using whey protein concentrate, sodium caseinate and glucose syrup as wall materials. The microencapsulated oil was then used to fortify bread and SEM pictures were taken of the crumb.

2.10.3 Gas chromatography

Gas chromatography is a technique that is used to separate and analyze volatile compounds. It is based on the principle of "partition chromatography", where the compounds are separated based on their partitioning behavior between a mobile phase (usually a gas) and a stationary phase. In gas chromatography, a sample is vaporized and then injected into a heated column. As the sample moves through the column, the compounds in the sample partition between the mobile phase (gas) and the stationary phase. The different compounds are separated based on their partitioning behavior, with some compounds spending more time in the stationary phase and others spending more time in the mobile phase. The separated compounds then reach a detector, which is usually a flame ionization detector (FID) or a mass spectrometer (MS). The detector measures the signal generated by each compound as it elutes from the column and provides information about the compound's identity and concentration. (Sparkman et al., 2011)

Gas chromatography is used in a wide range of applications, but with regards to this project is important for food analysis to measure the concentrations of various compounds in food, such as flavor and fragrance compounds, and to identify contaminants. There are different types of gas chromatography methods. The one used in this thesis was headspace gas chromatography (HS-GC). HS-GC is a type of GC that is used to analyze volatile compounds in a headspace sample. This type of GC is used for the analysis of volatile compounds in food, pharmaceuticals, and other samples. (Wang et al., 2008)

3. Materials and Methods

This section will describe the materials and methods used in this study. The structure of the procedures done in this study can be seen in figure 5 below.

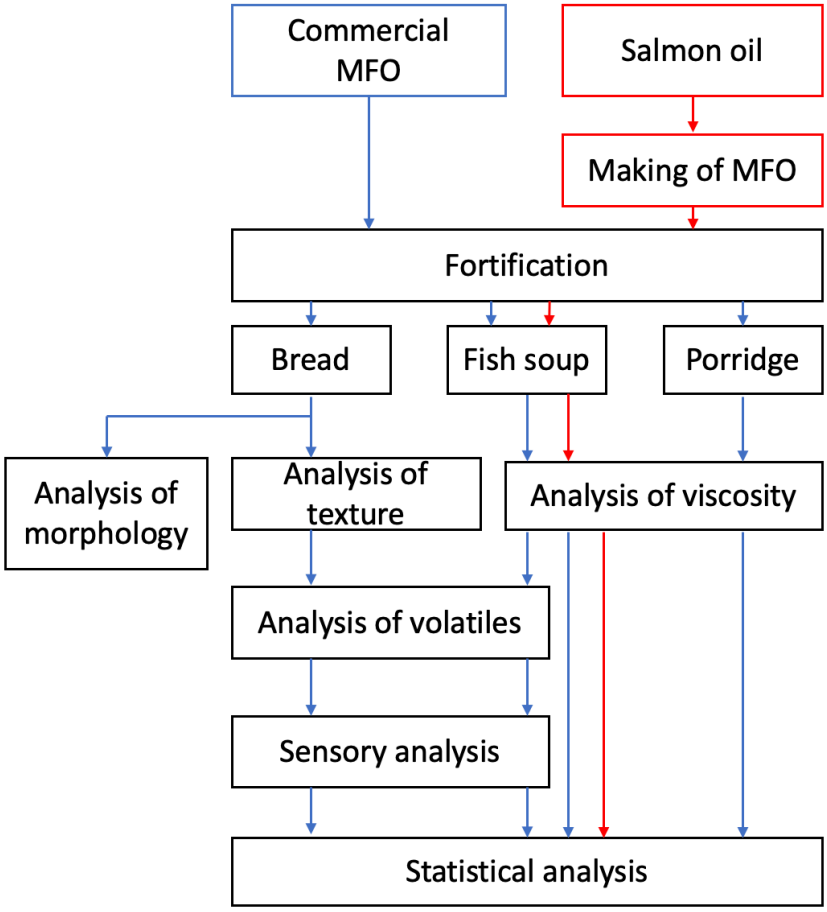


Figure 5: Flow chart showing the experimental design. Blue color indicates commercial MFO while red color indicates MFO-powder made with gelatin and gum acacia.

3.1 Making of microencapsulated fish oil powder (MFO-powder)

Commercial MFO-powder was used for all procedures in this study. The omega-3 content of this powder is 12% by weight. Additionally in order to see the effect of a different wall material commercial gelatin was used by SINTEF Industry together with gum acacia to encapsulate salmon oil. The powder was then kindly donated to this project.

3.2 Food formulation

EFSA's recommended daily intake (DRI) of EPA + DHA: 250 mg (Efsa, 2012).
This study aimed at covering 40-50% of DRI for each portion. The fish soup comes with a description of what constitutes 1 portion while the porridge comes in a 1-portion-size. 1 loaf of chosen bread had 18 slices. Dues to the characteristics of the MFO and the ratio between the powder and bread mixture, it was aimed at 40 mg EPA+DHA per 1 portion (2 slices).

3.3 Food models

Foods from Norwegian grocery stores chosen for this master's thesis were bread (69% wholegrain), fish soup, and barley porridge with raspberry sauce. These food models were chosen based on discussion through the project group with NTNU, SINTEF Industri and from study groups conducted by my supervisors where they got feedback from asking regular consumers what type of sort of fortified products they would be interested in trying.

3.4 Preparation of food models

3.4.1 Bread

The bread mixture came with instruction on what ingredients to use and methodology of preparing the loaf. Whole grain bread was chosen for this study as it a staple in the Norwegian diet, and is shown to be more healthy. (Bugge et al., 2008) Recipe used in this experiment is shown in table 1.

Ingredients: bread
- 500 grams Bread mix (69% wholegrain) - 300 ml water - 25 grams fresh baker's yeast

Table 1: Recipe for 1 loaf of bread.

Water was heated to 35 °C. Fresh baker's yeast was dissolved in warm water (35°C) and mixed in a bowl with the bread mix . Then the dough was kneaded for 5 minutes using a "Kenwood Major Titanium" (England) stand mixer on speed setting 5. After kneading the dough was left to rest for 45 minutes. The dough was placed in a 2000 ml bread pan (pre-greased with rapeseed oil) where it was left to rest for an additional 45 minutes until it doubled in size.
The dough was baked on the bottom rack in the oven for 40 min at 200 °C.

After baking the bread was left to completely cool down before being packaged and stored in the refrigerator.

The MFO-fortified bread was prepared using the same recipe and method, but 10 g of MFO-powder was sifted into the flour before the addition of water and yeast.

3.4.2 Fish soup

The fish soup-package came with a instruction on how to prepare the product. The recipe for fish soup is listed below. In this project it was targeted that 1 portion contains 50% DRI of EPA + DHA.

Ingredients: fish soup
20 grams of Fish soup mix
2 dl water
0,5 dl 1% fat milk

Table 2: Recipe for 1 portion of soup.

Soup mixture was added to a pot, together with water and milk. The pot was then heated on high stirring continually while the soup boiled for 5 minutes.

The MFO-fortified soup was prepared using the same recipe and method, where 2,5 grams of commercial MFO-powder was sifted into the dry soup-powder before liquids where added.

3.4.3 Barley porridge

The barley porridge is a instant food made to eat directly after opening. The product came with raspberry sauce. When preparing samples with MFO, the MFO-powder was added directly into the sauce and manually stirred until homogenous.

3.5 Analytical methods

3.5.1 Texture analysis

Bread firmness was measured using a texture analyzer (Stable Micro Systems TA-XT plus, United Kingdom). When performing this analysis, 15 mm thick slices of bread where used. The apparatus was calibrated using a 5 kg load cell. Each sample was compressed twice with a

cylindrical plunger probe of a 35 mm diameter with a return speed of 10 mm/sec and a contact force of 1 mm/sec. Measurements were performed 3 times on each slice for the whole loaf. Measurements were performed twice with 2 separates loafs of both regular and fortified bread.

In order to see how MFO-powder affects water retention in bread, 4 slices from both regular and fortified bread were stored for 7 days at 20 °C in aluminum-foil. After storage the slices where then measured using the same

3.5.2 Viscosity

Viscosity of porridge and soup were measured using a Rheometer (Anton Paar Physica MCR 301, Austria) with the use of a krebs spindle. The temperature, when measuring, for the porridge was 5 °C and the soup 25 °C. In order to determine the thixotropic (time-dependente) behavior of the how the MFO affects both the soup and porridge, the viscosity was measured in two ways:

In the first measurement the shear rate was progressively increased linearly from 0.1-500. Because of large gaps in the measurements when pretesting both products, this step was split into two intervals: Interval 1 lasted 200 seconds with a measuring point every 1 second and with a linearly increasing shear rate from 0,1-30 s^{-1}. Interval 2 lasted 600 seconds with a measuring point every 10 seconds and the shear rate linearly increasing from 30-500 s^{-1}. When processing the results only measurements for every 10 second from the first interval was used.

3.5.3 Volatiles

To analyse volatiles gas chromatography (GC) was performed using a “Teledyne Tekmar HT3^{TM}” Headspace autosampler (USA), “Agilent Technologies 7000 GC/MS Triple Quad” gas carrier (USA), and “Agilent Technologies 7890A” GC system (USA). The software package “MassLynx” from Waters Corporation (USA) was used to control the analytical equipment. The parameters set for the methodology is shown in table 3.

Variable	Value
Valve Oven Temperature	150ºC
Transfer Line Temperature	160ºC
Standby Flow Rate	50 mL/minute
Trap Standby Temperature	30ºC
Trap Sweep Temperature	0ºC
Platen/Sample Temperature	70ºC
Sample Preheat Time	10.00 minutes
Preheat Mixer	On
Preheat Mixing Level	Level 5
Preheat Mixing Time	2.00 minutes
Preheat Mixer Stabilize Time	1.00 minute
Sweep Flow Rate	50 mL/minute
Sweep Flow Time	10.00 minutes
Dry Purge Time	1.00 minute
Dry Purge Flow	50 mL/minute
Dry Purge Temperature	25ºC
Desorb Preheat	200ºC
Desorb Temperature	220ºC
Desorb Time	1.00 minute
Trap Bake Temperature	230ºC
Trap Bake Time	10.00 minutes
Trap Bake Flow	200 mL/min

Table 3: Parameters used when performing GC.

To see if there are detectable amounts of volatile coming from both the MFO-powder and the heat-treated products, it was measured using samples of MFO-powder, both regular and fortified bread & soup. Samples and codes used in the analysis is shown in table 4. For each sample 2 g of material was measured placed in 20 ml GC vials and sealed with septum crimp tops. For extraction of volatiles.

Sample	Code
Bread with MFO	Bw/MFO
Bread without MFO	Bwo/MFO
Soup with MFO	FSw/MFO
Soup without MFO	FSwo/MFO
Pure MFO-powder	MFO

Table 4: Codes for the samples used in GC-analysis.

For each sample 2 g of material was measured placed in 20 ml GC vials and sealed with septum crimp tops. For extraction of volatiles.

3.5.4 Scanning electron microscopy (SEM)

SEM was used to study the morphology of the MFO and the fortified bread. This was done in collaboration with NTNU Nanolab. When performing this step, 1 g of sample was dried using a heating oven at 60 °C. The samples were then coated with 10 nm of a platinum- and palladium-mix. The quantity ratio between these metals were 80 to 20 respectively. The samples were analyzed using a "Thermo Fisher/FEI SEM Apreo" microscope (USA).

3.5.5. Informal testing of sensory qualities

During the semester the opportunity arose to do an informal testing of the products at the Norway Seafood Festival. At the event a stand was set up.

During the breaks participants were sporadically recruited to test both the consistency and smell of the products (not eat/taste them!). They were represented with two samples each of the bread and the fish soup, where one of each food model contained MFO-powder. The participants where then asked which of the samples contained MFO-powder and to give input on what led to their decision.

3.5.5 Sensory analysis

Sensory analysis was performed using both hedonic scaling and "check all that apply" (CATA). Both were performed in the same session for each of the samples. Hedonic scaling was performed first and according to Jaeger et al. (2017) it is unlikely that this can result in hedonic bias when performing CATA later. The Panelists were recruited through advertisement at various campuses (posters shown in picture 13, appendix 1 and picture 14, appendix 2). All

participants gave consent in participating to the panel. The products chosen for sensory analysis was bread and fish soup. Codes for the samples of bread and soup is shown in table x.

The test-layout was designed using EyeQuestion version 5.5 (Netherlands). This program collects the data from the contestants after a test. The information can then be transferred to EyeOpnR where it can be processed using analytical tools. (EyeQuestion nd.) The sensory test was held at the sensory lab, Kalvskinnet Campus. It was structured such that the participants only tasted one food group at a time. The participants had to access mentioned questionnaire through QR-code before being presented with two samples, one being a reference and one being the fortified sample.

Sample	**Code**
Bread reference (without MFO)	525
Bread fortified (with MFO)	177
Soup reference (without MFO)	758
Soup fortified (with MFO)	396

Table 5: Codes for the samples used in the sensory analysis.

Beforehand, a pre-test was performed with 3 judges in order to find the most suitable product characteristics/terms for the sensory analysis. During the sensory analysis, the participants were tasked with taking a mouthful of the sample. The consumer where then tasked in rating the sample on how much they liked the product on a scale from 1-9. 1 being the lowest meaning "Don't like at all" and 9 being the highest meaning "Like very much". The consumer was then asked to take another mouthful of the same sample and tick a multiple-choice question for which characteristics or claims they thought fit the sample. List of terms used for bread and soup is shown in table 6 in appendix 3 and table 7 in appendix 4.

The data collected from this thesis would not be connected neither directly or indirectly to the participants devices or their personal data.

3.5.6 Statistical analysis

IBM SPSS Data Editor (vers. 28.0) was used to calculate p-values for texture analysis and viscosity. Since there are two sets of quantitative data to be compared, paired t-test was used to compute the p-values with a 5% level of significance.

Data from sensory analysis was processed using the analytical tool EyeOpenR within Eyequestion. Cochran & McNemar test was used to see whether there are significant differences between samples for different characteristics. Additionally, t-test was performed so see if there were significant differences in overall liking and purchase intentions.

4. Results & discussion

This chapter will present the findings from the analyses, followed by discussion.
It is comprised of 2 sections; food fortification with Omega MFO in the first chapter, and food fortification with Commercial MFO second. The composition of these MFO are quite different. Omega MFO contains gelatin and gum Acacia. The commercial MFO contains caseine and corn starch.

4.1 Food fortification with Omega MFO

4.1.1 Analysis of viscosity

Soup

The flow behavior of both fish soup without MFO and Omega MFO from SINTEF is illustrated in figure 6.

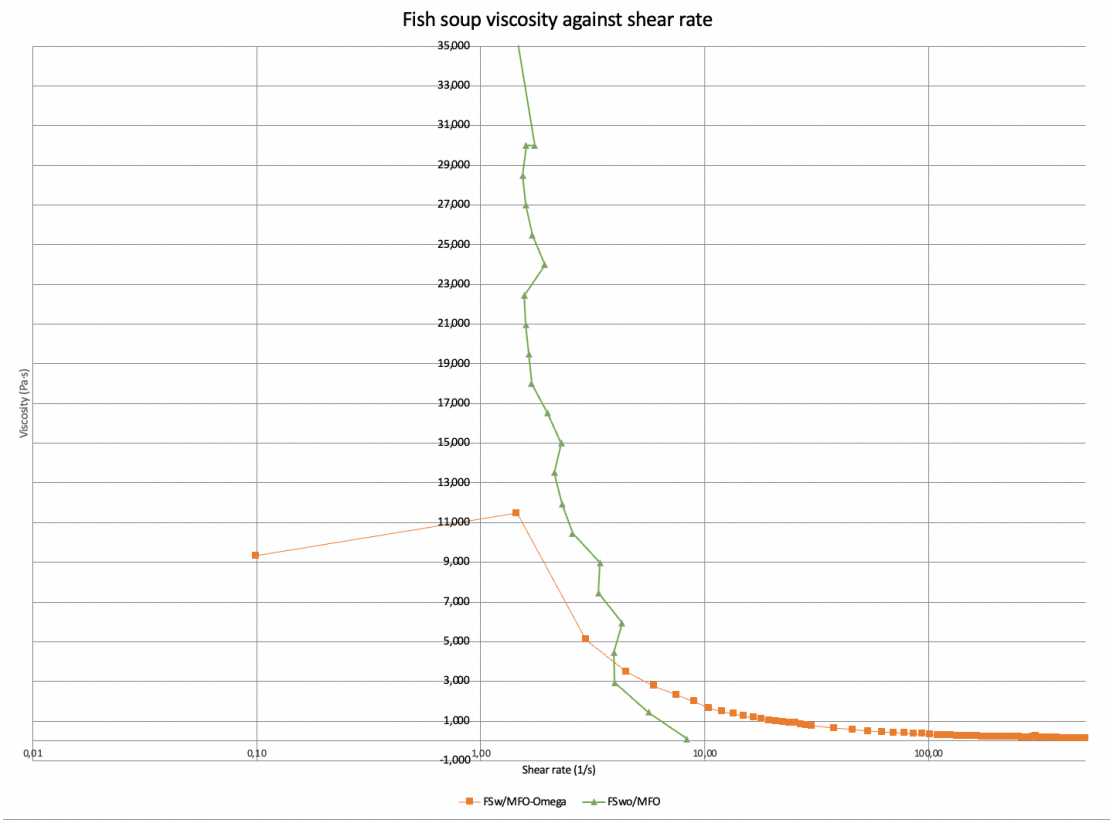


Figure 6: Viscosity against shear rate in fish soup without MFO (green) and with Omega MFO (orange).

The apparent viscosities of both samples decreased with increasing shear rate. In the first measurement the samples display quite dramatically different starting viscosities, but then narrows down. FSw/MFO reaches a flatline were the viscosity is near constant while FFSwo/MFO goes down to zero. This is an interesting find since, as discussed earlier, gelatin and gum acacia should be able to bind water due to its molecular structure and thus increase the apparent viscosity. There may be other factors contributing to this development. According to a study done by Wang et al. (2021), the viscosity of gelatin seems to be negatively correlated to the amount of salt in the liquid. They also observed that chaotropic-anions can promote polymer chains within the compound's matrix. Since the fish soup mixture contains salt, this might help explain the curve.

Gelatin and gum acacia chemically enhance water retention through their unique molecular structure and interactions with water molecules. Their structures allow formation of a three-dimensional network when dissolved in water. Additionally, gelatin has hydrophilic properties, meaning it has a high affinity for water. The amino acids present in gelatin contain polar functional groups, such as hydroxyl (-OH) and amide (-CONH-) groups, which readily interact with water molecules through hydrogen bonding. These hydrogen bonds between gelatin and water molecules contribute to the retention of water within the gelatin network. (Sang et al., 2022) This network might explain the flattening of the FSw/MFO-curve in figure 7.

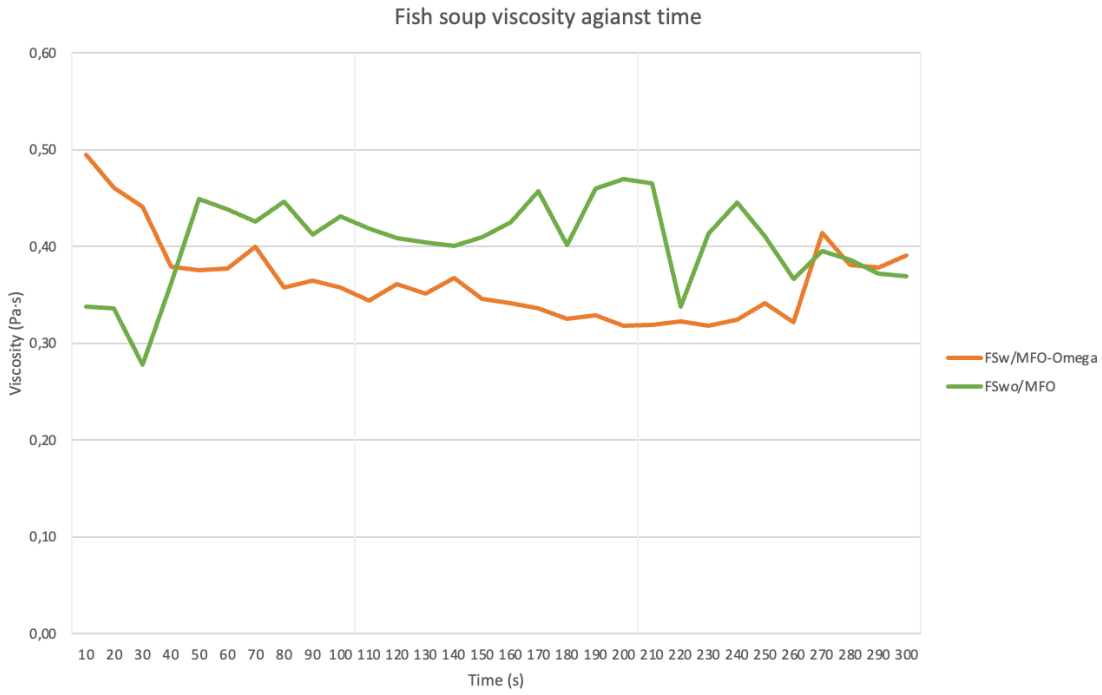


Figure 7: Viscosity against time in fish soup without MFO (green) with Omega MFO (orange).

Results from measuring viscosity against time in fish soup is shown in figure 10. The sample fortified with Omega-MFO proved to be significant from the regular fish soup (FSwo/MFO). The viscosity of all soups did hold relatively stable through the interval, ending between 0.32-0.37 Pa.s. What stand out is that the apparent viscosity of FSwo/MFO is higher than the fortified samples.

What may have happened here is that the gel formation of the gelatin is interrupted or negated. The soup is boiled for 5 minutes. This may affect the physiochemical properties of the soup. As observed in (Hayashi and Oh, 1983) gelatin denatures when exposed to heat. The protein structure in gelatin disrupts, in which leads to the break down the hydrogen bonds thus leading to a loss of its stability to form a gel, which in turn will make it loose its thickening and swelling properties (Bigi et al., 2004). The degree to which this happens varies depending on concentration of gelatin within the soup, cooking time and other ingredients that can intercept with the structural characteristics of gelatin. as discussed earlier.

4.2 Food Fortification with Commercial MFO

4.2.1 Analysis of viscosity

Porridge

Results from measuring viscosity against shear rate in porridge is shown in figure 8. There were differences in apparent viscosity between Pwo/MFO and Pw/MFO. Notably in the first measurement, Pw/MFO has almost twice as high apparent viscosity (2289.22 Pa.S) as Pwo/MFO (1348.78 Pa.s). However, as the shear rate increases the apparent viscosity dramatically decreases in both samples. From the shear rate 30 1/S, the viscosity between the samples appears to be similar. At 500 1/S the final viscosity of Pwo/MFO is at 5.03 while Pw/MFO is 5.56 1/S.

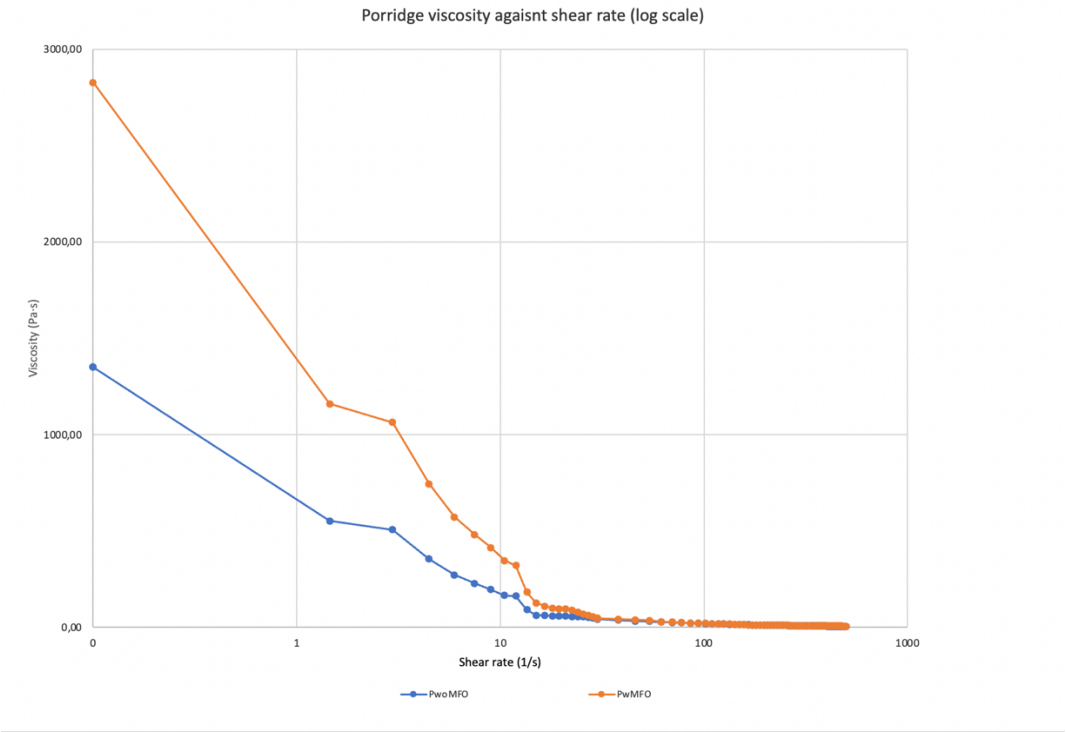


Figure 8: Measurement of viscosity against shear rate in porridge.

The commercial MFO was added to the porridge at 4 ºC. When MFO is added to a liquid and then heat treated, the individual molecules will begin to absorb water and begin to swell. E.g. for gelatin this usually happens at around 40 ºC. (Bigi et al., 2004, González et al., 2021). The commercial MFO however, contains both casein and starch. These compounds are also naturally found within the porridge; casein in the milk, and starch from the barley grains. Starch will start forming gels at around 60 ºC while casein has been shown in Horne (1998) to form gels at lower temepratures (5-10 ºC). The factors could potentially have been affecting the starting viscosity. The MFO was added to the raspberry sauce. When eating this raspberry sauce is to be added to the porridge just before eating. This is a very short contact time for the MFO to react with the products as a whole. Both of these factors might help explain why the viscosities are so close after 10 1/s, as lower degree of gel-network would result in a lower "resistance" towards mechanical stress from the krebs spindle.

Differences in apparent viscosity were also found when measuring the viscosity against time. The results are shown in figure 9. Again, Pw/MFO have in the measurement a viscosity of 45.42 Pa*S and Pwo/MFO 29.42 Pa*S. During the interval, they gradually close into one another, Pw/MFO having by the end a viscosity of 35.97 Pa*S and Pwo/MFO 30.81 Pa*S.

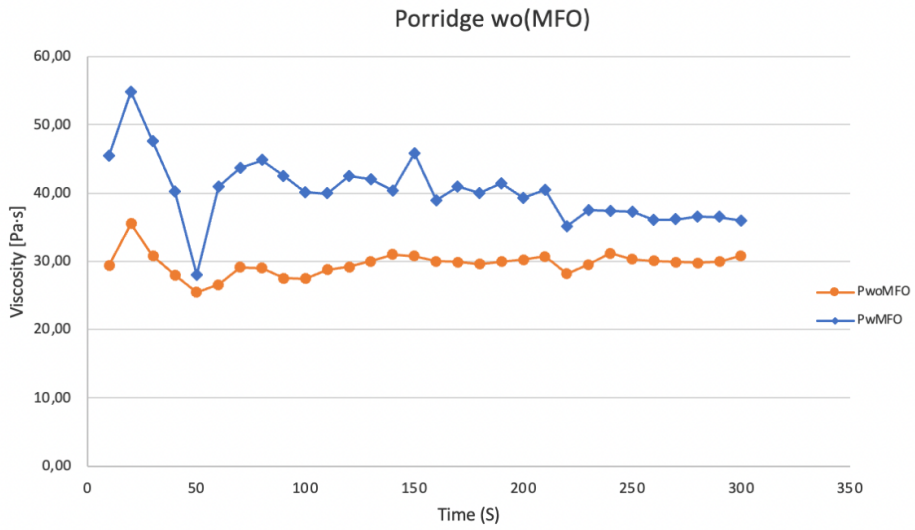


Figure 9: Measurement of viscosity against time in porridge.

The same pattern in figure 8 can been in figure 9, where the lines gradually align. Still the values are significant which means. When formulating a new recipe for the porridge, the polymer-containing ingredients (e.g. starch and casein) could be proportionally changed to the degree of which the MFO affects viscosity. This way the differences could be mitigated.

Based on the results from analyzing viscosity in porridge, it looks like MFO has potential as a suitable ingredient in porridge. With enough MFO to cover 40% of daily recommended intake per portion, the viscosities are not far apart. This could be mitigated when formulating a new product. The porridge has by default casein added into the mixture. Therefore, when formulating a new product, this ingredient could be proportionally reduced to make the viscosity of the fortified porridge match the original product.

Fish soup

The flow behavior of both fish soup without MFO and commercial MFO is illustrated in figure 10.

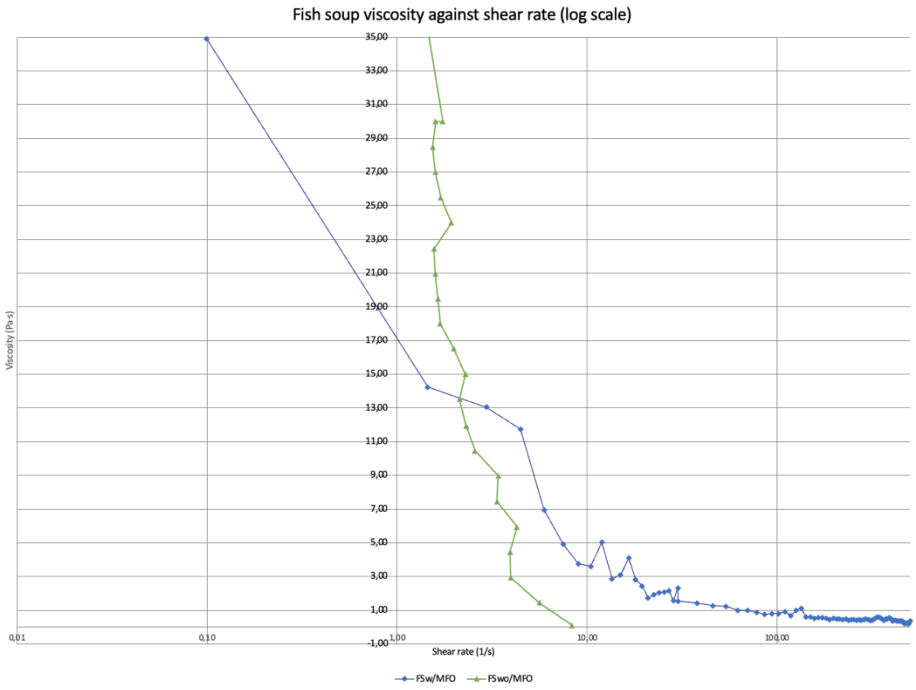


Figure 10: Viscosity against shear rate in fish soup without MFO (green) and with Omega MFO (orange).

Here, the apparent viscosities of both samples also decreased with increasing shear rate. The samples display quit different starting viscosities, but then narrows down. Viscosity of sample FSwo/MFO descends to 0 Pa*s at a shear rate of 9 1/s. FSw/MFO however, almost plateaus at 100 1/s where the viscosity stays in between 0-1 Pa*s. Though the samples proved to be significantly different from each other, the ends of the graph display not too different rheological qualities.

These findings are similar to that of Tavares and Noreña (2020), who conclude that the coacervates-phase acts viscous accordingly with agitation time. In this study however, they used both whey protein isolate with gum acacia, and chitosan with gum acacia respectively. The commercial MFO powder contains casein and starch. The soup was also boiled for 5 minutes. As discussed in chapter 4.1.1, the process of boiling has the potential to alter the structure of proteins. With caseine and starch, it has been found that they possess the ability to restructure gel-structure when cooled. Casein however, when exposed to temperatures above 90 ºC, it has been reported by Nicolai and Chassenieux (2021) to denature. Starch, however,

has been reported to be less affected by boiling. It been reported by Sagum and Arcot (2000) that heating of starch in excess water does not cause any dispersion, most likely because of gelatinization of the starch at high temperatures.

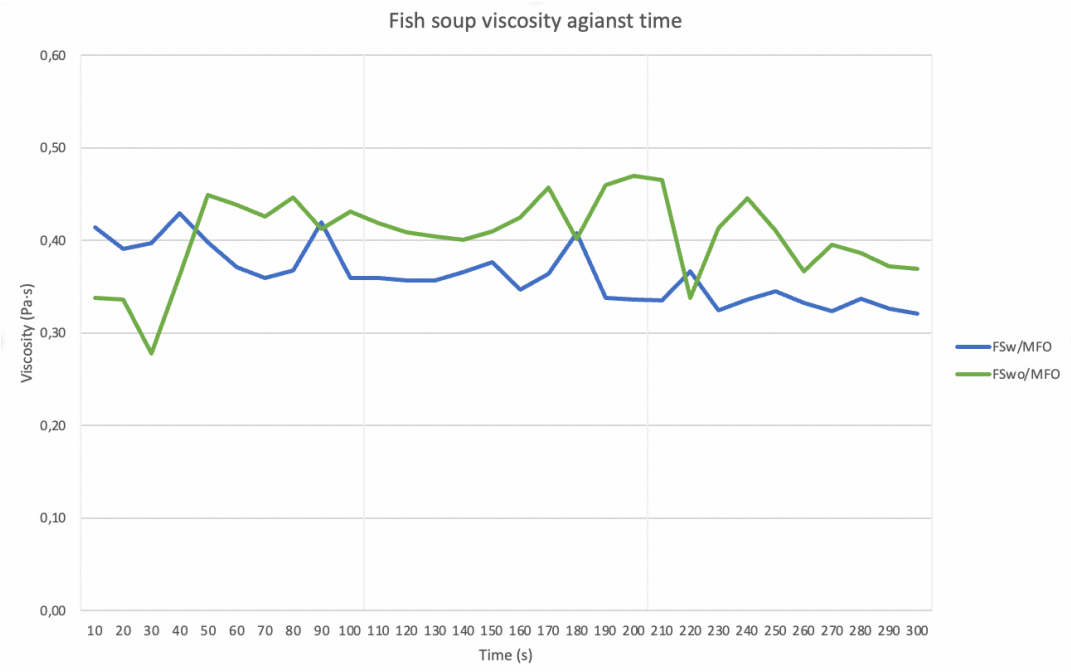


Figure 11: Viscosity against time in fish soup without MFO (green) with commercial MFO (blue).

Results from measuring viscosity against time in fish soup is shown in figure 11. The sample fortified with commercial MFO proved to be significant from the regular fish soup (FSwo/MFO). The viscosity of all soups did hold relatively stable through the interval, ending between 0.32-0.37 Pa*s. What stand out is that the apparent viscosity of FSwo/MFO is higher than the fortified samples.

Based on the results from analyzing viscosity it also looks like the commercial MFO possesses promising qualities as a functional ingredient in fish soup.

4.2.2 Analysis of texture

Results from the texture analyzer are shown in figure 12.

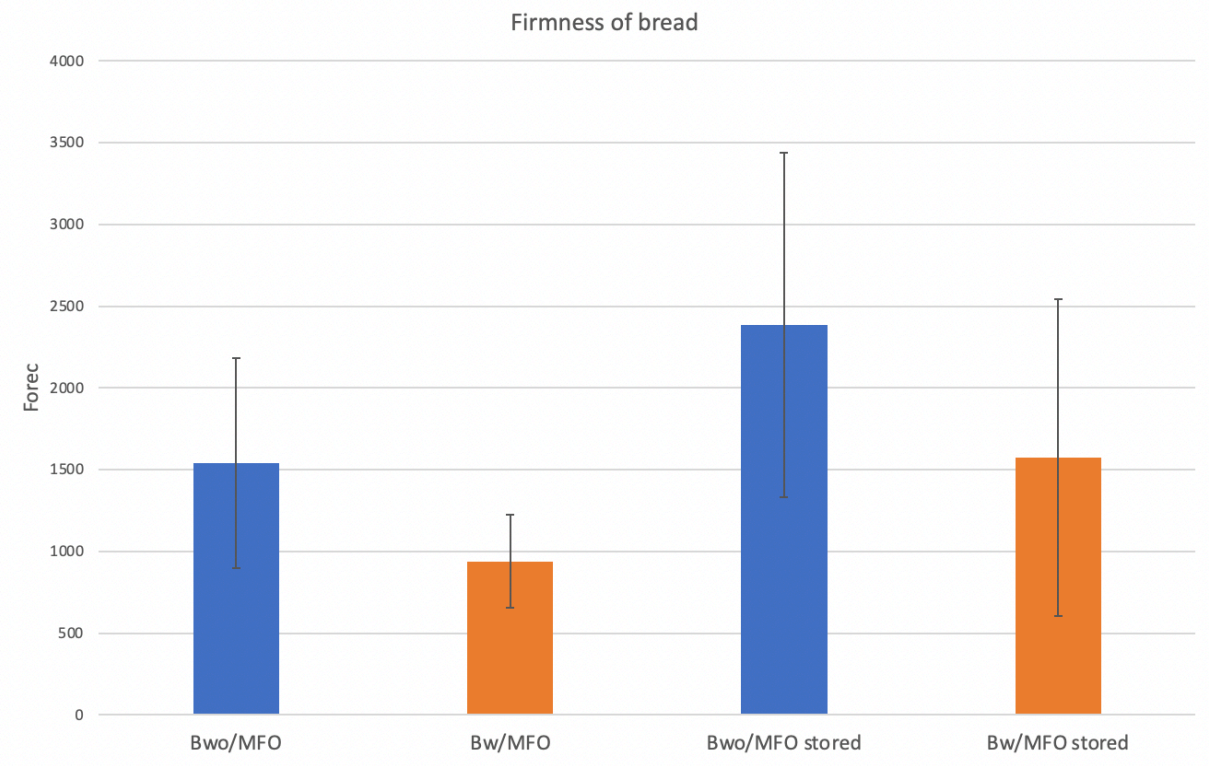


Figure 12: Results from texture analyzer. The two bars furthest to the left are the two samples of bread before storage while the two furthest to the right are stored 7 days at 20 ºC.'

When talking about firmness it is referred to as a textural attribute that refers to the resistance of a material to deformation. In the case of bread, firmness is associated with the structural integrity and density of the crumb that was measured. The presence of MFO had an impact on the development of firmness in the finished bread. Before storage the samples of Bread wo/MFO possessed greater firmness than Bread w/MFO, the former being almost 1/3 higher in firmness. After 7 days storage at 20 ºC firmness for both loaves increased. However, bread w/MFO that was stored had developed a firmness similar to that of bread wo/MFO before storage. Bread wo/MFO had the highest increase in firmness, which shows that the addition of MFO-powder possibly has an positive effect on the bread by decreasing the rate of staling.

When MFO is incorporated into bread dough, The wall material likely interacts and forms a gel-like network during baking. This network interacts with different components in the bread matrix, such as starch, proteins, and water. (Tolve et al., 2022) One way the powder can affect

the firmness of bread is by modifying the water-holding capacity. The powder contains among other things casein that have a high water-binding capacity, which allows it to trap and retain moisture within the network. By doing so, the MFO-powder theoretically helps to maintain the hydration of the bread crumb, preventing excessive moisture loss and maintaining its softness. This moisture retention contributes to a softer and less firm crumb texture. (Hadidi et al., 2021) Moreover, the commercial MFO-powder that was used could possibly also interact with starch molecules in the bread matrix. The powder can bind to starch granules, altering their behavior during gelatinization and retrogradation processes. Retrogradation of starch, which is the re-association of starch molecules after gelatinization. By interfering with starch retrogradation, gelatin can help maintain a more tender and less firm crumb structure. (Serna-Saldivar et al., 2006) Additionally, it has also been shown that this network formed in the bread dough can act as a physical barrier, restricting the movement of water and other molecules within the bread matrix.

By maintaining a more compact structure, the MFO could help prevent excessive softening and maintain the firmness of the bread crumb. It's important however to note that the specific effects of MFO on firmness can depend on various factors, including the type of wall-materials, concentration of the powder, the processing conditions, and the interactions with other ingredients in the bread formulation. As observed by González et al. (2018), bread samples that contained MFO actually had a higher migration of water. This might be because of the wall material being soy protein isolate and maltodextrin, which possess other characteristics than casein and starch.

Furthermore, the commercial MFO contains starch. Starch is naturally found in the bread mix, and as mentioned earlier can form complexes and absorb water. According to Onyango (2016), starch is the second most important fraction within flour for the formation of bread dough. Though starch is responsible for staling through retrogradation, modified starch has the ability to improve the quality of bread. With an increased amount of starch within the bread, it is possible this contributes to increased binding of water within the bread, through interaction with other

Casein is also a component in the commercial MFO. This on the other hand can affect firmness through its ability to form a cohesive and elastic protein network. Casein proteins have unique molecular characteristics that enable them to undergo structural changes, such as unfolding and

reassembling, under certain conditions. During the bread-making process, casein can form a network that contributes to the overall strength and firmness of the bread crumb. (Storck et al., 2013)

Moreover, casein can also influence the water-holding capacity of the bread dough. Casein proteins have hydrophilic regions that can bind and retain water within the bread matrix. By holding onto moisture, casein helps to maintain the hydration of the bread crumb, preventing excessive moisture loss and contributing to a softer and less firm texture. (Goel et al., 1999)
During baking, starch granules absorb water and undergo gelatinization, resulting in a gel-like structure. Casein has been shown to interact with starch molecules and modify the gelatinization process, potentially influencing the firmness of the resulting bread crumb. This is strongly dependent on moisture content and casein proportion to starch. (Fernández-Gutiérrez et al., 2004)

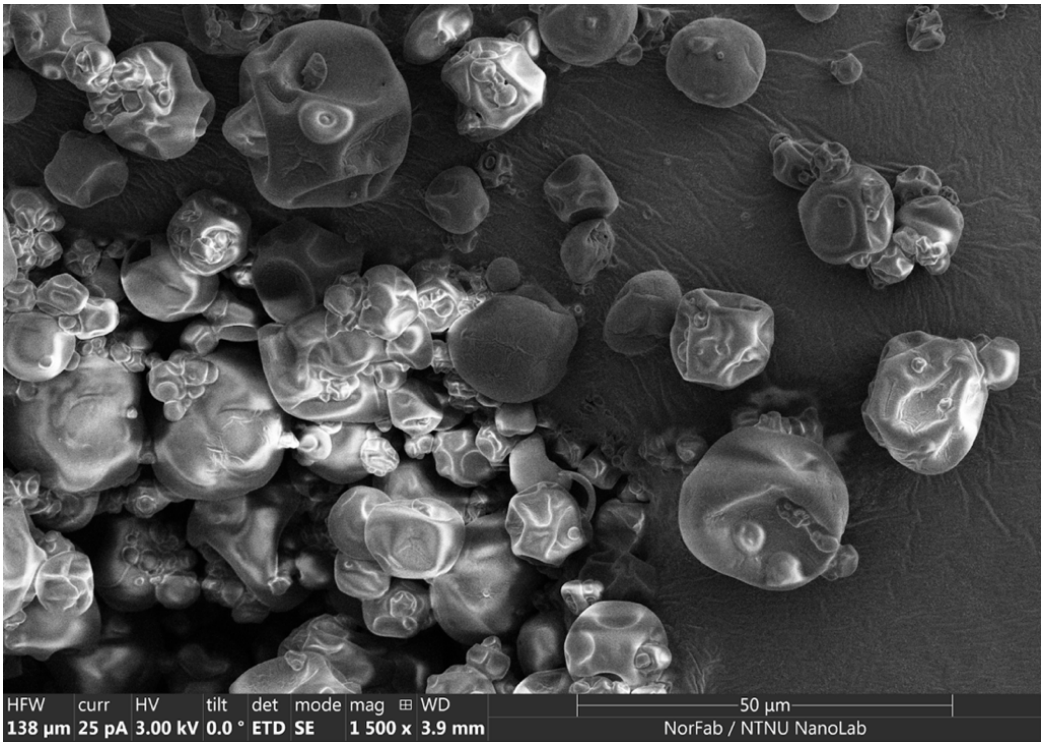
Even though the commercial MFO did affect the texture of the bread, making the texture less firm, it apparently halted staling. This makes for a promising ingredient, expanding the freshness of the bread, thus being suitable functional ingredient in this regard.

4.2.3 Analysis of morphology

SEM pictures obtained from the surfaces examined in this study are shown in pictures 2-12.

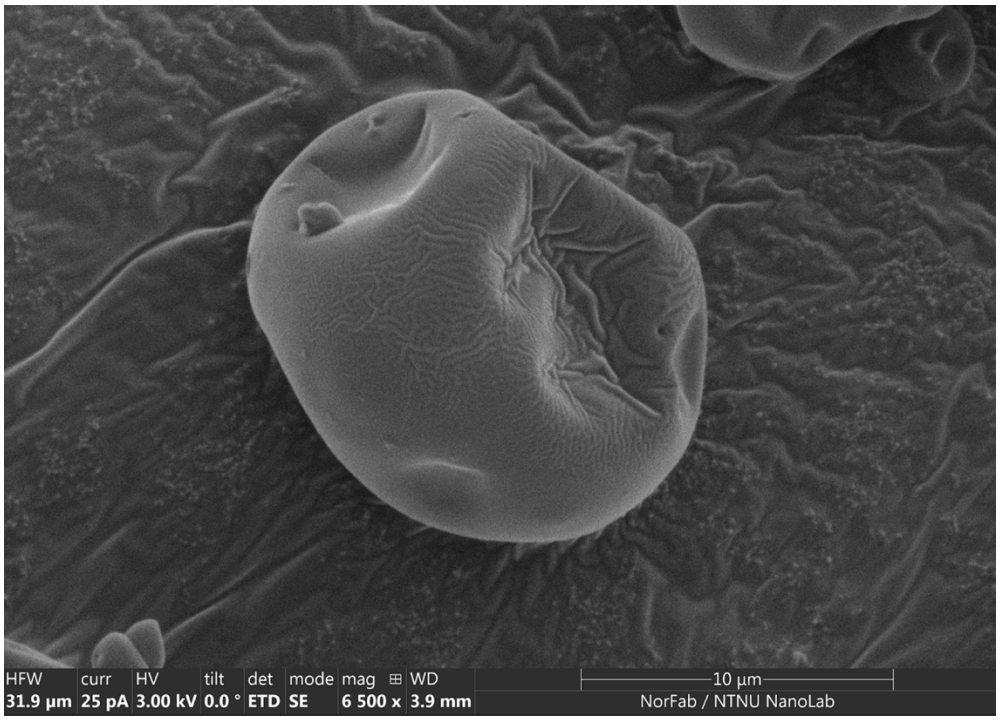
4.4.1 Picture of MFO-powder

Pictures 2 and 3 show Morphological characteristics of MFO-powder at 50 µm and 10 µm respectively.



Picture 2: Picture of MFO-powder at 50 µm. At this length it shows all the different shapes and sizes of granules that exist within the powder.

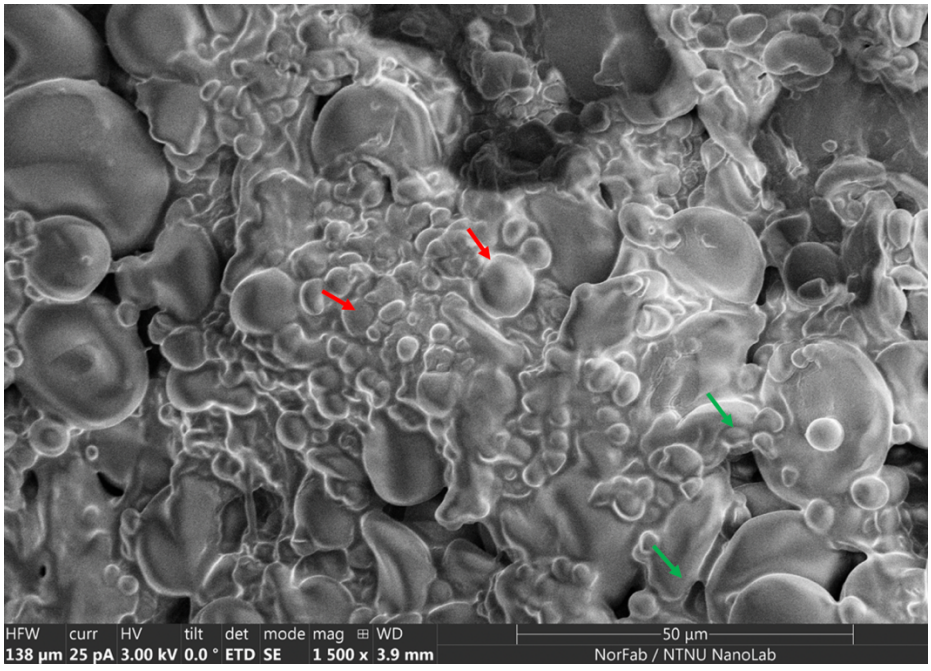
What can be observed in picture 2 are granules in various sizes. The SEM micrographs obtained from the surface show fine powder with particle sizes of 10-20 µm. They all appear to have a rough surface. The larger granules are covered with dents covering the surface area, while the smaller ones have a more uniform and smoother surface. Besides dents, all granules also seem to have small lumps sticking out from the walls. A zoom of one granule at 10 µm is shown in picture 3. In this picture one can see that around the dents one can see that the flat area around appears to have a wrinkled surface, which is an important marker when trying to identify the powder in the bread after baking.



Picture 3: MFO-granule at 10 µm. At this length one can more clearly see surface characteristics of the granule.

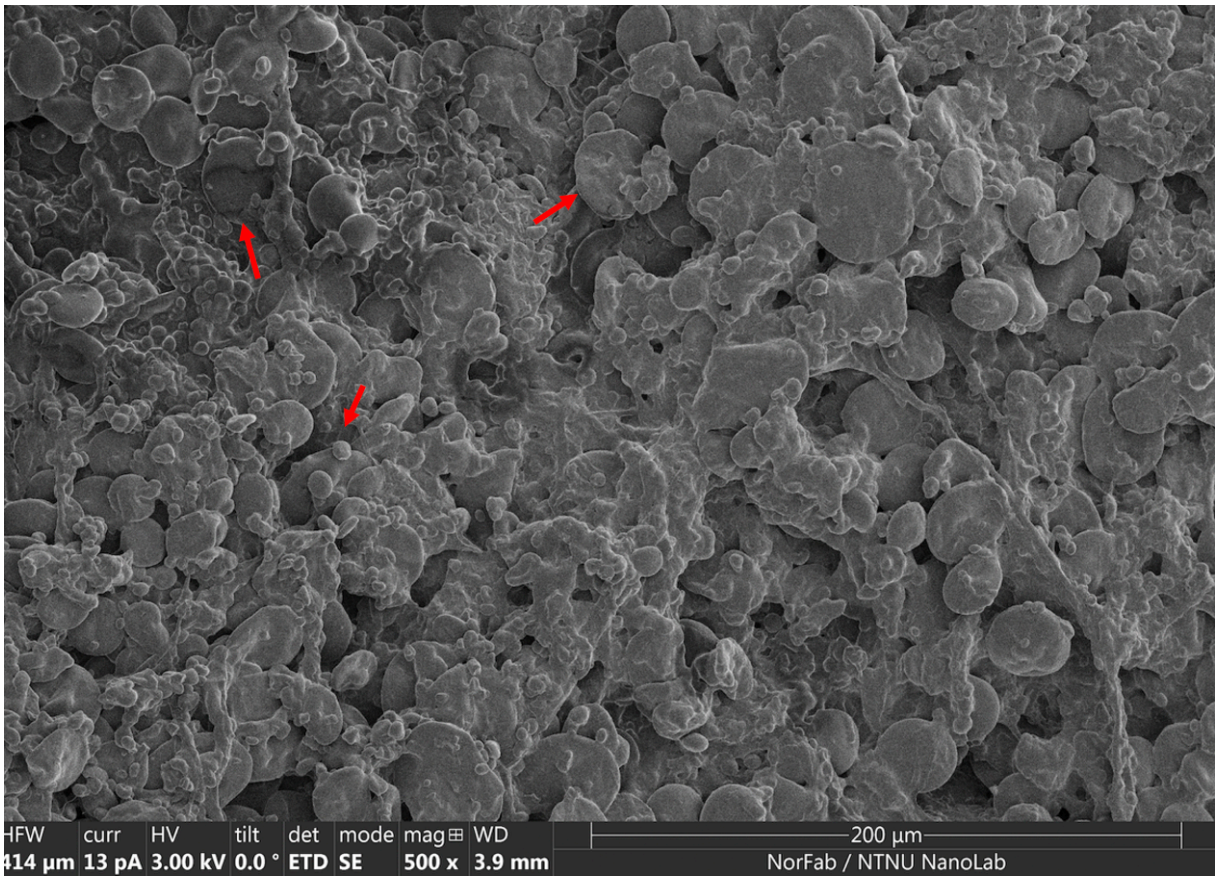
4.4.2 Pictures of bread without MFO

Picture 4 and 5 shows morphology characteristics of Bwo/MFO at 50 µm and 200 µm respectively.



Picture 4: Picture of sample Bwo/MFO at 50 µm. Red arrows points at what appears to be starch granules, while green points at air bubbles/vacuoles.

In picture 4, we can see starch granules which are densely distributed at this magnification (red arrows). Protein fibrils are also noticeable (which can been seen as strands within the dough). There are individual cells/compartments that resemble irregularly shaped structures and gas bubble or vacuoles noticeable which further adds to the roughness of the surface (green arrows). These are likely due to gelatinization which can cover the surface and make it look coarse. (Kim et al., 2003)

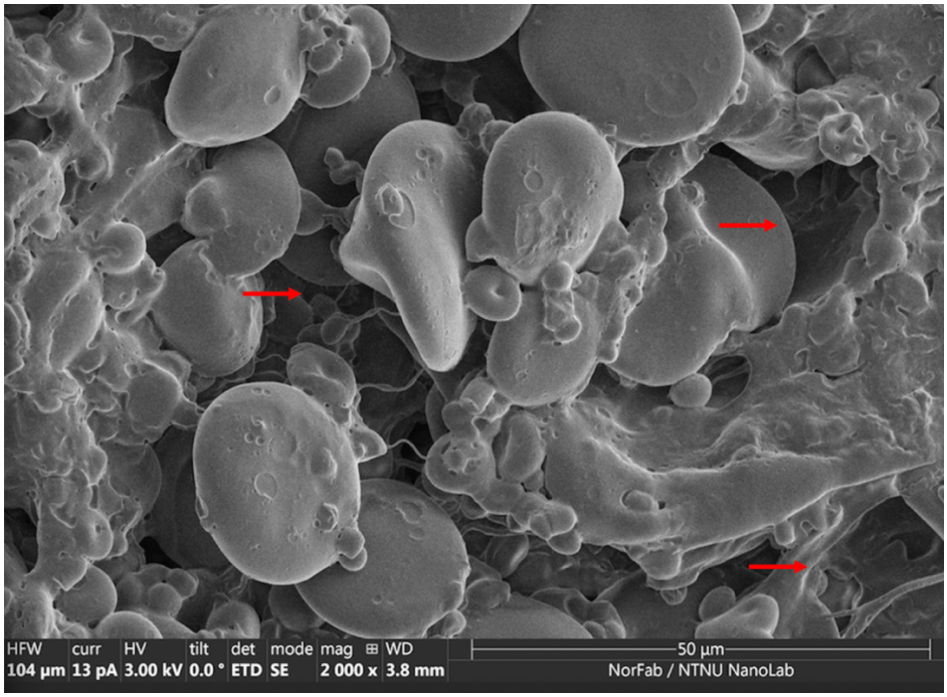


Picture 5: Picture of sample Bwo/MFO at 200 µm. Red arrows points at what appears to be starch granules.

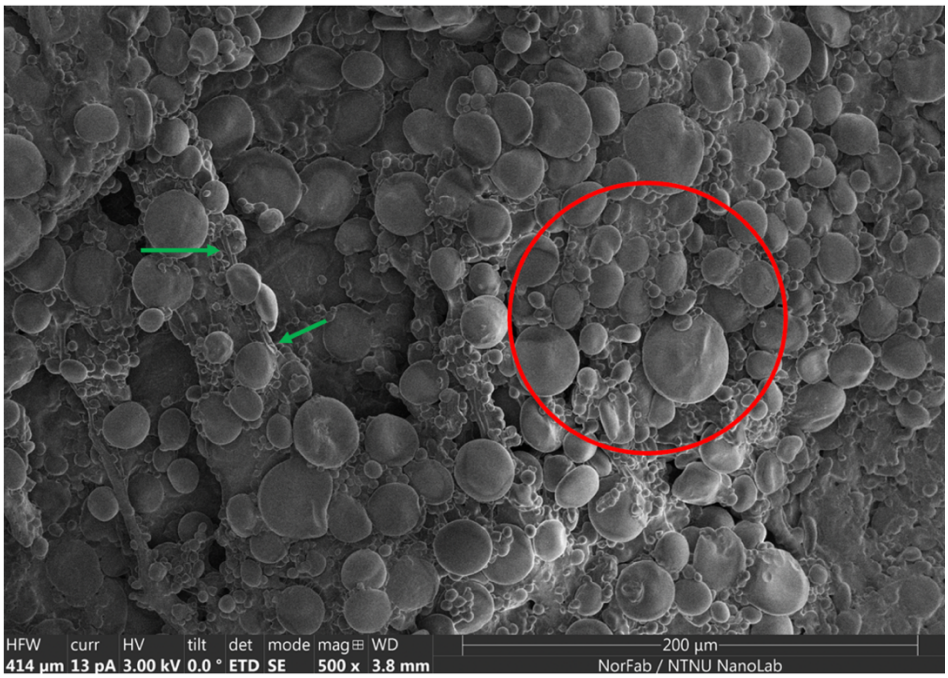
In picture 5 we get a clearer view of the bread crumb. Here spherical starch granules (red arrows) are distributed within a protein matrix. The shapes of the starch-granule vary radically in size. This is consistent with findings from Hayta and Hendek Ertop (2018) who reported a compact microstructure with protein network including starch granules.

4.4.3 Bread with MFO

Picture 6 and 7 shows structural characteristics of sample Bw/MFO at 50 µm and 200 µm respectively.

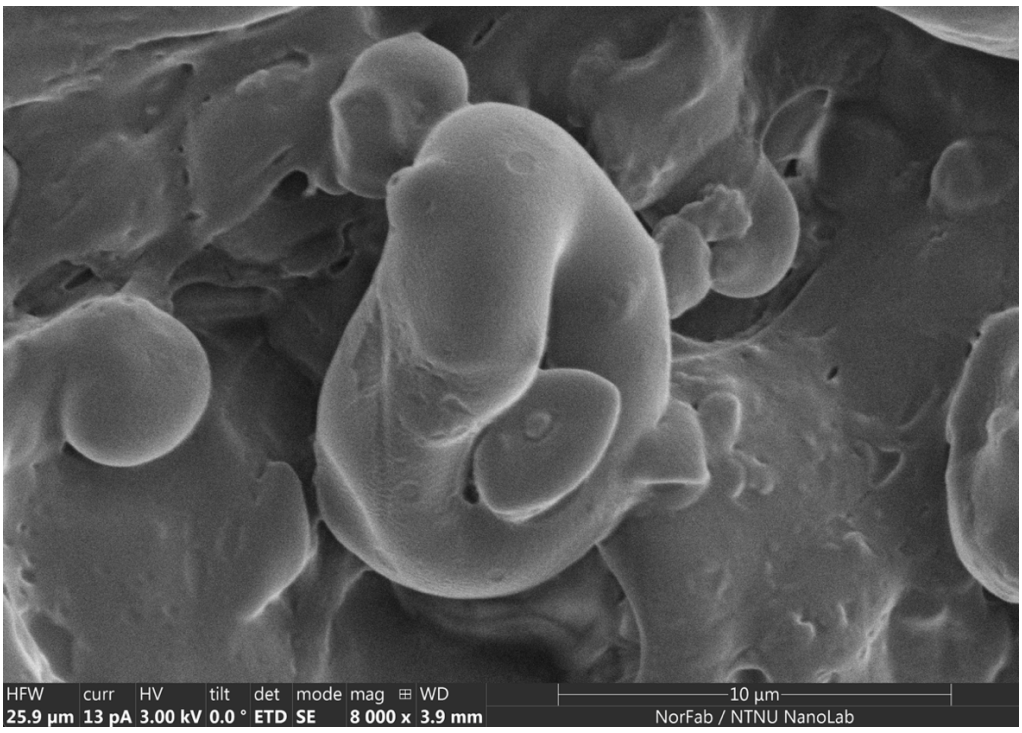


Picture 6: Picture of sample Bw/MFO at 50 µm. Red arrows points at what appears to be part of the protein matrix.



Picture 7: Picture of sample Bw/MFO at 200 µm. Red circle highlights what appears to be a bundle of starch molecules, while green arrows point at what appears to be protein fibrils.

In picture 6, similar characteristics to that of picture 4 can be seen. However, it appears here that the fortified bread looks more brittle. It was observed larger pockets of air within the crumb and less of a more cohesive protein matrix that cover the starch granules (red arrows). This is more clearly illustrated in picture 7. Here Starch granules are more defined and look more plentiful (red circle). It looks like the protein matrix is lessened, though protein fibrils are still possible to see (green arrows). Even though only 20 grams of MFO was added, it seems to have a dramatic effect on the structure of the bread. This may be linked to the findings in chapter 4.1. Since this bread has been baked one cannot discard the possibility that the MFO dissolved during the process of kneading and baking the bread, even though intact MFO-granule was observed in the bread (picture 8). The granule has kept its shape, which is promising. This was also observed by Akhtar et al. (2022), which could mean a good omega fatty acid retention within the bread.



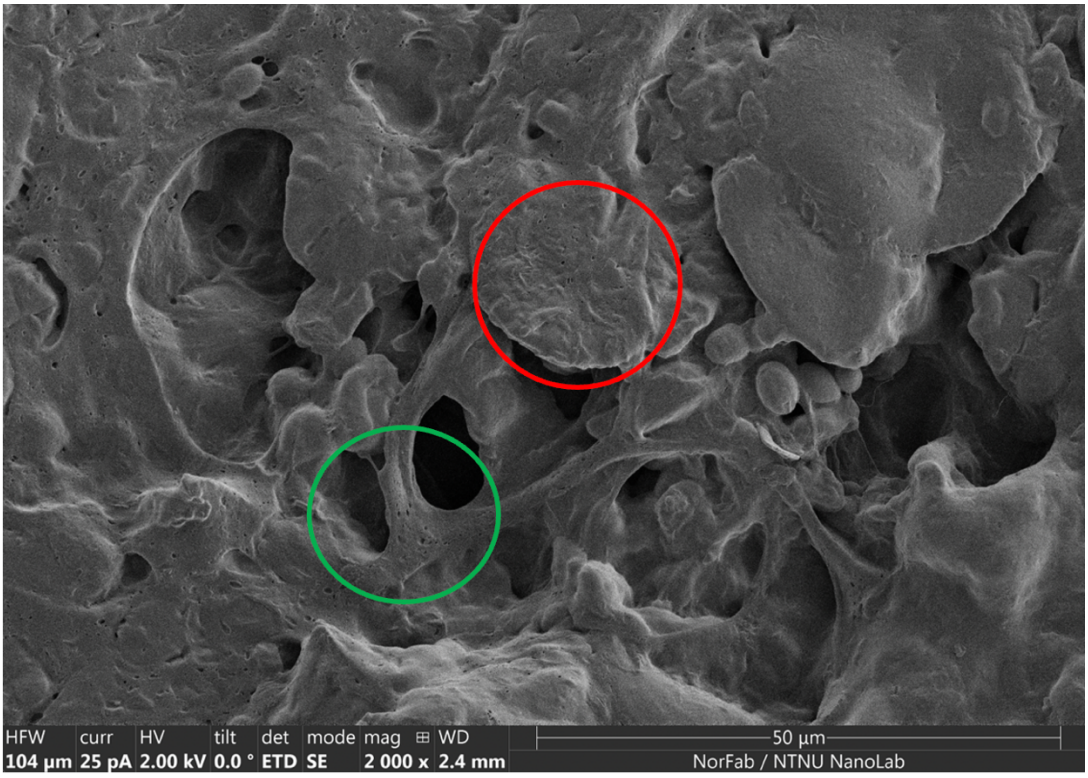
Picture 8: Picture of MFO-granule found in sample Bw/MFO (after baking).

If this was not the case and the granules have to a large enough structurally disintegrated, then this might be the cause of apparent weak protein-development. The oil can also lubricate the protein matrix, which in turn makes it more difficult to for gluten proteins to form strong bonds thus reducing elasticity of and weakening the structure of the bread. This may also help explain the results from the texture analyzer as this in turn will make the bread become more crumbly. (Verbauwhede et al., 2018) The bread was made using water at 35 ºC. Additionally, the bread

was kneaded, which causes friction and can potentially increase the temperature of the dough even further. The oil which is inside the MFO-might therefore be the reason that the degree of protein development is not as large as in Bwo/MFO. In a study done by Conforti and Strait (1999) they observed that fat can interfere with gluten development. What might therefore have happened is that when fat was introduced into the dough, it can impede the gluten structure.

4.4.4 Bread after 7-day storage at 20 ºC

Picture 9 and 10 shows morphological characteristics of sample Bwo/MFO at 50 µm and 200 µm respectively.

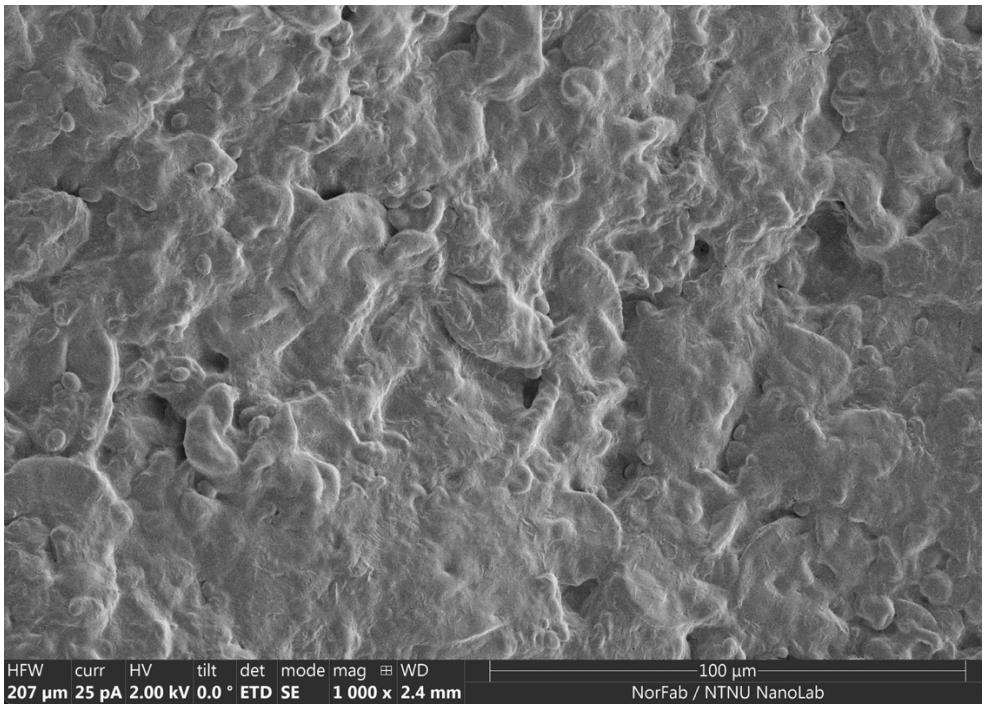


Picture 9:Picture of sample Bwo/ MFO (stored 7 days at 20 ºC*) at 50 µm. Green circle highlights what appears to be a porous protein fibril. Red circle highlights what appears to be wrinkly starch granules.*

Comparing picture 9 with picture 4 one can see that after 7 days of storage, the bread looks more compact. Both starch granules and protein fibrils seem to have retracted. The surface of the starch granules have developed wrinkles (red circle) while the protein fibrils have developed pores (green circle), which might be because of structural changes that happens during storage.

During storage, the gluten network in bread can undergo changes that affect its structure and texture. One of these changes is retrogradation, which is a process in which the starches in bread undergo structural changes. This can occur especially in bread that contains a high proportion of amylose. Retrogradation leads to the recrystallization of amylose molecules, causing them to become more tightly packed. As a result, the bread's crumb becomes firmer. (Hayta and Hendek Ertop, 2018)

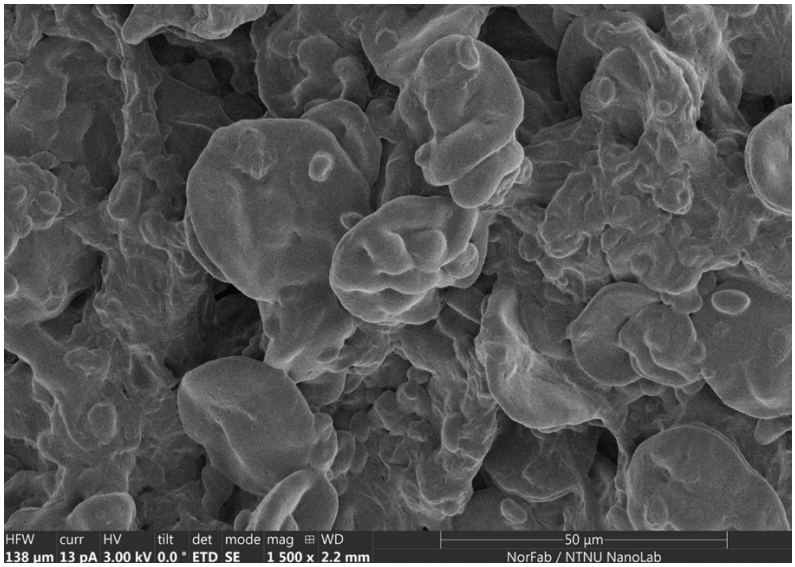
Another way the gluten network can be affected during storage is through moisture migration. Moisture migration refers to the movement of water within the bread. Over time, moisture tends to migrate from the bread's crumb to the crust, which can lead to a loss of moisture in the crumb. This loss of moisture can result in a staling effect, causing the bread to become dry and stale. (Breaden and Willhofr, 1971)



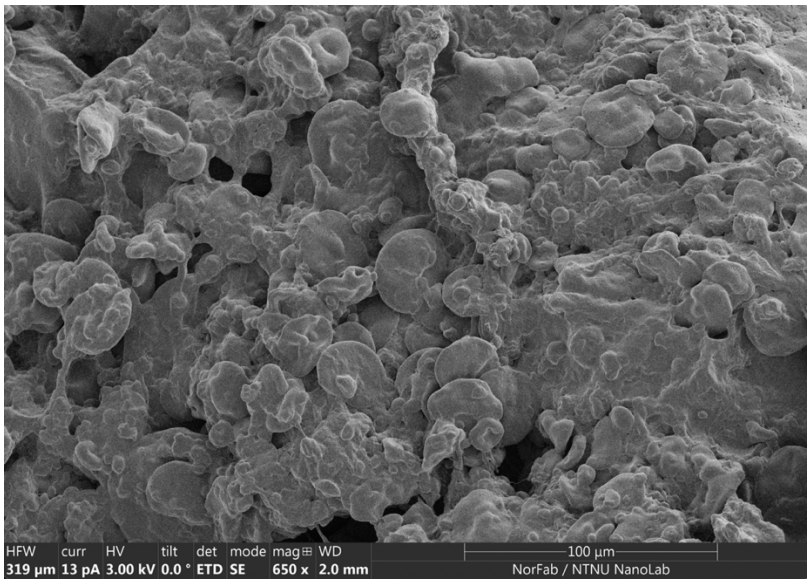
Picture 10: Picture of sample Bwo/MFO (stored 7 days at 20 ºC*) at 100 µm.*

Picture 11 and 12 shows structural characteristics of sample Bw/MFO at 50 µm and 200 µm respectively. Bw/MFO on the other hand seems to have minimally changed their appearance, when compared to before storage (picture 6 & 7). MFO was not possible to observe in this sample since as mentioned earlier the starch-granules had gotten wrinkles on their surfaces, which make it harder to decide if structures observed are MFOgranules.

The reason for this might be that the microcapsules act as small reservoirs of moisture within the bread. As the bread ages and starts to lose moisture, the microcapsules release small amounts of fish oil. This oil interacts with the surrounding environment, primarily the bread's crumb, and helps to retain moisture. In a study done by Smith and Johansson (2004), they found that fat slows down the moisture migration from the crumb to the crust. The oil might therefore have the ability to trap moisture within the bread. This might explain the lower degree of textural change.



Picture 11: Picture of sample Bw/MFO (stored 7 days at 20 ºC) at 50 µm.



Picture 12: *Picture of sample Bw/MFO (stored 7 days at 20 ºC)* at 100 *µm.*

Still, it appears that the MFO is relatively stable when added to bread. Even though the amount added into the dough was low, it was still possible to observe a granule. It seems to have kept its integrity even after baking. This is a good thing a sit indicates that bread is a suitable oh having omega-3 microcapsules as a functional ingredient.

4.2.4 Analysis of volatiles

MFO

Results from GC for MFO-sample is shown in figure 13. When compared to the Mass Spectral library, there were peaks matched(over 90 % match) for hexanal (retention time ~12-13), 1-penten-3-ol (retention time- ~9.2), 1-pentanol (retention time ~11) and heptanal (retention time~15.7. With 90 % matched scores it is highly possible that the correct compound that is registered. (Song et al., 2004)

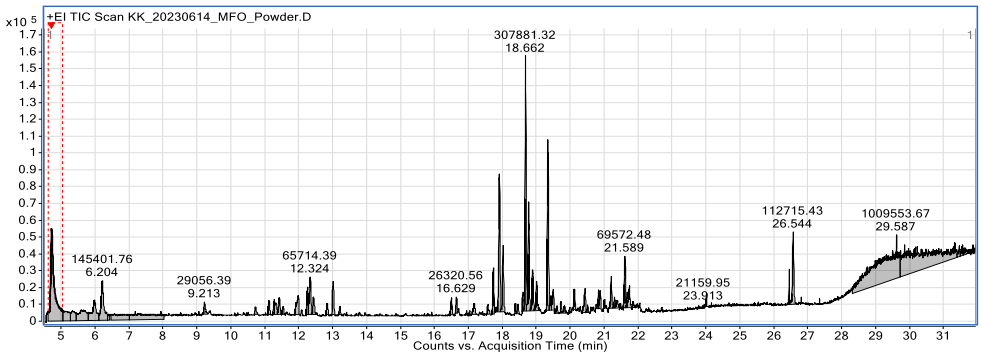


Figure 13: Gas Chromatogram of MFO-powder. The upper number for each peak shows peak area, while the lower number shows retention time. Hexanal is believed to have a retention time at 12.324, 1-penten-3-ol at 9.213, and 1-pentanol around 11.

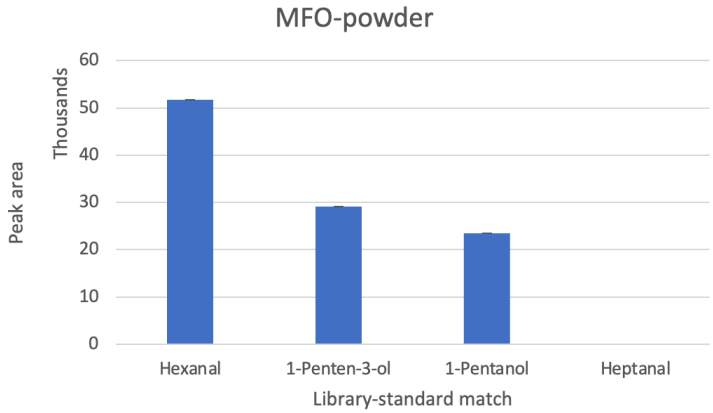


Figure 14: Volatile analysis of commercial MFO.

When analyzing MFO; Hexanal, 1-penten-3-ol and 1-pentanol were present in the samples (shown in figure 14). Hexanal increased with the fortified bread sample (figure 22), indicating the addition of MFO increases formation of hexanal. Hexanal was also detected in the fish soup samples (figure 17), though the difference between the reference and the fortified sample were not large.

Soup

Results from GC for reference sample (FSwo/MFO) and fortified sample (FSw/MFO) is shown in fidure 15 and 16. When compared to the Mass Spectral library, there were peaks matched (over 90 % match) for hexanal (retention time ~12-13), 1-pentanol (retention time ~11) and heptanal (retention time~15.7).

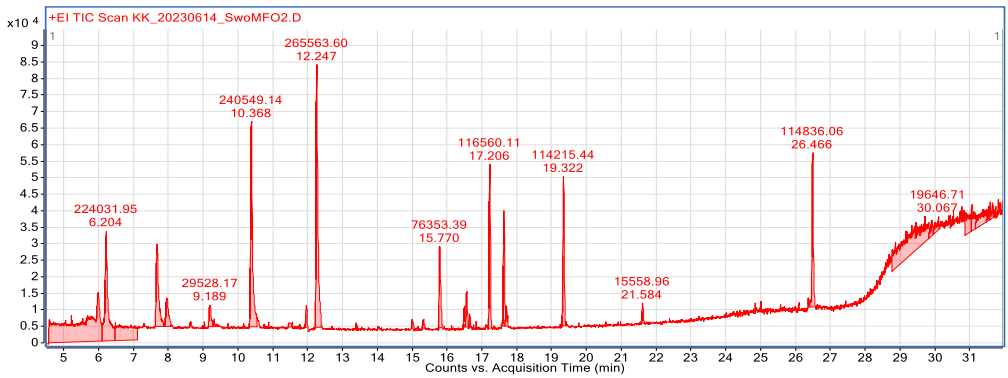


Figure 15: Gas Chromatogram of FSwo/MFO. The upper number for each peak shows peak area, while the lower number shows retention time. Hexanal is believed to have a retention time at 12.247, 1-penten-3-ol at 9.189, 1-pentanol at around 11, and heptanal at 15.77.

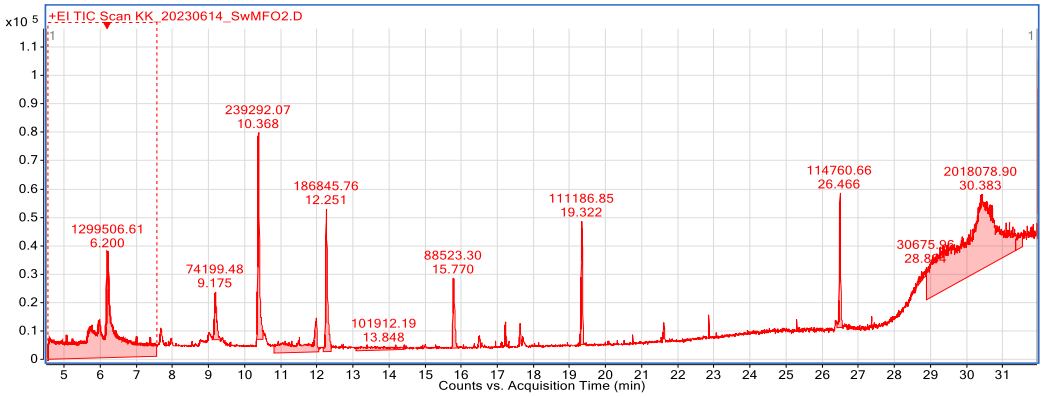


Figure 16: Gas Chromatogram of FSw/MFO. The upper number for each peak shows peak area, while the lower number shows retention time. Hexanal is believed to have a retention time at 12.251, 1-penten-3-ol at 9.175, 1-pentanol at around 11, and heptanal at 15.77.

When looking at figure 17, hexanal is lower in fish soup with MFO (FSw/MFO). In figure 18, fish soup without MFO (FSwo/MFO) has a higher 1-pentanol peak area than FSw/MFO. The amount of heptanal was almost double in FSw/MFO.

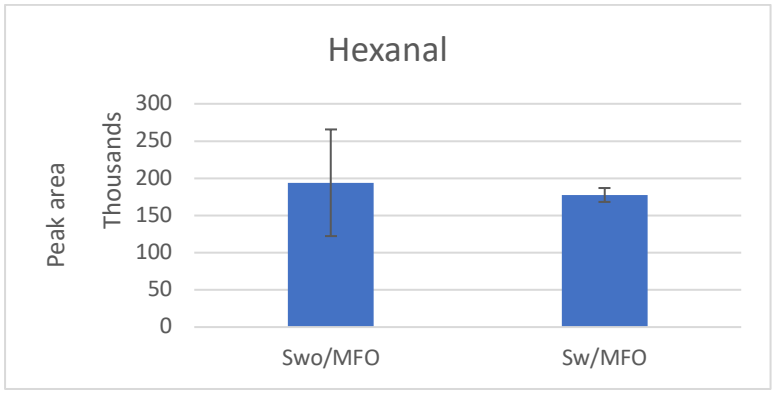


Figure 17: Volatile Analysis of fish soup-samples. Peak was found to match library-standard with hexanal. Due to high standard deviation, standard error was used for the error bars.

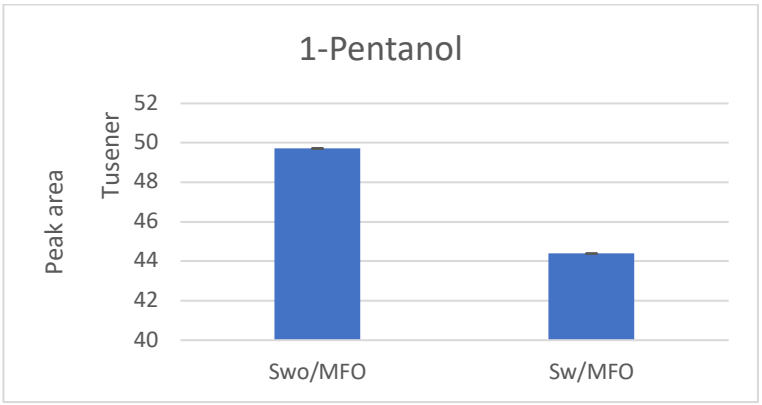


Figure 18: Volatile analysis of fish soup-samples.

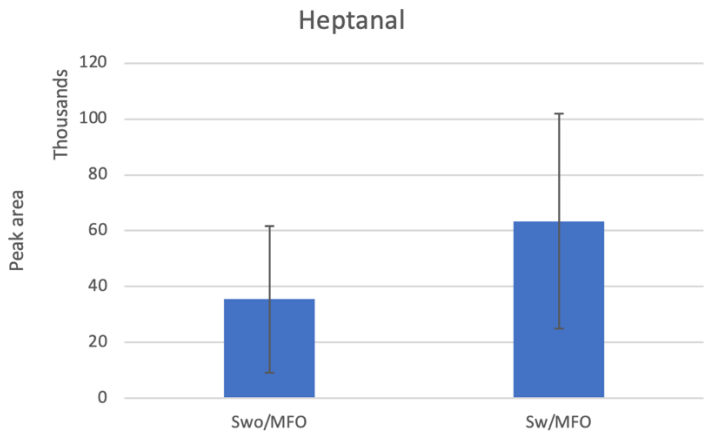


Figure 19: Volatile analysis of fish soup-samples. Peak was found to match library-standard with Heptanal. Due to high standard deviation, standard error was used for the error bars.

Even though the results from measuring of volatiles shows that the content of both hexanal and 1-pentanol is higher in the samples without MFO. This might be due to a few things. Firstly there are only 2 parallels for each sample, which might be the reason for such high error bars. Secondly, there could be a due to contamination of other oils within the sample. The fish soup mix has by default rapeseed oil added into it.

Volatile compunds present in fortified samples were also present in samples without fortification this could be due to the presence of other ingredients in the bread and soup. The more prominent ingredient which is already within both the bread- and fish soup-mix is Rapeseed oil.

Rapeseed oil can leave residue on the GC equipment. This residue could potentially adsorb analytes, reducing their availability for separation and detection. It may cause ghost peaks or disturbances in the baseline of the chromatogram, making it difficult to identify and quantify target analytes. (Sato, 1994) Another issue is the potential for rapeseed oil to foul the GC column. Rapeseed oil contains fatty acids, which can coat the stationary phase of the column. This coating reduces the separation efficiency and alters peak shapes. As a result, poor resolution, peak tailing, or increased retention times can occur. (Wang et al., 1997) Rapeseed oil also has a complex matrix, containing various compounds such as triglycerides, fatty acids, sterols, and impurities. When rapeseed oil is added to a sample, these components can elute at similar retention times as the analytes of interest. This co-elution can result in overlapping peaks in the chromatogram, making it challenging to accurately identify and quantify the target analytes. (Ballesteros et al., 1996) Another effect is the distortion of peaks in the chromatogram. The presence of rapeseed oil in the sample can cause peak distortion, altering the shape and integrity of the analyte peaks. This distortion can manifest as peak broadening, splitting, or tailing. Distorted peaks can lead to inaccuracies in peak integration and quantification, affecting the reliability of the GC results.

The impact of rapeseed oil on GC results however, depend on different factors, such as the concentration of oil in the sample, the specific compounds being analyzed, the composition of the rapeseed oil itself, and the chromatographic conditions used. To mitigate these effects, it is possible to set up appropriate controls and validation procedures. This can include using blank samples without any rapeseed oil as a reference, conducting calibration curves using standard solutions that mimic the sample matrix, and employing suitable sample preparation techniques

to minimize the interference from rapeseed oil. Additionally, optimizing chromatographic conditions, such as adjusting column selectivity or using different detectors, may help mitigate the impact of rapeseed oil on the GC results. (Beens et al., 2000, Mostafa et al., 2012, Richter and Schellenberg, 2007)

Bread

Results from GC for reference sample (Bwo/MFO) and fortified sample (Bw/MFO) is shown in figure 20 and 21. When compared to the Mass Spectral library, there were peaks matched (over 90 % match) for hexanal (retention time ~12-13), 1-penten-3-ol (retention time- ~9.2), 1-pentanol (retention time ~11) and heptanal (retention time~15.7).

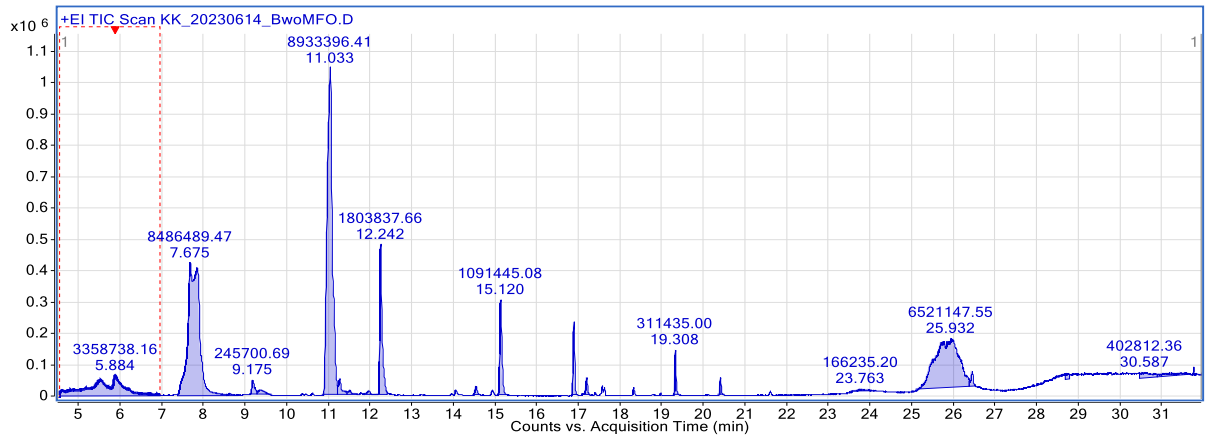


Figure 20: Gas Chromatogram of Bwo/MFO. The upper number for each peak shows peak area, while the lower number shows retention time. Hexanal is believed to have a retention time at 12.242, 1-penten-3-ol at 9.175, 1-pentanol at 11.033, and heptanal at 15.12.

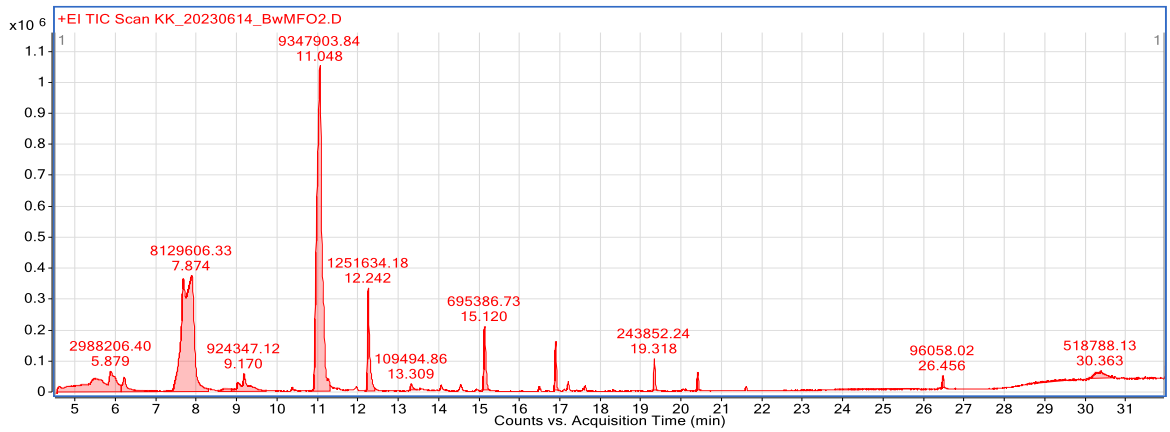


Figure 21: Gas Chromatogram of Bw/MFO. The upper number for each peak shows peak area, while the lower number shows retention time. Hexanal is believed to have a retention time at 12.242, 1-penten-3-ol at 9.17, 1-pentanol at 11048, and heptanal at 15.12.

In figure 22, formation of 1-penten-3-ol also increased with the fortified bread. This indicates that MFO increased the formation of 1-penten-3-ol.

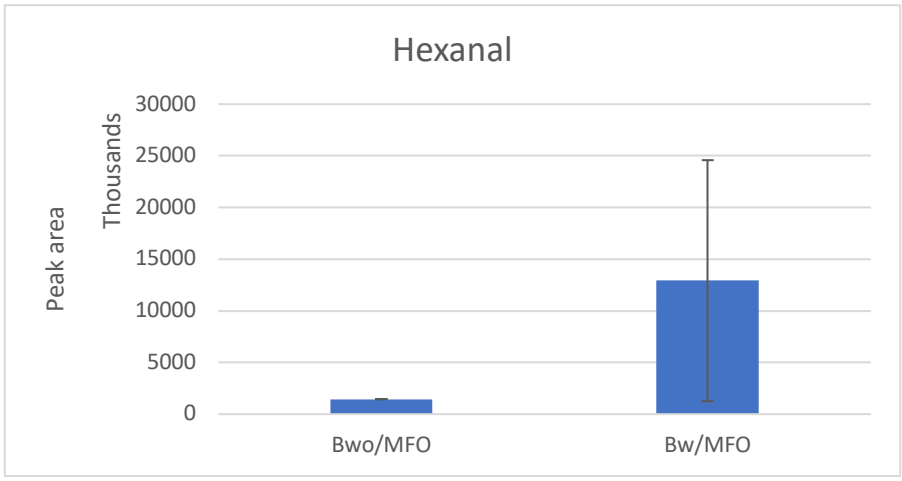


Figure 22: Volatile analysis of bread-samples. Due to high standard deviation, standard error was used for the error bar.

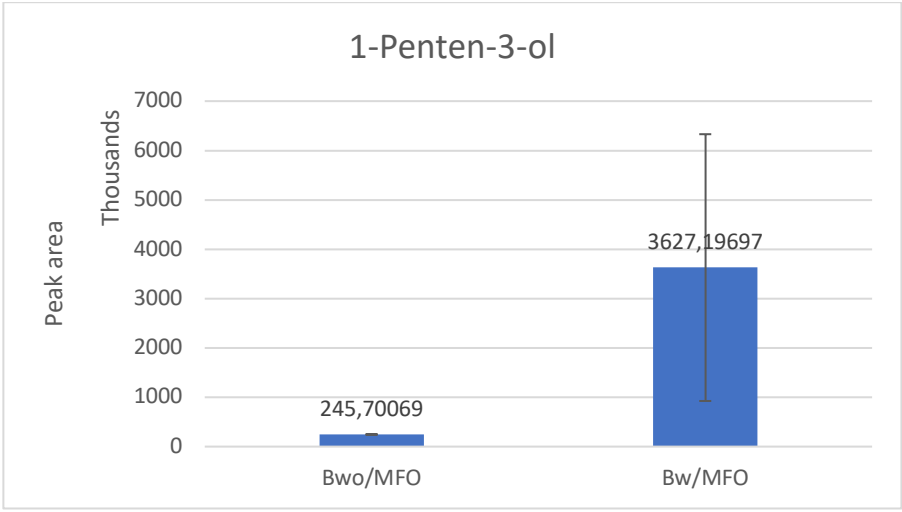


Figure 23: Volatile Analysis of bread-samples. Due to high standard deviation, standard error was used for the error bar.

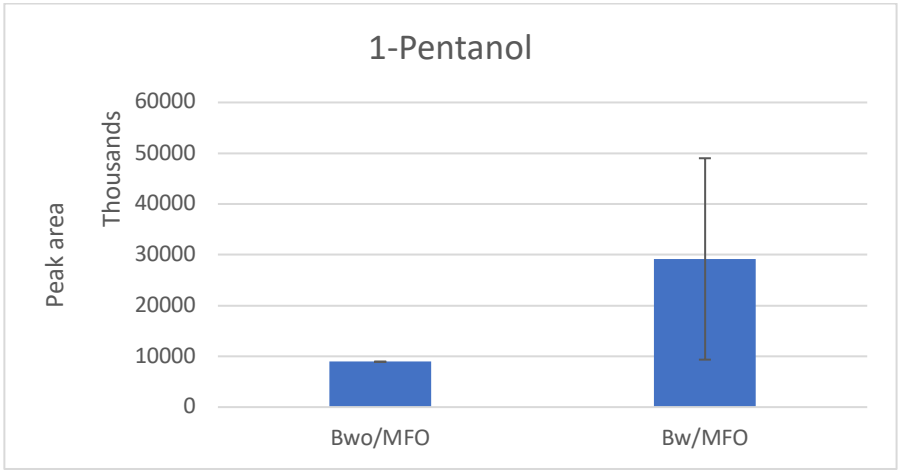


Figure 24: Volatile analysis of bread-samples. Due to high standard deviation, standard error was used for the error bar.

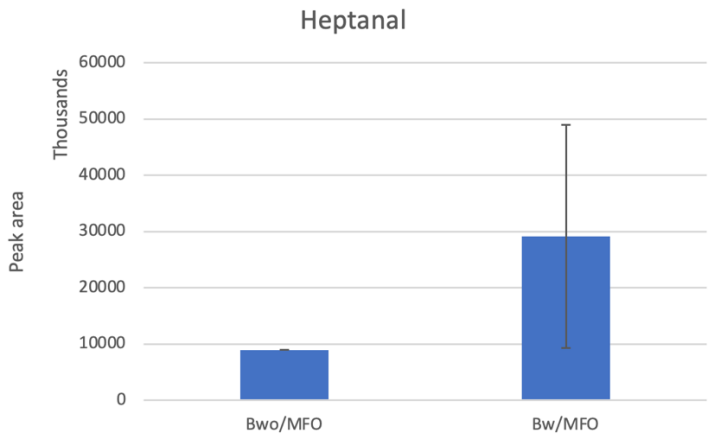


Figure 25: Volatile analysis of bread-samples. Due to high standard deviation, standard error was used for the error bar.

From figure 24, Bw/MFO has a higher 1-pentanol peak area than Bwo/MFO. For Heptanal, both food models (figure 25 & 26) displayed higher peak areas in fortified samples.

Based on the results of analyzing volatiles in bread, the stability of the MFO seems to be affecting the chemical quality of the bread. Though figure 22-25 have quite the large error bars, similar results were reported by Takeungwongtrakul et al. (2015a), who observed that the addition of microencapsulated shrimp oil did have a negative effect on the bread. The bread used in this study was stored for 3 days, while the bread in this study was mad the day beforehand.

4.2.5 Informal tasting

The results from this part of the thesis will be discussed together in chapter 4.6, together with the sensory analysis.

Input was received from 27 people. Firstly, the soup. Interestingly, FSwo/MFO was reported to smell more than FSw/MFO. It was also reported that FSwo/MFO has a more undesirable color.

Secondly the bread. Bwo/MFO was reported to be denser, have a darker color and has a subtle smell. Bw/MFO was reported to look more appetizing, have a lighter color, and a fresher smell.

4.2.6 Sensory analysis

Bread

Consumers liked the reference sample (Bwo/MFO) more than the fortified sample (Bw/MFO) (see figure 26). On a scale from 1-9, both samples received a score of over 6,00. This indicated that the consumers were more satisfied than dissatisfied with both samples.

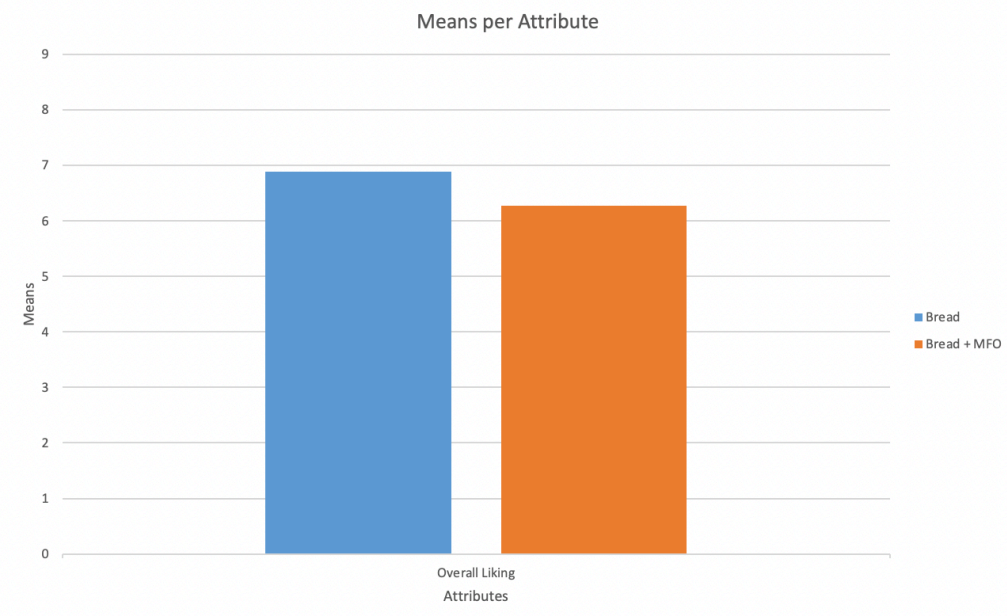


Figure 26: Consumer acceptance indicated on a 9-point scale.

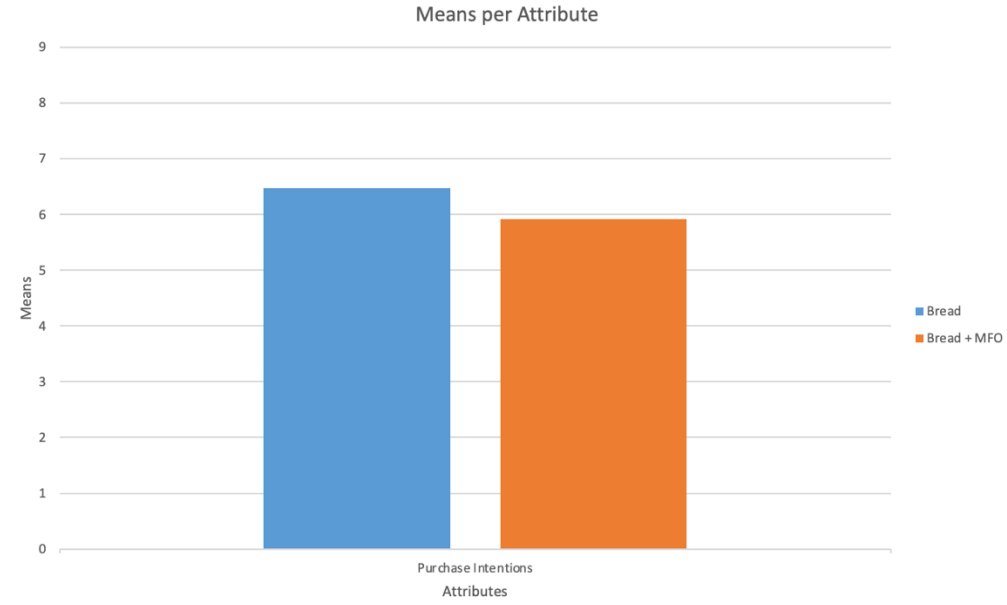


Figure 27: Purchase intentions indicated on a 9-point scale.

These results are supported by the results from the t-test, showing significant difference in liking at 5% level of significance. Consumers also reported to be more willing to buy the reference sample (Bwo/MFO) than the fortified sample (Bw/MFO) (see figure 27). Both samples scored a bit lower on this 9-point scale. Results from t-test showed significant differences in purchase intentions as well. The results here show that consumers have a stronger proclivity towards the reference sample.

When looking at the results from CATA, Only "Off-taste" and "Off-odor" where found to be significantly different at 5% level of significance. The options for characteristics consumers could choose to describe the sample are shown in figure 28.

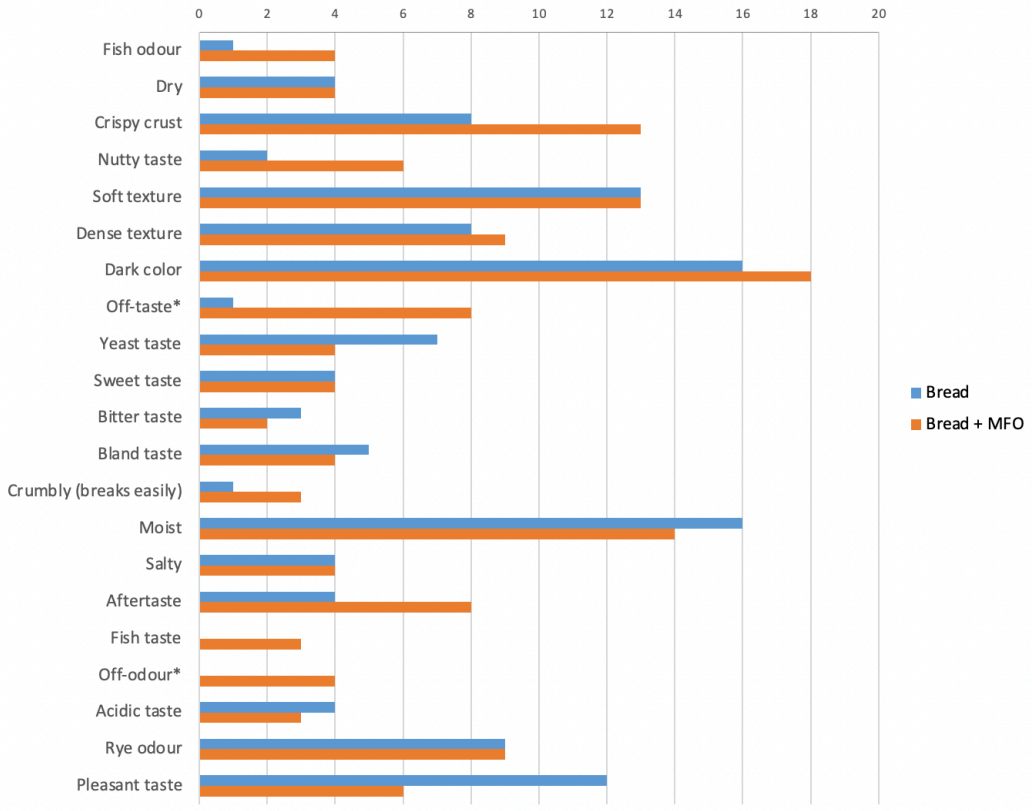


*Figure 28: Frequency of ticks (%) for the alternatives in the sensory analysis for bread that was carried out by 25 consumers to describe the two samples. *Noticeable difference between the samples at 5% level of significance.*

Based on these findings, it clearly shows that the MFO affects the aroma and taste of the bread. Fish taste was also a descriptor that on the graph in figure 30, appears to be perceived to a much bigger extend in sample Bw/MFO (though it was not found to be significant). Bw/MFO was also reported to have a crispier crust, nuttier taste, more dense texture, darker color, be crumblier and have a stronger aftertaste. The MFO used in this study had been stored for some time before

usage, which might explain the significant attributes mentioned since MFO has been shown to degrade with time and exposure to environmental factors. (Silva et al., 2012) However, despite the consumer ratings, the MFO seems to enhance attributes of the bread. Additionally, the idea of enriched bread has in previous studies been found to have a high acceptability in the population depending on the formulation (Cox et al., 2011, Hobbs et al., 2014)

Soup

Consumers liked the reference sample (FSwo/MFO more than the fortified sample (FSw/MFO) (see figure 29). On a scale from 1-9, both samples received a score of over 6,00. This indicated that the consumers were more satisfied than dissatisfied with both samples.

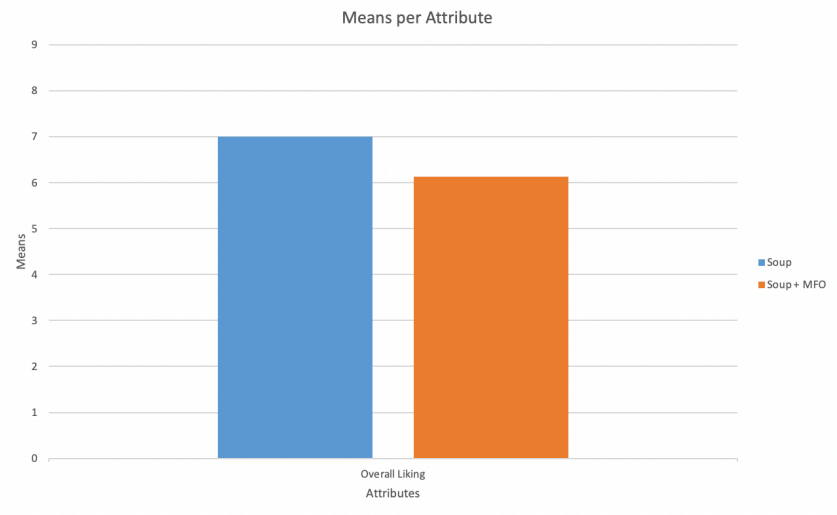


Figure 29: Consumer acceptance indicated on a 9-point scale.

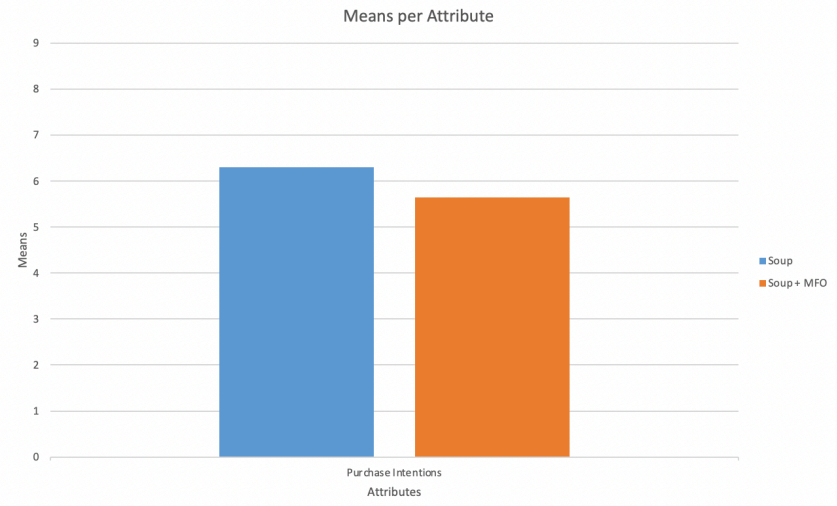


Figure 30: Purchase intentions indicated on a 9-point scale

These results are supported by the results from the t-test, showing significant difference in liking at 5% level of significance. Consumers also reported to be more willing to buy the reference sample (Bwo/MFO) than the fortified sample (Bw/MFO) (see figure 30). Both samples scored lower on this 9-point scale. Results from t-test showed no significant differences in purchase intentions. This is a good indication that despite using "aged" powder and being boiled, the soup may be a suitable food model to fortify with MFO.

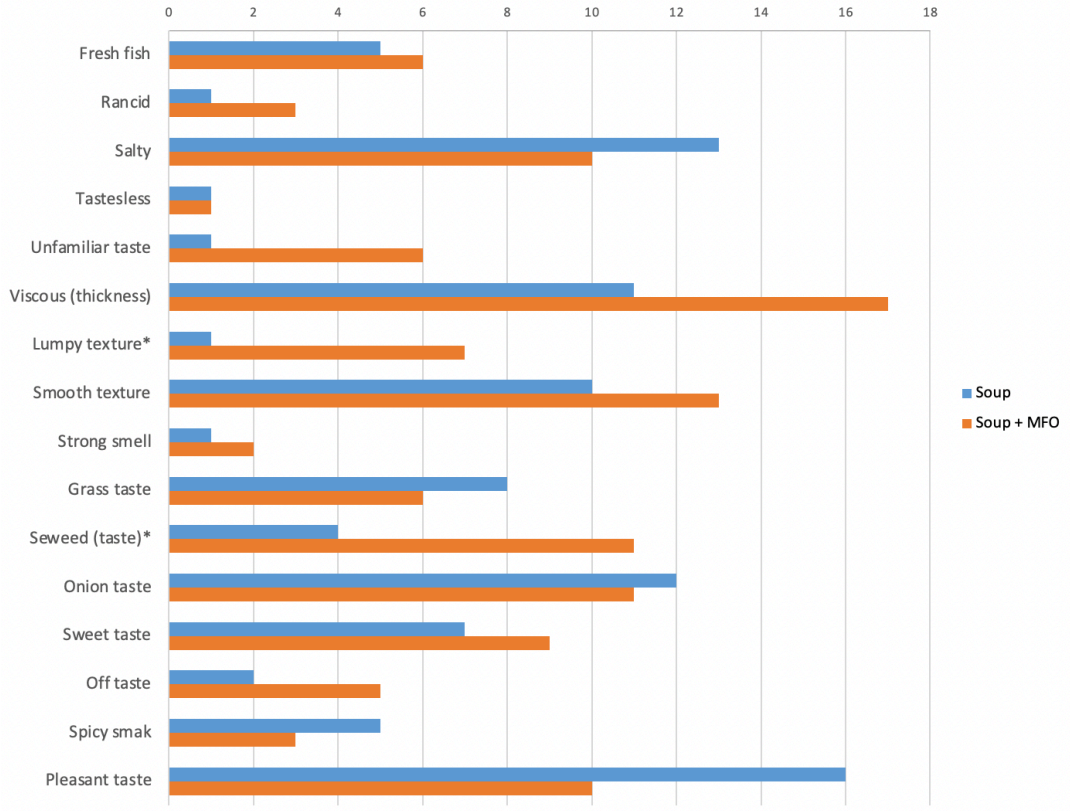


*Figure 31: Frequency of ticks (%) for the alternatives in the sensory analysis for fish soup that was carried out by 23 consumers to describe the two samples. *Noticeable difference between the samples at 5% level of significance.*

The CATA results showed significant differences in the attributes "Lumpy texture" and "Seaweed (taste)". The consumers noted that the sample FSw/MFO had lots of small lumps, which is most likely because of the MFO powder. Based on the results from figure 31, FSw/MFO appears to be more viscous (which matches with the results from chapter 4.2.1). This seems generally to be a problem that MFO when added in high amounts can form lumps (regardless of wall materials). This might be because of the deposition of oil (from when boiling) on the wall of the MFO can lead to plasticizing of the structure, thus forming lumps that do not dissipate. (Domian et al., 2015, Soliman et al., 2013)

5. Conclusion

The incorporation of microencapsulated fish oil into food products has been shown to have a significant impact on the chosen food models. Both commercial- and Omega-MFO changed the physical parameters of viscosity in both fish soup and porridge, as well as texture in bread. Results from CATA showed consumers could detect differences in specific attributes, which likely also affected overall liking and purchase intentions. However, based on the input from the informal testing and the results from storing bread, it was discovered that commercial MFO does have a positive impact on the color of soup and texture of bread. Results from analysis of volatiles in fish soup had mixed results. This was probably coming from the intervention of other ingredients within the products. Results from analysis of volatiles in bread showed higher amounts in fortified bread.

Overall, the results of using MFO showed a promising strategy to enhance the nutritional value. Continued research and innovation on how to mitigate significant differences when applying the powder into foods is necessary. Especially when wanting to cover both a percentage of recommended daily intake and at the same time have a high consumer acceptance of food products.

6. Further work

Further scientific work on using microencapsulated fish oil in food holds potential. Focusing on various aspects to advance its application in food products are an important priority. Encapsulation techniques play an important role in enhancing the stability and controlled release properties of microencapsulated fish oil (MFO).

The choice of encapsulation materials is a crucial area of research. Investigating other different compounds such as alginate, chitosan, would provide insights into their encapsulation capabilities, bioavailability enhancement, and ability to prevent oxidation. Also, understanding the interactions between encapsulation materials and fish oil could help identify the most effective combinations. Evaluating the effect storage conditions, pH, and food composition in other food models, would help guide the selection of appropriate processing, packaging and storage methods, ensuring better functionality and bioavailability of MFO over extended periods.

Additionally, it would also be interesting to investigate the release kinetics. Understanding the factors influencing the release rate and designing a encapsulation system that enable controlled and sustained release would enhance the bioavailability of omega-3 fatty acids and ensure optimal health benefits.

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Appendix 1: Poster for sensory analysis

Hey you!

Want to contribute to the development of sustainable food?

If you love fish soup and bread, you are welcome to participate in a tasting between May and June 2023

Please sign up only if you meet the following criteria:

- Age: 20-55
- Speak Norwegian/English
- Eat bread and fish soup
- Are not allergic to berries, dairy, gluten or fish.

The sensory analysis will take place at NTNU Department of Biotechnology and Food Science, in the sensory lab TU1.633. The address is Sverres gate 10 (Kalvskinnet).

QR-code

Hvis du er interessert, vennligst bruk denne lenken (lenke) eller skann QR-koden.
Har du noen spørsmål om prosjektet kan du kontakte oss på e-post (e-post) merket med »Navn»

NTNU
Department of Biotechnology and Food Science

Picture 13: Poster in English used to recruit participants for sensory analysis

Appendix 2: Poster for sensory analysis (Norwegian)

Hei du!

Lyst til å bidra til utviklingen av bærekraftige mat?

Om du er glad i suppe og brød er du hjertelig velkommen til å delta på å smake mellom Mai og Juni 2023

Registrer deg kun hvis du oppfyller følgende krav:

- Alder: 20-55
- Snakker norsk/engelsk
- Spiser brød og fiskesuppe
- Er ikke allergisk mot bær, meieriprodukter, gluten eller fisk..

Den sensoriske analysen vil ta sted ved NTNU Institutt for bioteknologi og matvitenskap, i sensorikklaboratoriet TU1.633. Adressen er Sverres Gate 10 (Kalvskinnet).

QR-code

If you are interested, please use this link (Link) or scan the QR code.

I you have any questions about the project you can contact us by email (email) marked with "Name".

NTNU
Department of Biotechnology and Food Science

Picture 14: Poster in Norwegian used to recruit participants for sensory analysis

Appendix 3: Terms for Bread

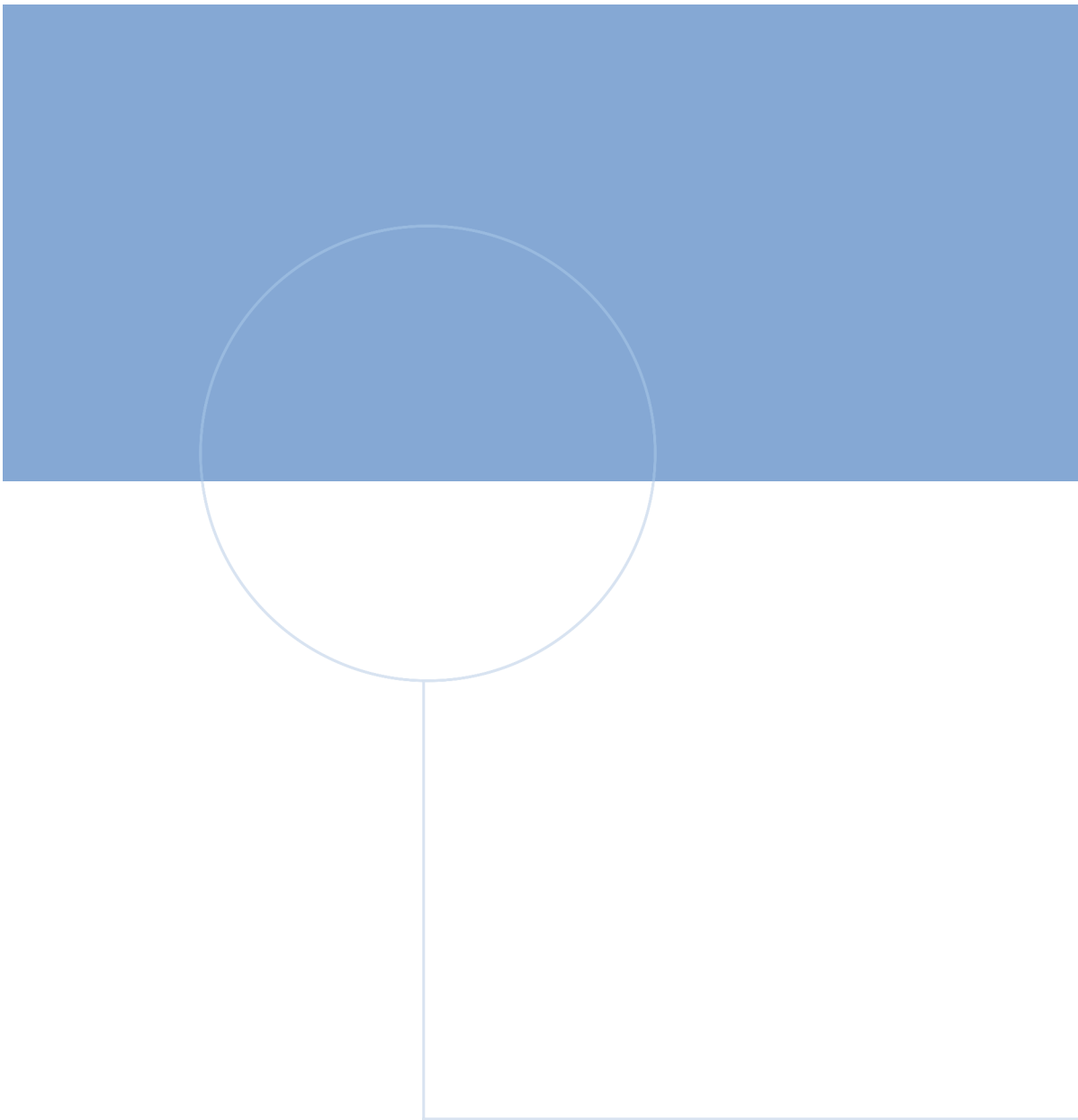
English	Norwegian
Fish odour	Fiskelukt
Dry	Tørr
Crispy crust	Sprø skorpe
Nutty taste	Nøttaktig smak
Soft texture	Myk tekstur
Dense texture	Kompakt tekstur
Dark color	Mørk farge
Off-taste	Bismak
Yeast taste	Gjærsmak
Sweet taste	Søtsmak
Bitter taste	Bittersmak
Bland taste	Tam smak
Crumbly (Breaks easily)	Smuldrete/Smulete
Moist	Saftig
Salty	Salt
Aftertaste	Ettersmak
Fish taste	Fiskesmak
Off odour	Bilukt
Acidic taste	Syrlig smak
Rye odour	Ruglukt
Pleasant taste	Behagelig smak

Table 6: Terms for bread used when performing CATA, with English words on the left and Norwegian words on the right.

Appendix 4: Terms for Fish soup

English	Norwegian
Fresh fish	Fersk fisk
Rancid	Harsk
Salty	Salt
Tasteless	Smakløs
Unfamiliar taste	Ukjent smak
Viscous (Thickness)	Viskøs (Tyktflytende)
Lumpy texture	Klumpete tekstur
Smooth texture	Glatt tekstur
Strong smell	Sterk Lukt
Grass taste	Gressmak
Seaweed (taste)	Sjøtang (smak)
Onion taste	Løksmak
Sweet taste	Søtsmak
Off-taste	Bismak
Spicy taste	Krydet smak
Pleasant taste	Behagelig smak

Table 7: *Terms for fish soup used when performing CATA, with English words on the left and Norwegian words on the right.*



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