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Study of influence of fatty acid profile on properties of emulsions stabilized with bacterial cellulose

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ABSTRACT

One of the applications of bacterial cellulose that is currently raising attention is its use as an emulsion stabilizing agent. This project analyzes how the structure of the fatty acids in the oil forming the emulsion affects the stability created by bacterial cellulose. Four types of oil-in-water emulsions with different fatty acids composition (canola oil, olive oil, coconut oil and pork lard) are prepared using the sonication technique and the stabilizing effect of bacterial cellulose in each one is compared by various methods including microscopy and analytical tools. The results obtained show that long-chained unsaturated fatty acids contribute to higher emulsion stability, while solid fats with shorter chains show the worst performance as to stability, although bacterial cellulose seems to prevent the fats from oxidation during storage. While liquid oil emulsions stabilized with bacterial cellulose are likely to play an important role in the food industry, oxidation-resistant solid fat emulsions have a notable potential in long shelf-life cosmetics.

Keywords: Bacterial cellulose; emulsion stability; vegetable oil emulsions; animal fat emulsions.

1. INTRODUCTION

1.1 EMULSION

An emulsion is a mixture of two immiscible liquids, known as 'water' and 'oil', where one of the phases is scattered in the other in form of microscopic droplets (Binks, 1998). There are two main groups of emulsions based on which phase is dispersed in the other: oil-in-water emulsions (or o/w) and water-in-oil emulsions (or w/o).

When the droplets are dispersed by traditional mechanical methods, such as agitation, the emulsion formed is highly unstable and the immiscible phases tend to separate because of increased superficial tension in the interface. That is why surface-active agents are used to stabilise the emulsion and prevent the droplets from joining together (Binks, 1998).

Emulsions are of great interest in modern industrial processes, such as food technology, cosmetics production, and the paint manufacturing industry (Leal-Calderon, 2006). That is why the study of emulsion stability plays an important role in modern research.

1.1.1 Oils and Fatty Acids

Lipids can be defined as non-polar water-insoluble biological macromolecules present in nature, both in plants and in animals, as structural elements and energy storage compounds (Gajera, 2008). It is a diverse group of carbohydrate structures with different properties and purposes.

Fatty acids are molecules built of carbohydrate units joined together in a long chain with carboxylic acid moiety at the head of the chain. There are two groups of fatty acids, based on the bonds between carbon units in their structure: saturated fatty acids, which only exhibit single bonds between carbons, and unsaturated fatty acids, which exhibit one or more double bonds between carbons. For example, oleic acid, present in olive oil, is a monounsaturated fatty acid, while palmitic acid, present in palm oil, is saturated (Table 1). In their chemical geometry, unsaturated fatty acids sometimes are bent at the point of a double bond (known as *cis*-bond). When *trans*-bond occurs, the unsaturated fatty acid remains unbent, while saturated fatty acids are always straight molecules since they do not have double bonds.

Table 1. Examples of common fatty acids, their nomenclature, and melting point (Recreated from Wynn, 2011).

Common name	Systematic name	Numerical designation	Melting point (°C)	Appearance at room temperature
Myristic acid	Tetradecanoic acid	14:0	58.8	Solid (fat)
Stearic acid	Octadecanoic acid	18:0	69.6	Solid (fat)
Oleic acid	Octadeca-9-enoic acid	18:1	14	Liquid (oil)
α-Linolenic acid	Octadeca-9,12,15-trienoic acid	18:3 n-3	−11	Liquid (oil)

However, fatty acids are rarely found as a chemical entity. They are usually part of various lipids, e.g., triacylglycerols or phospholipids. Triacylglycerols are esters synthesized with one molecule of glycerol and three fatty acids (Figure 1). Triacylglycerols that are solid at room temperature are referred to as fats, while the triacylglycerols that remain liquid are called oils (Gajera, 2008).

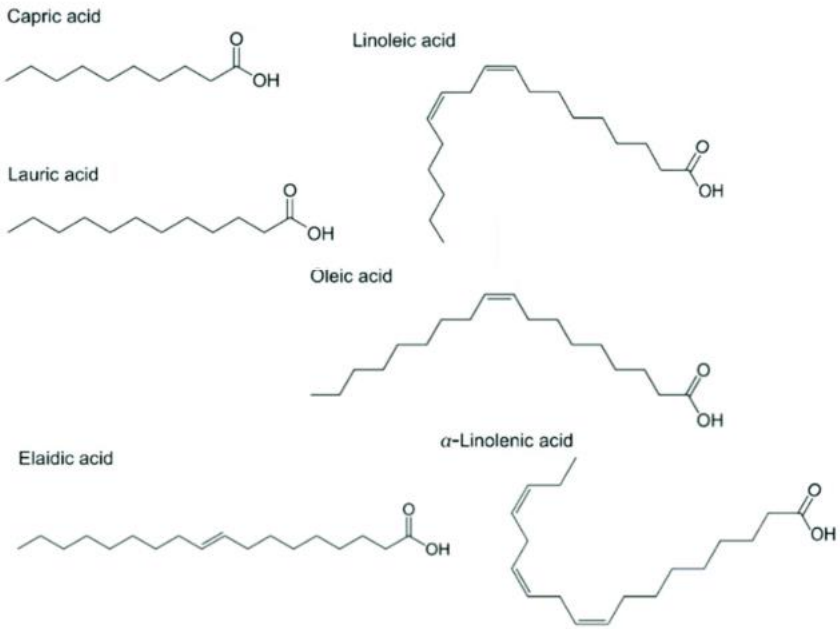


Figure 1. Examples of some common saturated and unsaturated fatty acids and their structure. Saturated: Capric acid (C10:0), Lauric acid (C12:0). Unsaturated: Elaidic acid (trans-C18:1), Linoleic acid (C18:2), Oleic acid (C18:1), α-Linolenic acid (C18:3) (Recreated from Yoon, 2018).

As to nomenclature, there are four systems of naming fatty acids (Table 2).

1. **n-nomenclature** (former omega-nomenclature). The fatty acid is designated by a number of carbons in the chain followed by the number of unsaturations separated by the colon. In the case of unsaturated fatty acids, the carbon of the last methyl unit in the chain is designated with the letter n followed by a number that indicates the position of the nearest double bond in relation to the n-carbon. Formerly, the Greek symbol ω (omega) was used instead of n.
2. **Delta-nomenclature**. This system is similar to n-nomenclature, however, in this case, the carbons are counted from the carboxylic acid group of the fatty acid. Instead of n, the Greek letter Δ (delta) is used and the positions of all double bonds with reference to the carboxyl group are written as a superscript separated by a comma.
3. **Common name**. The fatty acid is known by a name.
4. **Systematic name**. According to the standard IUPAC Rules for the Nomenclature of Organic Chemistry.

Table 2. Example of different nomenclatures on the same fatty acid.

n-nomenclature	Delta-nomenclature	Common name	Systematic name
18:1 n-9	18:1 Δ^{9}	Oleic Acid	Octadeca-9-enoic acid
18:3 n-3	18:3 $\Delta^{9,12,15}$	α-Linolenic Acid	Octadeca-9,12,15-trienoic acid

1.1.2 Emulsion Stability

The emulsion that maintains its homogeneous properties is referred to as stable. When the emulsion is stable, the droplets are dispersed and animated by Brownian motion in a continuous medium (Figure 2a). However, some physicochemical processes impair the emulsion stability and favor phase separation (Figure 2b-f).

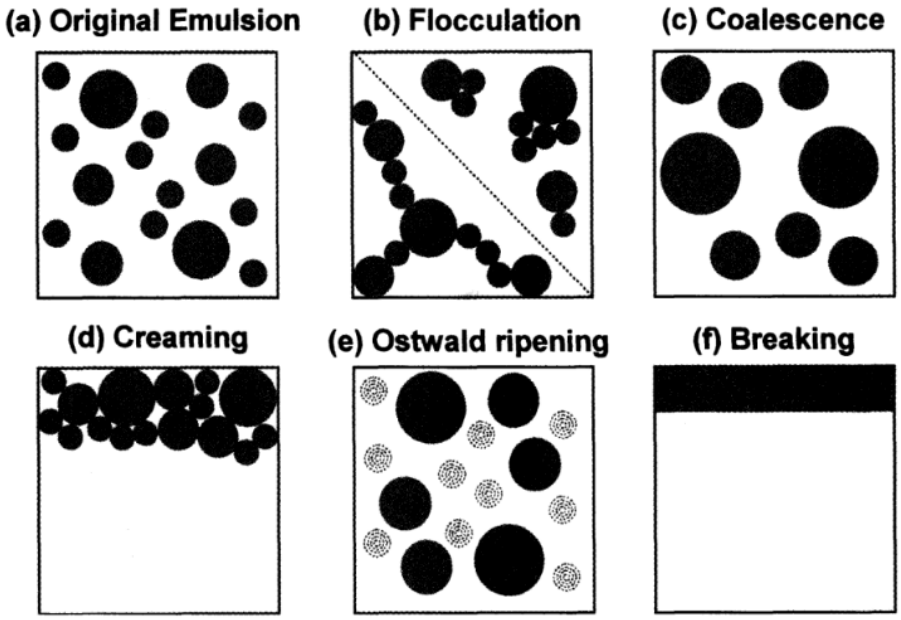


Figure 2. Emulsion instability processes (Binks, 1998).

Flocculation (Figure 2b) happens when the droplets are submitted to a net attractive force between them that is stronger than the existing Brownian force in the medium and persistent droplet aggregation occurs. The droplets form flocs and stay close together without merging into a bigger droplet. The process of droplets flowing together and creating one larger droplet is referred to as coalescence (Figure 2c).

Both aforementioned processes require droplet encounters to take place, however, it is not the case for creaming (Figure 2d) and Ostwald ripening (Figure 2e). Creaming occurs when the densities of water and oil phases are not similar and the droplets are pushed to the top of the emulsion by buoyancy or centripetal force if the centrifuge is used (McClements et al., 2004). In the case of water-in-oil emulsions, a similar process called sedimentation occurs, when more dense particles deposit on the bottom of the emulsion following Stoke's Law.

Finally, Ostwald ripening (Figure 2e) takes place when the dispersed phase spontaneously travels through the continuous phase over time, from smaller particles to larger ones, that are more favored energetically.

The last process depicted in Figure 2f is the equilibrium of the two phases when they eventually separate, also referred to as the breaking of the emulsion. It occurs over time and for some emulsions, it can take a few minutes to reach this state, while for others it may take several years (Binks, 1998).

To overcome these instability factors emulsifying agents, also known as surfactants, are used. These substances adsorb to the interface between oil and water phases and exchange monomers in the solution continuously (Figure 3b). When the homogenization technique is applied, the oil phase is broken into small droplets coated in surfactant, which prevents the droplets from coalescence and other instability processes due to interfacial repulsion (Figure 3c and d) (Mason et al., 2006).

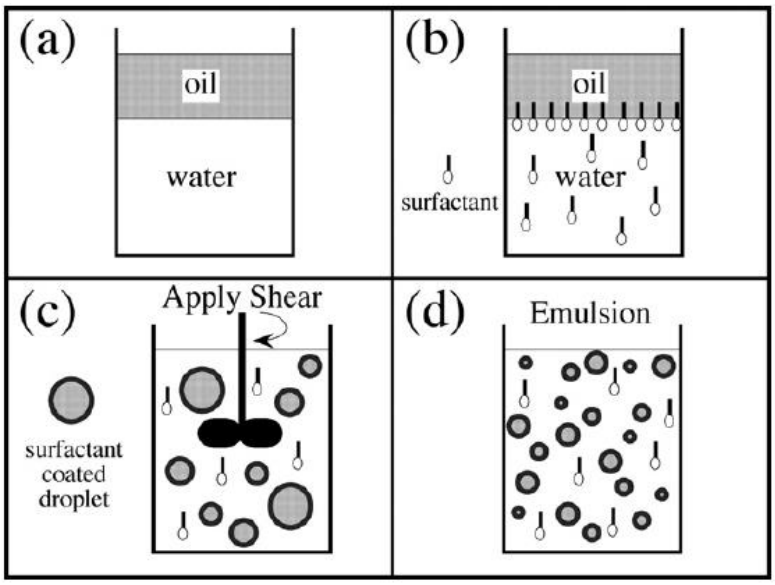


Figure 3. Formation of emulsion stabilized with surfactant (Mason et al., 2006).

Different kinds of surfactants are used for different purposes based on their properties and structure. One of the stabilizing agents that are arousing particular interest nowadays is nano/microparticles that are used in stabilizing what is called Pickering emulsions.

1.1.3 Polysaccharide-based Pickering Emulsions

In recent years, numerous advantages of Pickering emulsions have been discovered. Unlike traditional surfactants which act by decreasing interfacial tension between the phases, micro/nanoparticles provide higher resistance to coalescence and Ostwald ripening by creating physical barriers on the interface (Pang et al., 2021). In Figure 4, this difference is graphically presented.

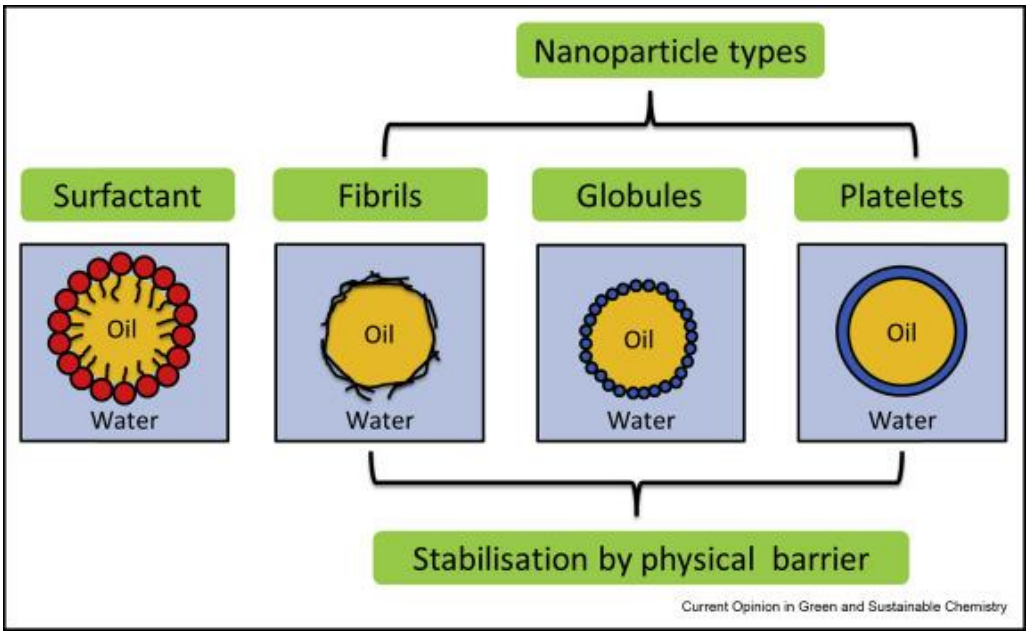


Figure 4. Graphic comparison between surfactant-stabilized emulsion and particle-stabilized emulsion (Calabrese et al., 2018).

Among the most used materials for stabilizing Pickering, emulsions are minerals (e.g. Cloisite clay nanoparticles (Agarwal et al., 2012) and modified kaolinite nanotubes (Grechishcheva et al., 2017), polysaccharides, and proteins.

Polysaccharides such as cellulose and chitin are particularly interesting materials because of their biodegradability (Klemm et al., 2011) and the possibility to be strategically modified to control their behavior in the emulsion, thus creating on-demand emulsions with specific properties (Calabrese et al., 2018). Such changes as hydrophobicity adjustment as well as surface charge can be induced to enhance dispersibility (Klemm et al., 2011).

As to future prospects, polysaccharides as emulsion stabilizers represent a potential eco-friendly alternative to conventional surfactants aiming to reduce the environmental hazards caused by the latter (Capron et al., 2013).

1.1.4 Effect of Fatty Acid Chain Length and Saturation on Emulsion Stability

In addition to selecting a proper emulsifier, it is crucial to know the influence of the fatty acid structure on emulsion stability. Zheng et al. investigated this influence in protein-stabilized emulsions and discovered that medium chain fatty acids, monounsaturated fatty acids and saturated fatty acids did not create much stability because of the weakening of interfacial protein film, which contributed to increased coalescence. Moreover, it negatively affected the coverage of oil drops by the protein and resulted into an uneven particle distribution (Zheng et al., 2021)

On the other hand, according to the same authors, unsaturated fatty acids performed better than their counterparts contributing to higher stability. Unsaturated fatty acids enhanced the surface charge of particles in the emulsion as a consequence of increasing repulsion between them and improving the attraction strength (Zheng et al, 2021).

1.1.5 Industrial Importance of Emulsions

Emulsions have a particularly attractive characteristic that gives them multiple potential uses in different industries, and that is their two-phase composition. Emulsions possess both hydrophobic and hydrophilic components, which is why they are great solubilizers for both polar and nonpolar substances. Thanks to this advantageous property emulsions are exploited in the food industry, cosmetics, textiles, and soil remediation (Binks, 1998).

Food industry

One of the most common food emulsions is the mayonnaise which has been used for centuries as dressing for dishes. Nowadays, a great variety of salad dressings with unique properties and flavors are commercially available to satisfy consumers' tastes and the majority of them are w/o emulsions.

Mayonnaise is a semisolid emulsion made of egg yolk, vegetable oil, and acetic or citric acid. Usually, flavoring ingredients such as salt, spices or spices oils or natural sweeteners are added to improve the taste. Egg yolk acts as an emulsifier and gives the product its characteristic pale yellow color. The proportion of oil in mayonnaise must be at least 65% by weight, and the product must contain a minimum 2.5% of acid by weight (Yang et al., 2003).

More examples of well-known o/w emulsions are found in dairy products such as milk, cream, and ice cream. Those emulsions are stabilized by proteins and low molecular weight surfactants. The most widely used protein emulsifier for o/w emulsions is sodium caseinate (O'Kennedy, 2011). Usually, this type of emulsion is produced with high-energy homogenization method and the oil droplets are 0.5 μm large on average (Krog, 2011).

Finally, w/o emulsions can be also found in popular food products such as butter, margarine, and table spreads. They are stabilized by a fat-based emulsifiers, milk protein, and artificial stabilizers and are produced using low-energy agitation methods. As a result, water droplets are dispersed in oil, their diameter varying between 5 to 50 μm (Krog, 2011).

Cosmetics

Most skincare products are emulsions because the surface of human skin has a hydrophobic behavior due to natural oils produced in the tissue, which makes it inconvenient to use only aqueous solutions for skincare. W/O volume ratio in the emulsion must be thoroughly determined for the product to have a pleasant texture and suitable viscosity and spreading properties.

Emulsions used in cosmetics usually contain between 65% and 80% of water. The aqueous phase may incorporate polar components such as moisturizers (e.g., glycerol, urea) or cell regenerative agents (e.g., α-hydroxy acids). On the other hand, the oil phase may contain oil-soluble active components (e.g., vitamin E) (Binks, 1998).

Moreover, microemulsions stabilized with alkyl polyglycoside (APG) arouse important interest in skin cleansing products. APGs are nonionic surfactants with great dermatological benefices that are also eco-friendly and, combined with proper cosurfactants, produce stable microemulsions with high efficiency. These emulsions are generally incorporated into facial cleansing products with a caring effect (Binks, 1998).

Textiles

One of the uses of microemulsions in the textile sector is to wet the fabric quicker. This application is possible as a result of the high interfacial activity of those microemulsions. Fabrics are impregnated in emulsions stabilized with active agents that apart from increasing the wettability of the fabric provide certain characteristics to the fabric, such as antistatic effect (Binks, 1998), increased comfort indexes, or antibacterial properties (Zaharia et al., 2020).

Other uses of emulsions in the textile sector are based on their cleaning capacity provided by surfactants. Emulsions are used to remove soil from fabric, both hydrophilic (cotton) and hydrophobic (polyester), by solubilizing oily stains in the cleaning solution (Binks, 1998).

Soil remediation

In recent years, due to alarming agricultural pollution, soil remediation with surfactants has aroused interest in the investigation of eco-friendly cleaning solutions that provide the best efficiency. Surfactants are well-known for their capacity to solubilize the highly hydrophobic components such as polycyclic aromatic hydrocarbons (PAH) (Binks, 1998) and persistent toxic pesticides such as dichlorodiphenyltrichloroethane (DDT) and lindane (Chang et al., 2021).

1.2 Bacterial Cellulose

Bacterial cellulose (BC) is a polysaccharide produced by microbiological organisms that nowadays is gaining interest in industry. It is mostly used in food production due to its beneficial properties and eco-friendliness. However, it possesses a wide range of qualities that extend its use to multiple areas. It is used as a thickening agent, organic emulsifier, as well as biodegradable material for alternative packages (Shi et al., 2014).

Moreover, it is non-toxic (Jeong et al., 2010). It is considered safe (GRAS) and was accepted as a dietary fiber by the USA Food and Drug Administration in 1992 (Shi et al., 2014).

Bacterial cellulose is produced by various species of Gram-negative bacteria such as *Acetobacter, Aerobacter, and Escherichia*, among others (Shi et al., 2014). Nowadays, one of the most prominent varieties of bacteria for cellulose production is *Komagataeibacter xylinus (*former names: *Glucoacetobacter xylinus* and *Acetobacter xylinum).*

When compared to plant-derived cellulose, bacterial cellulose possesses an interesting advantage which is that it does not require a sophisticated purification process, because it is free of lignin, hemicelluloses, and pectin (Huang et al., 2014). Moreover, bacterial cellulose has a higher degree of polymerization, surprising tensile properties due to its web-like network structure (Iguchi et al, 2000) as well as the higher specific area (Sulaeva et al., 2015).

1.2.1 Structure of Bacterial Cellulose

Bacterial cellulose is a polymer compound with the formula $(C_6H_{10}O_5)_n$ that contains sets of parallel β-1,4-glucan chains connected with regular intra- and intermolecular hydrogen bonds (Jeong et al., 2010). The chemical structure of bacterial cellulose is comparable to that of plant cellulose, however, it has special physical, mechanical, and chemical properties such as high

strength, elasticity, gas permeability, high water-holding capacity, and porosity (Volova et al., 2018).

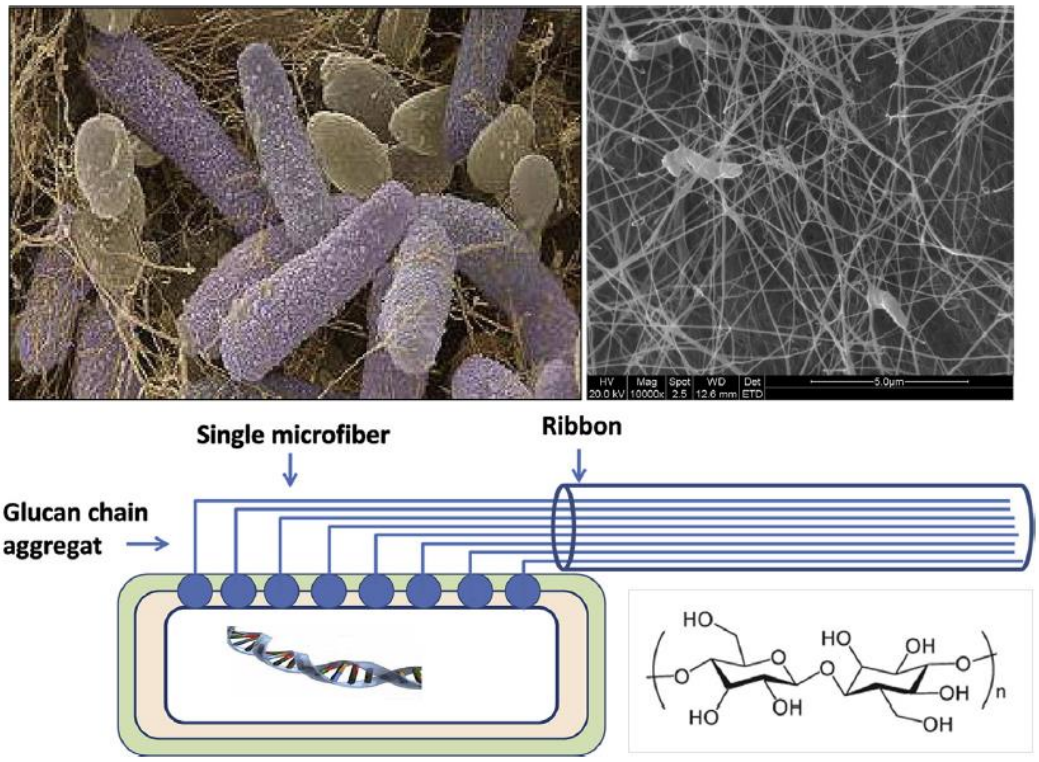


Figure 5. SEM capture Komagataeibacter xylinus and its structure (Brown, 1992).

Hiroshi et al. compared the action of BC from static (St-BC) and agitated (Ag-BC) culture with other materials such as microcrystalline cellulose (MCC) and microfibrilated cellulose (MFC) as well as xanthan gum, and sorbitan monolaurate. The result of the experiment stated that Ag-BC scored the highest emulsion stability index (ESI) among the cellulosic stabilizers and also that it affects in the same way emulsions made with vegetable oil and those made using kerosene as oil phase. In addition, BC showed the finest fibrils of all the materials studied and this particular property directly influences the stabilization of emulsions.

As to the mechanism behind the process of stabilization, it is considered that colloidal microfibrils of BC form a network adsorbed on the medium surface that prevents the oil droplets from coalescence acting as a mechanical barrier (Hiroshi et al., 1997). The smaller the width of the fibrils the stronger the mechanical barrier is formed, therefore, the more stable the emulsion is.

Finally, one of the many strong points of using BC as an emulsion stabilizing agent is that its effect is not influenced by changes in the medium such as pH, temperature, or ionic strength (Paximada et al., 2016), which can be a downside for other types of surfactants.

1.2.2 Industrial Applications

Bacterial cellulose is mainly used in the food industry. It is an organic dietary fiber known for its health benefits. It can help to decrease the danger of chronic illnesses such as

cardiovascular diseases and diabetes, as well as obesity and diverticulitis (Cho, 2012). When used as a food additive, it presents an important value for dietary products, which is that it cannot be assimilated by the human digestive system, thus it improves intestinal transit (Azeredo et al., 2019).

Bacterial cellulose can be found in different forms based on its direct application purpose and production method, its shape varying from intact membranes to disassembled cellulose and nanocrystals (BCNC). In the food industry, it is conveniently mixed with additives (Shi et al., 2014).

1.2.2.1 Food Applications

Raw product

There is a type of dessert originating from the Phillippines that uses bacterial cellulose gel called *Nata* made by fermentation of coconut water (*nata-de-coco*) or pine juice (*nata-de-piña*). The gel is evenly cut into one-centimeter-thick cubes that are immersed in sugar syrup (Iguchi et al, 2000). It has a smooth mouthfeel, and its fabricating process is straightforward, which is why this dessert has become exceptionally well known and is now rapidly growing in popularity worldwide (Shi et al., 2014).

Food ingredient

Bacterial cellulose, in low concentrations, can be added to food products to enhance their stability, health benefits and modify rheology.

In one study, it has been used as a fat replacer to make healthier meatballs where half of the fats were replaced by bacterial cellulose without affecting the visual aspect and cooking conditions of the meatballs. Also, another study showed that a surimi product with partially replaced fats with bacterial cellulose fibers presented a higher water-holding capacity due to the unique structure of surimi-BC (Lin et al., 2004).

Moreover, bacterial cellulose complex has been implemented in lowering cholesterol levels in foods and showed remarkable success, providing reasons for further research (Stephens, 1990). There is a potential possibility to use bacterial cellulose along with *Monascus*, a natural red pigment, to produce artificial vegetarian meat, because, as studies show, the BC-Monascus complex is stable and tastes like meat (Sheu et al., 2004).

1.2.2.2 Material for food packing

Nowadays biodegradable packing is becoming more and more in demand due to global ecological awareness. Packaging material for food must be mechanically resistant and produce no changes in the product. One of the organic materials for food packing is the bacterial cellulose membrane combined with other components (Shi et al., 2014).

Thus, Xiao et al. merged polylactic acid (PLA) with bacterial cellulose and the combination of those elements provided decent mechanical properties, transparency, and eco-friendliness (Xiao et al., 2012).

Moreover, there are several studies regarding combining antimicrobial agents with the membrane of bacterial cellulose in food films in order to improve the safety of processed meats and prolong their storage time. On the one hand, nisin, which is an antibacterial polypeptide

produced by *Lactococcus lactis*, was incorporated into the BC membrane to control *Listeria monocytogenes* and other pathogens on the surface of the frankfurter sausages (Jipa et al., 2012). On the other hand, ε-Poly-L-lysine built into BC membrane also demonstrated remarkable antimicrobial properties as sausage packing material (Zhu et al., 2010).

1.2.2.3 Health care

Cosmetics

In the cosmetic industry, the non-toxicity of the product plays an important role when it comes to customers' choices and well-being. As was already stated, bacterial cellulose shows remarkable emulsion stabilizing properties, it is non-toxic and non-allergenic, which is why one of the potential uses for this material is cosmetics surfactant (Ullah et al., 2016).

One of the applications of BC in the cosmetic industry is face masks, due to its amazing moisturizing properties, as shown in the study carried out by Amnuaikit et al. in 2011. The facial mask containing BC is already patented and is being used for extended beautifying treatment (Zhong et al., 2008).

Moreover, BC can be used for natural facial scrub (Hasan et al., 2012) and skin cleansing products (Heath et al., 2012), therefore achieving great interest and multiple future perspectives within the cosmetic industry.

Drug delivery

Due to its remarkable moisturizing and absorbing properties, bacterial cellulose applications are being studied in the health care industry. Studies show that combined with antimicrobial agents, like benzalkonium chloride (Wei et al., 2011), can be used as a potential wound dressing supply.

Furthermore, other studies suggest BC-based dosage release systems be used for controlled drug delivery. Amin et al. looked at a method of administrating paracetamol by BC-coated tablets, which resulted in a slower drug release time compared to uncoated tablets (Amin et al., 2012). Also, hydrogels of bacterial cellulose for controlled drug release methods are capturing researchers' attention. In acidic media, BC hydrogel begins to swell (Pavaloiu et al., 2015) and the drug release rate slows down, making the hydrogel suitable for drug delivery to the gastrointestinal tract (Amin et al., 2014).

Finally, among other applications, BC is being investigated in dental drug delivery as material for root canal treatment (Yoshino et al., 2013), and transdermal drug delivery (Ullah et al., 2016).

2. PROJECT OBJECTIVES

The main concern of the present project is to study the effect of the oil phase composition on the stability of emulsions with bacterial cellulose utilized as an emulsifier. To achieve the main objective, two lateral basic goals can be distinguished.

Firstly, the composition of four different oil samples, both of plant and animal origin, will be studied. For this purpose, the samples will be analyzed using gas chromatography of fatty acid methyl esters, obtained directly from the oil samples.

Secondly, the stability of different emulsions made with oil samples, both with bacterial cellulose and without it, will be analyzed in order to confirm the stabilizing effect of bacterial cellulose, as well as to determine the sample with the best characteristics.

As a result, the influence of fatty acid length and amount of unsaturation will be determined by comparing the stabilities of all samples as well as the effect that bacterial cellulose produces on emulsions created with vegetable oil, and those made using animal fat will be contrasted.

3. MATERIALS AND METHODS

3.1 DETERMINATION OF OIL SAMPLE COMPOSITION

During the project four different types of oils will be analyzed and later used for making emulsions: refined canola oil (Kujawski, Zakłady Tłuszczowe Kruszwica, Poland), refined olive pomace oil (LugliO, MEDSOL SRL, Italy), refined coconut oil (Intenson sp. z o.o., Karczew, Poland) and pork lard (made available as a courtesy), as showed in Figure 6.

Only refined fats were selected for this study to exclude the presence of substances present in the crude oils that stabilize the emulsion, e.g. phospholipids.

Figure 6. Oils used in the experiments: a) canola oil, b) olive oil, c) coconut oil, d) pork lard.

At room temperature, canola oil appears as clear yellow liquid, while olive oil is also liquid but has a darker and more saturated color. Both of them are vegetable oils that remain liquid at room temperature, which indicates that they are rich in triacylglycerols with unsaturated fatty acids.

On contrast, coconut oil, which is also an oil from plant origin, is solid at room temperature. In solid form it appears as white opaque block of fat, and when it is melted, it takes a slightly yellow color. It is expected that coconut oil is rich in saturated fatty acids with shorter chains.

Finally, pork lard is animal fat extracted from the pork meat in a rendering process. At room temperature it is solid, white, and easy to scoop out. When it is melted, it takes the appearance of colorless transparent liquid. It is expected that pork lard is rich in saturated fatty acids with longer chains, due to its animal origin.

The oils were stored in the fridge at around 6ºC in the commercial packings.

3.1.1 Chemicals Used

- KOH (POCH)
- Methanol (POCH)
- Glycerol (POCH)
- Hexane (POCH)
- $SOCl_2$ (POCH)
- NaCl saturated solution (POCH)

3.1.2 Transesterification of the Fatty Acids

Fatty acids are characterized by the low volatility which makes them hard to be detected by gas chromatography. Also, they are prone to oxidizing. That is why transesterification of fatty acids is frequently used to obtain the fatty acid profile of a certain oil. As a result of this technique, methyl esters of the fatty acids present in the oil mixture are obtained. Fatty Acid Methyl Esters (FAMEs) are chemically more stable and more volatile, due to acylation, and allow good results to be obtained by GC.

Therefore, the derivatization of fatty acids is carried out by transesterification of fats with methanol, catalyzed by bases or strong acids (Figure 7). The result of the procedure is glycerol and fatty acid methyl esters. The methyl esters are separated by extraction with nonpolar organic solvents.

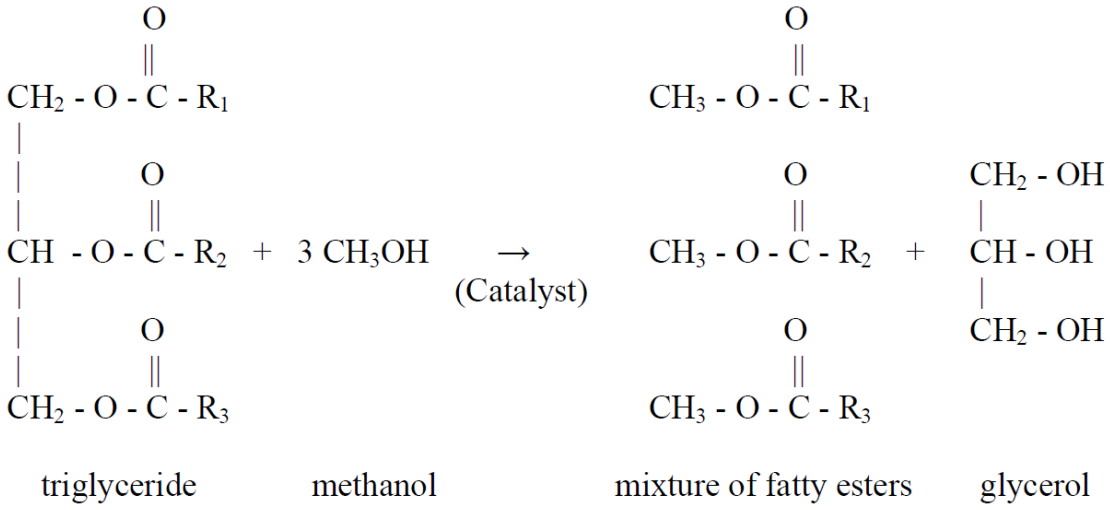


Figure 7. Schematic representation of transesterification reaction (Van Gerpen et al., 2004).

1. Preparation of oil samples

The first step of the procedure is to weigh 0.1 gram of each oil type in a glass ampoule. To do so, coconut oil and pork lard, which had turned solid during storage, are previously melted in the microwave for 5 minutes at various power ranges to avoid boiling.

2. Saponification reaction

The oil samples are then incorporated into the experimental setup shown in Figure 8. The setup consists of a magnetic heating plate (1), a bath of glycerin (2) with a stirrer (3) that transfers heat to the samples (4), and cooling pipes (5) joined to the ampoules. Each of the samples of oil also contain a piece of kaolin to accelerate boiling. The glycerin bath is set to 80ºC and 170 rpm stirring.

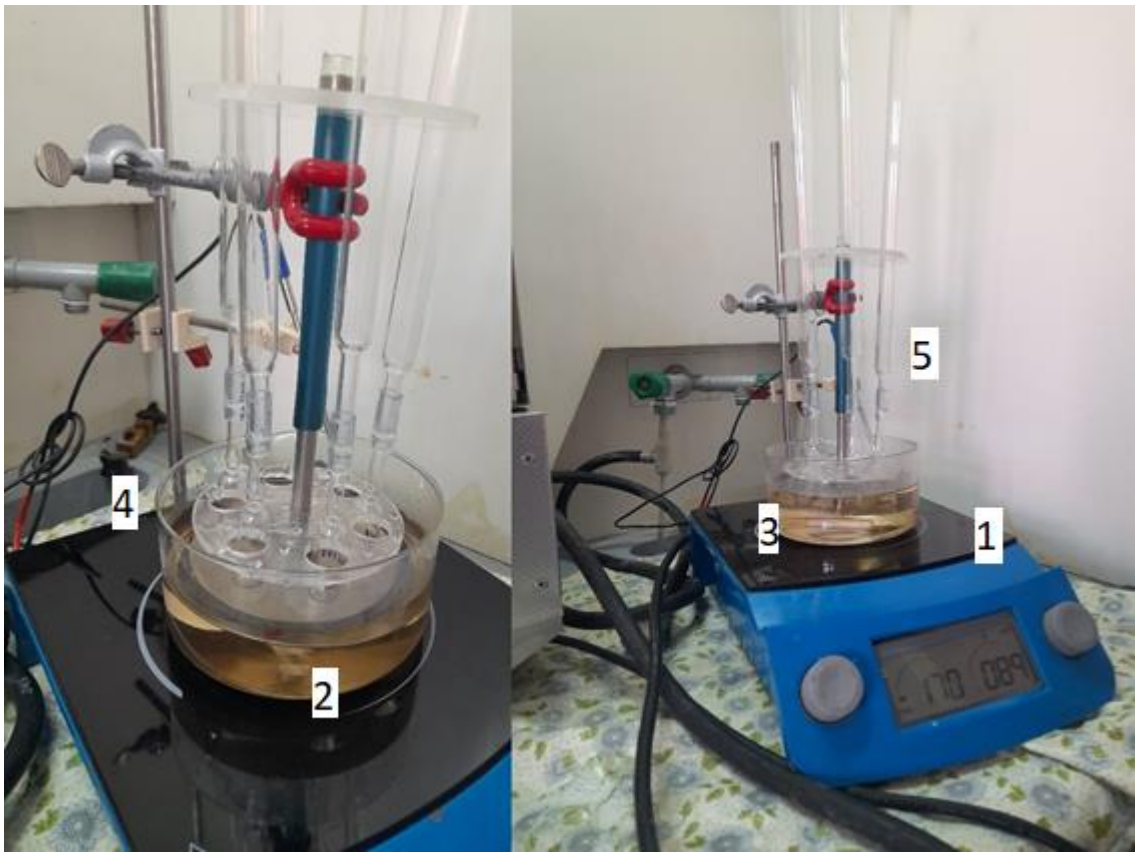


Figure 8. Experimental setup for transesterification process.

To each of the samples 1 ml of 0.5 M KOH/methanol solution is added to start the saponification reaction.

3. Esterification reaction

After 10 minutes 2 mL of 2% $SOCl_2$/methanol solution as well as a fresh piece of kaolin is added to each oil sample. At that point, the esterification reaction takes off. After 10 minutes the samples are taken out of the setup and cooled down at room temperature.

4. Preparation of the ester samples for gas chromatography.

When the samples are cool, 2 mL of hexane is added to each ampoule. Hexane will serve as a nonpolar solvent for esters in gas chromatography. Finally, a saturated NaCl solution is added to each sample to fill the ampoule.

The samples are covered with aluminum foil and stored in a cold place.

3.1.3 Gas Chromatography

Gas chromatography is a technique used in analytical chemistry in which the sample is volatilized and injected into the head of a chromatographic column oven. The setup for this part of the experiment is presented in Figure 9.

Figure 9. Gas chromatography setup.

The elution is produced by the flow of an inert gas mobile phase. Unlike the other types of chromatography, the mobile phase does not interact with analyte molecules; its only function is to transport the analyte through the column. The inert gas can be either nitrogen, helium, argon, and sometimes hydrogen or air. In Figure 10, a graphic scheme of the gas chromatograph is presented.

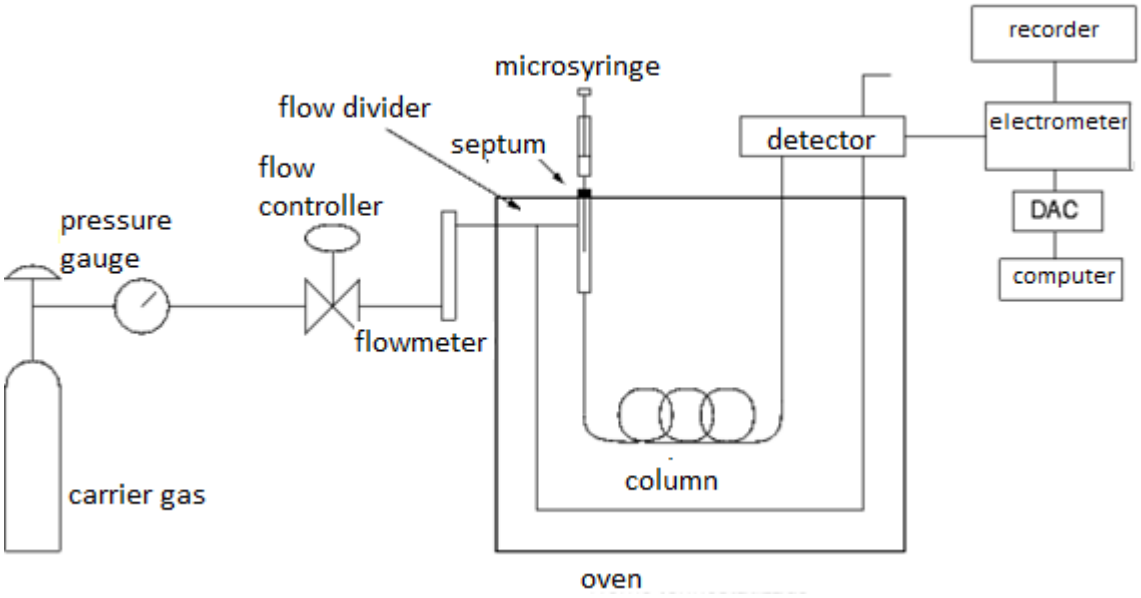


Figure 10. General diagram of the operation of a gas chromatography equipment. Taken and adapted from Castro, 2021.

As to operation conditions of the setup, 1 µL of solution is taken and subjected to GC analysis in a PerkinElmer Autosystem XL gas chromatograph equipped with an injector with a stream divider (60:1) and a Flame Ionization Detector (FID) using the Total Chrom computer software. The chromatographic separation of each sample lasts 40 minutes and is carried out in a GC CP-Sil 88, 50 m×0.25 mm×0.2 µm chromatography column (Agilent Technology, USA). The carrier gas is helium, and the flow rate is 1mL / min. The temperature of the injector and detector

is 250°C, while the temperature of the oven with the column is 170°C. Two replications are performed for each test. The collected results in the form of chromatograms are then processed using the Equivalent Chain Length (ECL).

As a result, a chromatography report is obtained after the separation stops.

3.2 Analysis of Emulsion Stability

3.2.1 Preparation of Bacterial Cellulose

The process begins with propagation of microorganisms. The species used for the production of bacterial cellulose is *Gluconacetobacter xylinus* LOCK 89 (Pure Culture Collection of the Institute of Fermentation Technology and Microbiology, Lodz University of Technology, Poland) which is grown in stationary conditions using a Herstin–Schramm medium, composed of 2% (w/v) anhydrous glucose (ACS), 0.27% (w/v) anhydrous Na_2HPO_4 (pure P.A), 0.115% (w/v) monohydrate citric acid (pure P.A) (POCH, Poland), 0.5% (w/v) yeast extract (for microbiology), 0.5% (w/v) peptone K (for microbiology) (BTL, Poland), and 1% (v/v) ethanol (PUH Chemirol, Poland). The incubation is carried out at 28°C for 7 days.

For purification, the resulting membrane is rinsed in tap water to withdraw a residual nutrient solution, boiled in a 5% (w/v) NaOH (pure P.A.) (POCH, Poland) solution for 1 hour to inactivate the bacterial cells, rinsed again in tap water to reach neutral pH and shaken (150 rpm) with distilled water for 1 hour.

Then the BC membrane immersed in distilled water is sterilized in an autoclave and stored in tightly closed vessels at 4°C.

Finally, the membranes are crushed into a paste with a blender, freeze-dried at -80°C in vacuum plastic bags and labelled as native BC (Sommer et al., 2020).

3.2.2 Preparation of Emulsions

A total of eight different 20% O/W emulsions of 30 mL volume is prepared. Four of them (E1-E4), are without bacterial cellulose and the rest of them (E5-E8) contain 0.3% of bacterial cellulose. The quantities and types of the emulsion components are shown in Table 3. Coconut oil and pork lard are previously melted in the microwave, as they were stored in their solid form.

Table 3. Emulsion composition summary.

Emulsion	Type of oil	Amount of oil	Amount of water	Amount of bacterial cellulose
E1	Canola oil	6 mL	24 mL	-
E2	Olive oil			
E3	Coconut oil			
E4	Pork lard			
E5	Canola oil			0.09 g
E6	Olive oil			
E7	Coconut oil			
E8	Pork lard			

The mixtures that are obtained consist of a water phase on the bottom and the oil phase floating on top. Bacterial cellulose must be fragmented into small particles to obtain a homogeneous suspension of the polymer in the aqueous phase of the emulsion. For that purpose, a cutting grinder is used, as shown in Figure 11.

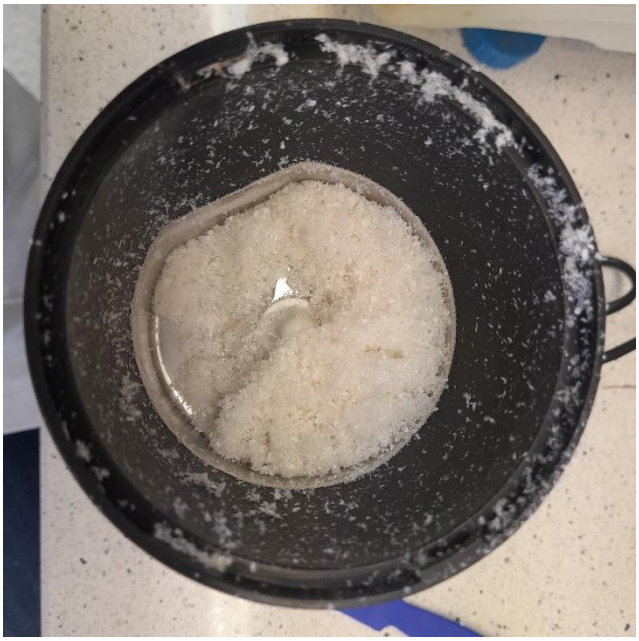


Figure 11. Dry bacterial cellulose grinded.

3.2.3 Sonication

The oil-water mixtures are then homogenized by sonication, which is a physical process that agitates the liquid medium by using sound waves (Berovič et al., 2011). The particles in the sample are broken into smaller particles (mostly nanoparticles) by physical vibrations provided by the energy of sound waves. The intermolecular forces are interrupted by the vibration and the particles are dispersed in the solution, therefore creating an emulsion (Ghauri et al., 2020).

Figure 12. Sonicator apparatus.

Each sample is submitted to sonication for 4 minutes, as shown in Figure 12. The sonicator operating parameters are: power 500W, pulse duration 1 min, pulse 2x5, amplitude 80%.

It can be noticed that instantly the mixture turns opaque and white, indicating the formation of the emulsion. After each sample the apparatus tip is purged with ethanol, running the process for ten to twenty seconds.

The emulsions are then stored at room temperature in plastic flasks, creating a replicant of each type for further comparison (Figure 13).

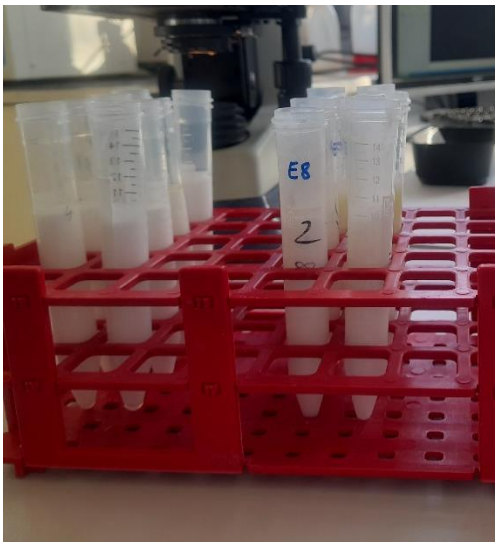


Figure 13. Emulsions stored in conical flasks.

3.2.4 Optical Microscopy

After a week, the samples are analyzed with an optical microscope that is equipped with a camera connected to the software (Figure 14). High Power Objective Lens (40x) is used for that purpose. Photos of droplets of oil are taken in different zones of the sample drop, both at the edge of two phases and in the inside of the emulsion.

Figure 14. Optical microscope equipped with a camera.

3.2.5 ESI

Emulsion Stability Index (ESI) is another way of assessing the stability of emulsions. One replicant of each sample stored for one week at room temperature is centrifuged at 10000 rpm for 5 minutes and the height of the phases is measured with a digital caliper. On the other hand, the samples that have not been centrifuged are submitted to the same measuring procedure. ESI is then calculated according to the formula:

$$ESI\% = \frac{H_E}{H_T} \cdot 100\%,$$

where H_E is the height if emulsion layer and H_T is the total height of the sample.

3.2.6 Fat Phase Oxidative Stability

The final assessment of emulsion stability consists of analyzing absorption spectra at 232 nm wavelength of solutions containing fresh oils and oil phase of emulsions with BC and without BC. Such solutions are prepared dissolving 0.01 gram of oil in 25 ml of hexane. For analyzing the spectra a spectrophotometer Spectrouant® Pharo 300 MERCK is used. The spectra of emulsions are then compared to those of fresh oils to assess how the bacterial cellulose affects fat's oxidation during storage. The numerical value of stability index is calculated according to the formula:

$$E_{1\,cm}^{1\%} = \frac{A}{B \cdot C},$$

where A is absorption value at 232 nm, B is optical path length equal to 1 cm (the length of the cubit wall) and C is oil concentration expressed in g/100mL.

4. RESULTS

4.1 FATTY ACIDS COMPOSITION

4.1.1 Canola Oil

The chromatography report for canola oil obtained lists all the compounds that form the solution with their retention time and area. The chromatogram for canola oil is presented in Figure 15.

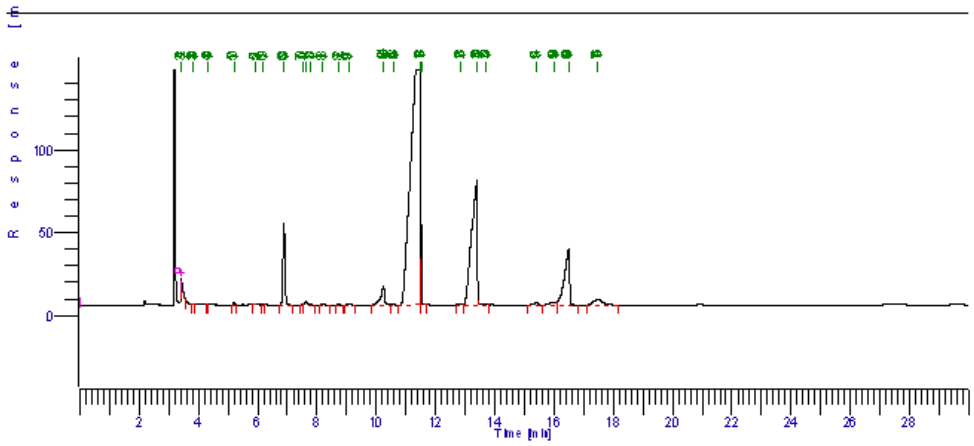


Figure 15. Gas chromatography results for canola oil.

In Table 4 the results from the report are shown. The sum of saturated fatty acids (SFA), total unsaturated fatty acids (UFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) is also calculated and shown below.

Table 4. Fatty acid composition in canola oil.

Retention time (min)	Fatty acid	Common name	FA content
6.887	C16:0	Palmitic acid	4.80%
10.241	C18:0	Stearic acid	2.06%
11.478	C18:c1(n-9)	Oleic acid	72.84%
13.392	C18:3(n-3)	α-Linolenic acid	20.30%
		Σ SFA	6.86%
		Σ MUFA	72.84%
		Σ PUFA	20.30%
		Σ UFA	93.14%
		ΣSFA/ΣUFA	0.07

According to the chromatography report, canola oil is mostly formed by unsaturated fatty acids. Among unsaturated fatty acids, oleic acid is the most abundant. The abundance of unsaturated acids is expected both in canola oil and olive oil because these oils remain liquid at temperatures lower than room temperature.

It is convenient to compare the results with literature values obtained by other authors. Lee et al. determined fatty acid content in various vegetable oils, including canola oil, olive oil and coconut oil. In the case of canola oil, the total content of UFA is 90.12%, being oleic acid the most

abundant acid (57.09%). Also, the study confirmed that canola oil is not rich in SFA, since ΣSFA/ΣUFA ratio is only 0.11 (Lee et al., 1998).

4.1.2 Olive Oil

Similar results can be observed in olive oil. The chromatography report for this sample looks similar to the previous sample and the summary of the results is shown in Table 5.

Table 5. Fatty acid composition in olive oil.

Retention time (min)	Fatty acid	Common name	FA content
6.917	C16:0	Palmitic acid	12.22%
10.184	C18:0	Stearic acid	2.84%
11.422	C18:1	Oleic acid	73.99%
13.285	C18:2(n-6)	Linoleic acid	10.96%
		Σ SFA	15.05%
		Σ MUFA	73.99%
		Σ PUFA	10.96%
		Σ UFA	84.95%
		ΣSFA/ΣUFA	0.18

Olive oil is also rich in unsaturated fatty acids, however in a slightly lower proportion than canola oil (85% versus 93% respectively). However, oleic acid presents almost the same fraction in olive oil as in canola oil. The second most abundant fatty acid in olive oil is saturated palmitic acid.

According to Lee et al., ΣSFA/ΣUFA ratio 0.19, which is close to the value shown in Table 5 (0.18). Moreover, oleic acid content is the highest in olive oil reaching 73.6%. The proportion of total SFA, according to the study, is 15.82%, which is also similar to the results from the table above (Lee et al., 1998).

4.1.3 Coconut Oil

For coconut oil, totally different results are expected, because at room temperature this oil is solid, which indicates that it is rich in saturated fatty acids. The results from the report are summarized in Table 6.

Table 6. Fatty acid composition in coconut oil.

Retention time (min)	Fatty acid	Common name	FA content
10.192	C8:0	Caprylic acid	1.96%
11.424	C12:0	Lauric acid	70.04%
13.349	C14:0	Myristic acid	19.58%
16.446	C16:0	Palmitic acid	8.41%
		Σ SFA	100.00%

As expected, coconut oil is formed by saturated fatty acids only, being lauric acid the most abundant one. It is worth mentioning that the fatty acids listed in the table above have a relatively short chain (lauric acid, for example, only has 12 carbons in the chain).

When these results are compared with literature, some significant differences are found. According to the study carried out by Lee et al., ΣSFA/ΣUFA ratio of coconut oil is between 1.27

1.53, depending on the origin of the sample. Moreover, the content of UFA in the study is around 40% and oleic acid is the most abundant acid within this group (Lee et al., 1998). The discrepancy with the results shown in Table 6 can be explained by the quality of the oil used and the process of refining to which it has been submitted.

4.1.4 Pork Lard

Finally, pork lard is the most interesting sample of all, because of its animal origin and properties that are quite different to the previous samples. It can be seen at first glance that pork lard is formed by a mixture of acids but the most abundant one does not have a high area, as in previous examples, and is only around 40%. All the acids are identified and listed in Table 7.

Table 7. Fatty acid composition in pork lard.

Retention time (min)	Fatty acid	Common name	FA content
5.159	C14:0	Myristic acid	1.46%
6.884	C16:0	Palmitic acid	27.39%
10.113	C18:0	Stearic acid	16.43%
11.196	C18:1	Oleic acid	45.00%
13.129	C18:2	Linoleic acid	9.72%
		Σ SFA	45.28%
		Σ MUFA	45.00%
		Σ PUFA	9.72%
		Σ UFA	54.72%
		ΣSFA/ΣUFA	0.83

Although pork lard is solid at room temperature, just like coconut oil, it is formed by an almost equal mixture of saturated and unsaturated acids, with an unsaturated acid proportion being even higher than its counterpart. The most abundant fatty acid in pork lard is oleic acid, the same as in canola and olive oil. This information makes pork lard an interesting product to analyze within the context of this research.

García Olmo et al. determined the composition of Iberian pork fat in their study and concluded that nearly 35% of pork fat is formed by SFA (mostly palmitic and stearic acids), while around 60% corresponds to UFA (mostly oleic and linolenic acids). It is worth mentioning that this study only considers the main fatty acids (C16:0, C18:0, C18:1 and C18:2) for the calculations. ΣSFA/ΣUFA ratios, according to the study, is approximately 0.56 (García Olmo et al., 2002).

Summing up all the results, the average chain length and ΣUFA/ΣSFA ratio for each sample are calculated and presented in Table 8 below.

Table 8. Summary of the descriptive parameters based on fatty acid content for each sample.

Sample	Average chain length	ΣUFA/ΣSFA ratio
Canola oil	17.90	13.58
Olive oil	17.76	5.64
Coconut oil	12.65	0.00
Pork lard	17.39	1.21

As it can be noticed from the table, coconut oil is rich in fatty acids with the shortest chain. In the following chapters it will be determined whether it has any influence on the stability on emulsions.

4.2 Microscopic Photos of the Emulsion

4.2.1 Canola Oil

The images for the first sample of emulsions without bacterial cellulose are presented below. In Figures 17 and 19 the appearance of the bulk of emulsion can be seen. It looks like an almost uniform phase with occasional dots which correspond to small droplets of oil dispersed in the liquid.

Edge between two phases

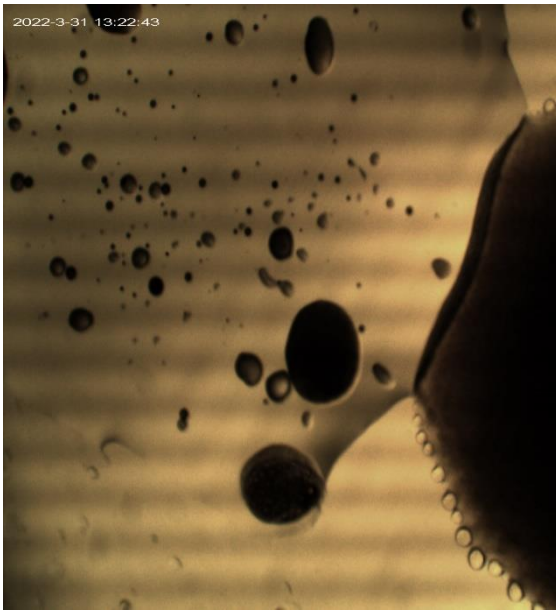


Figure 16

Inside the emulsion

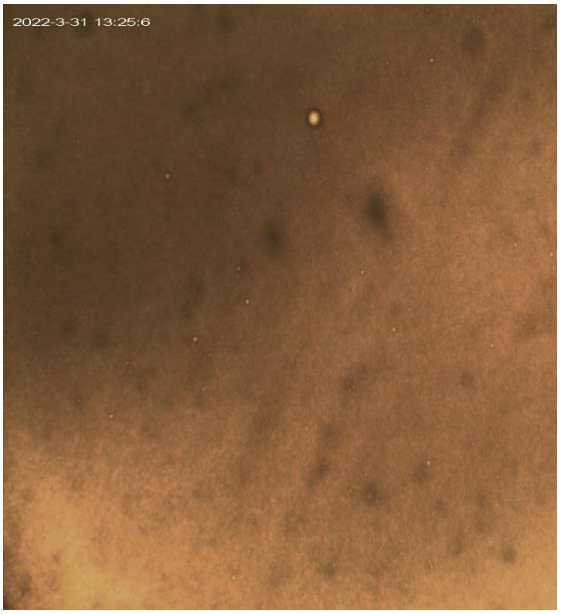


Figure 17

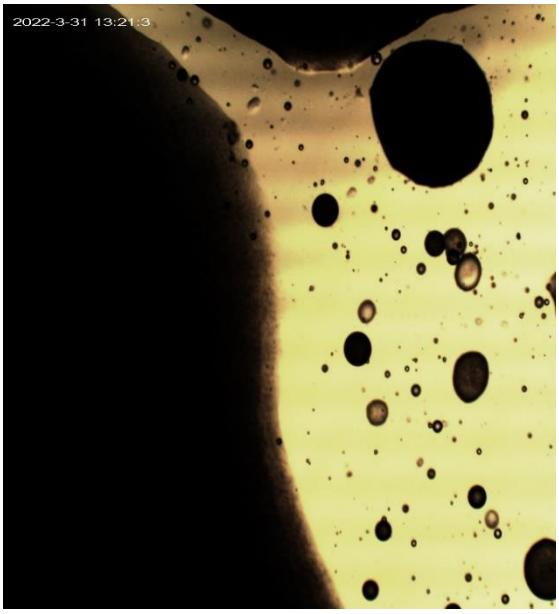


Figure 18

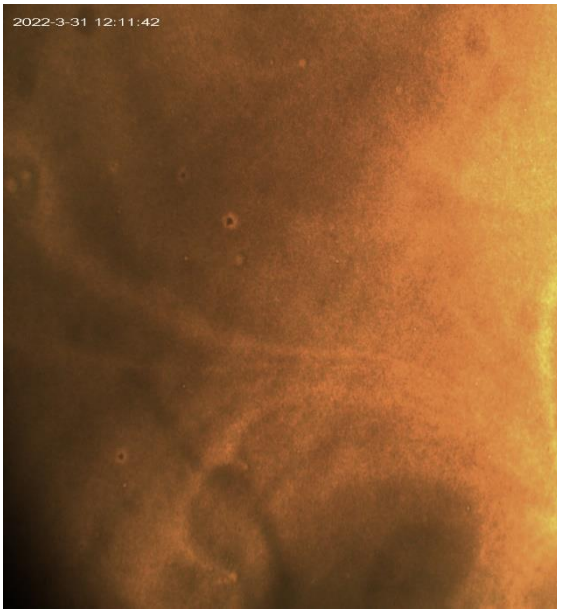


Figure 19

Figures 16-19. Microscopic images for canola oil emulsion with no BC.

On the edge between the phases (Figures 16 and 18) is presented the area where the emulsion breaks. Therefore, larger droplets of oil are seen clearly floating in the water. The size

of the droplets varies based on the zone that is captured. Moreover, emulsion instability phenomena such as flocculation and coalescence are present.

On the other hand, when the bacterial cellulose is added to the emulsion, the microscopic appearance changes significantly, as can be seen in Figures 20 to 23.

Edge between two phases **Inside the emulsion**

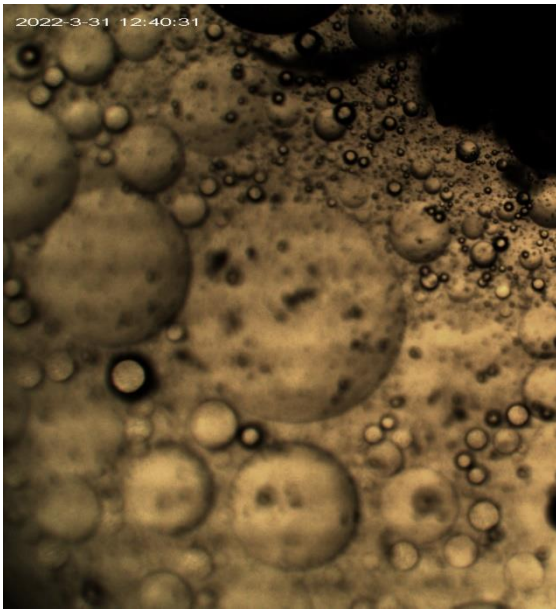


Figure 20

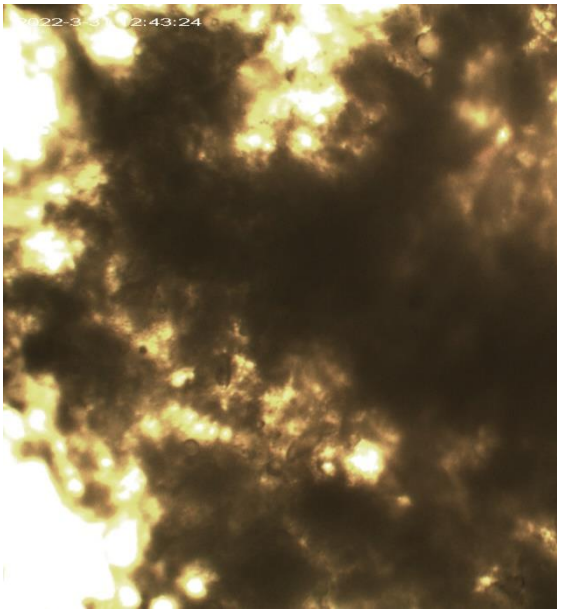


Figure 21

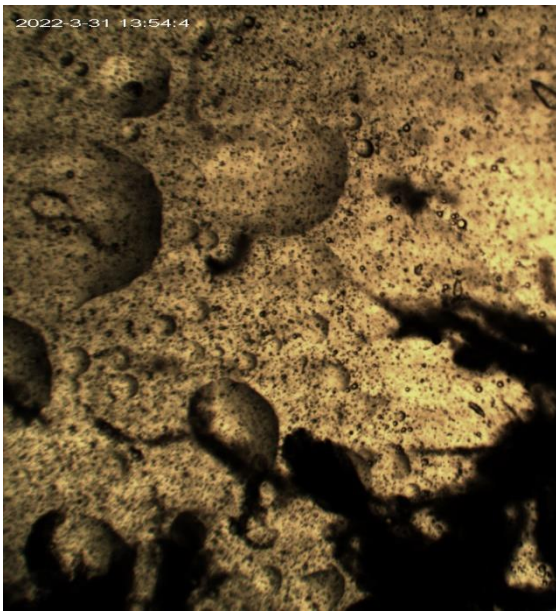


Figure 22

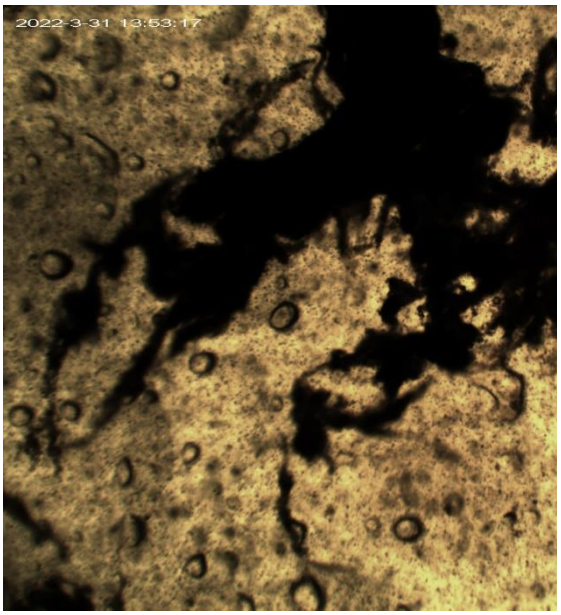


Figure 23

Figures 20-23. Microscopic images for canola oil emulsion with BC.

Inside the bulk of emulsion (Figures 21 and 23) the homogeneous phase is no longer present, due to large chunks and branches of bacterial cellulose spread over the liquid. It is difficult to appreciate the size of droplets of oil because they are so small that cannot be seen under the lens used for the analysis. Occasionally some larger droplets are distinguished in places where the emulsion is not stable. On the edge of the emulsion, however, large droplets of oil are

continuously colliding and are clearly visible (Figure 20). Moreover, black branches of cellulose are also present (Figure 22).

4.2.2 Olive Oil

The images for olive oil emulsions depicted below (Figures 24-27).

Edge between two phases **Inside the emulsion**

Without BC

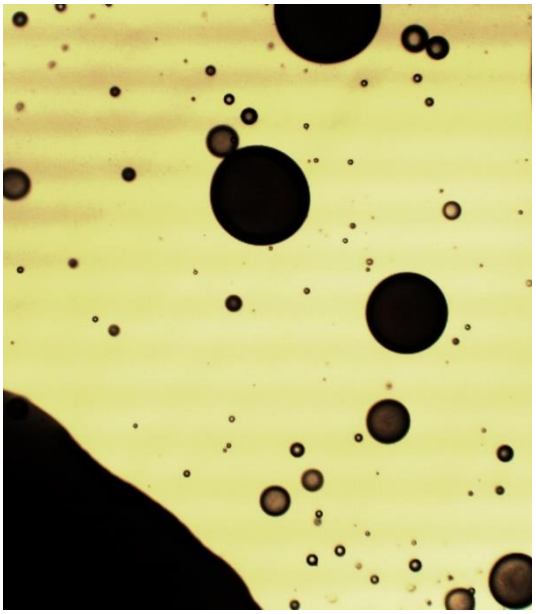


Figure 24

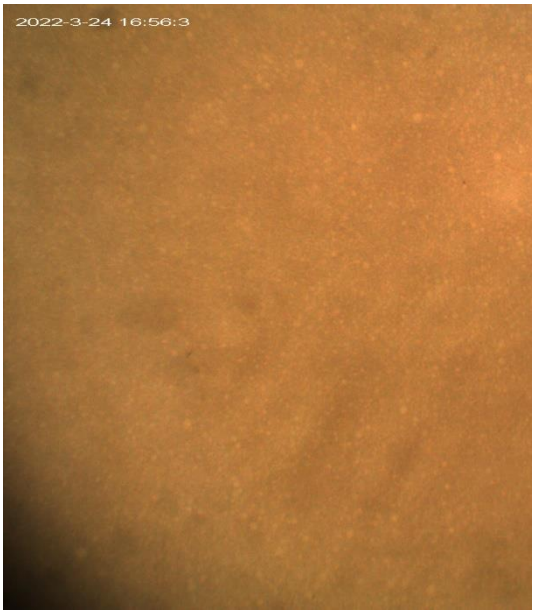


Figure 25

With BC

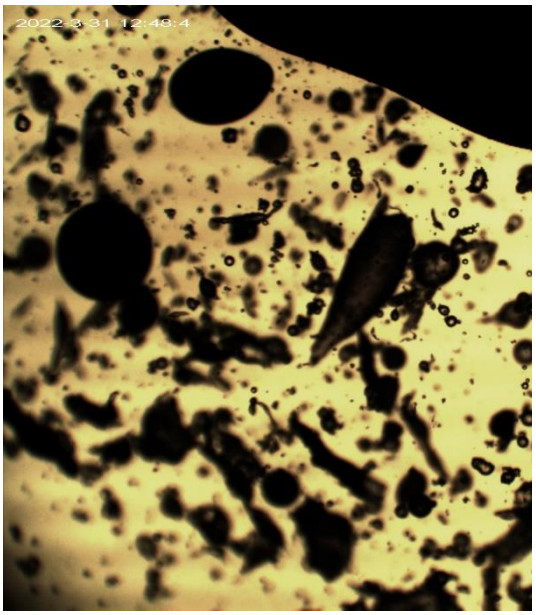


Figure 26

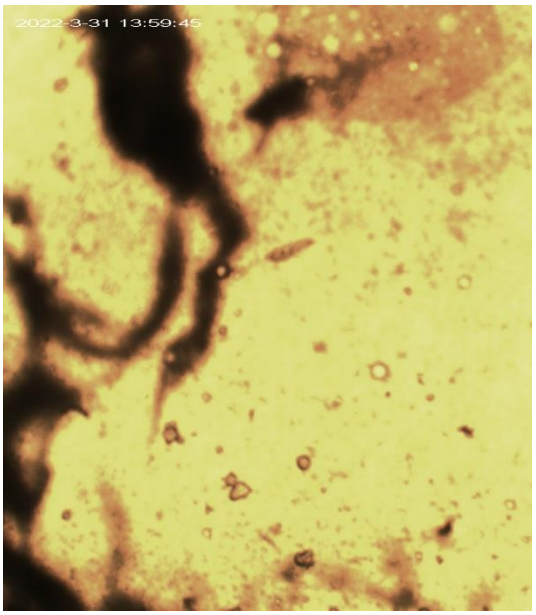


Figure 27

Figures 24-27. Microscopic images for olive oil emulsion.

It is worth mentioning that at the edge of the emulsion with BC (Figure 26) the droplets of oil are not as large as in the previous sample (e.g., Figure 20) and the BC looks more dispersed with smaller chunks. However, the samples of olive oil emulsion without BC (Figures 24 and 25) look similar to those of canola oil emulsion, although in Figure 25 it can be seen that there are more droplets in the bulk emulsion compared to Figures 17 and 19. Based on that observations,

it can be said that olive oil emulsion with BC is more stable than canola oil emulsion with BC, however, the result for emulsions without BC is the opposite.

4.2.3 Coconut Oil

The samples of coconut oil emulsions, however, do not have the same aspect as the previous examples. In the samples without BC, the coalescence at the edge is stronger than in canola oil and olive oil emulsions because the droplets tend to form a larger droplets, as observed in Figure 28.

Edge between two phases **Inside the emulsion**

Without BC

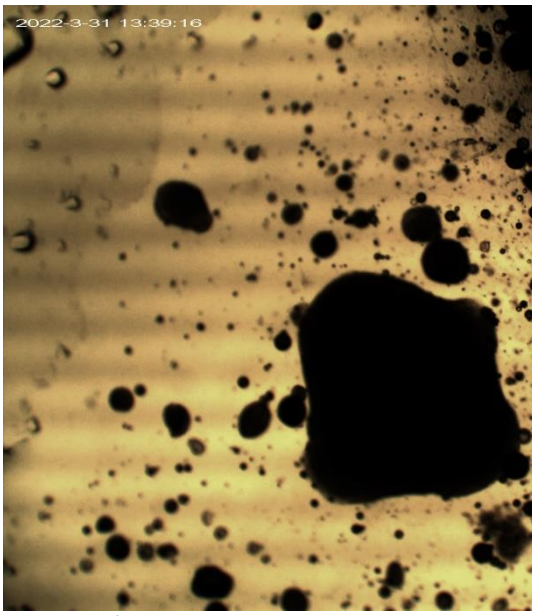


Figure 28

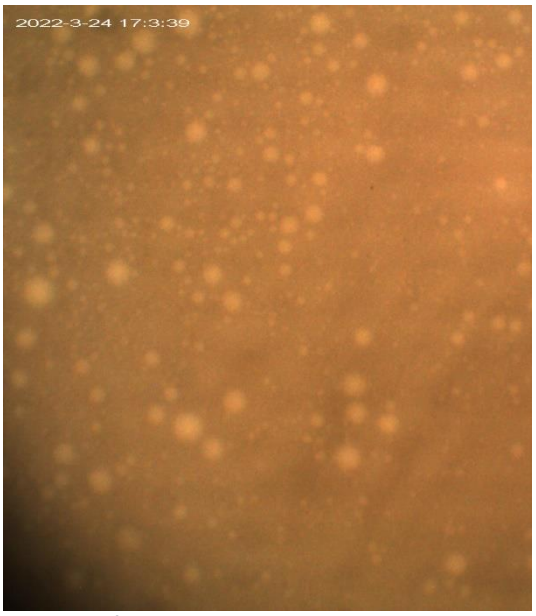


Figure 29

With BC

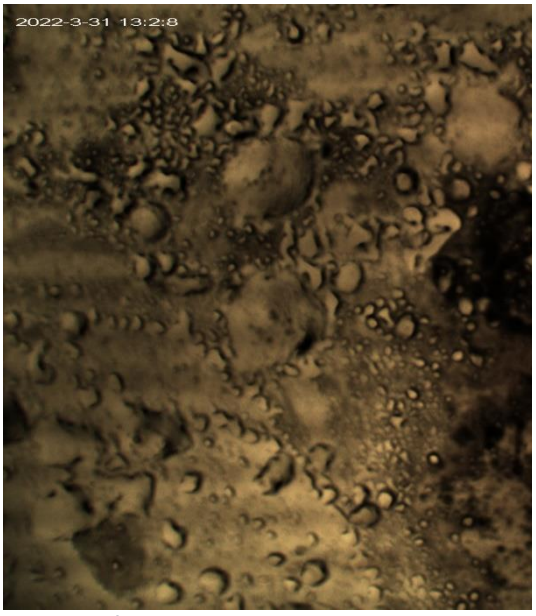


Figure 30

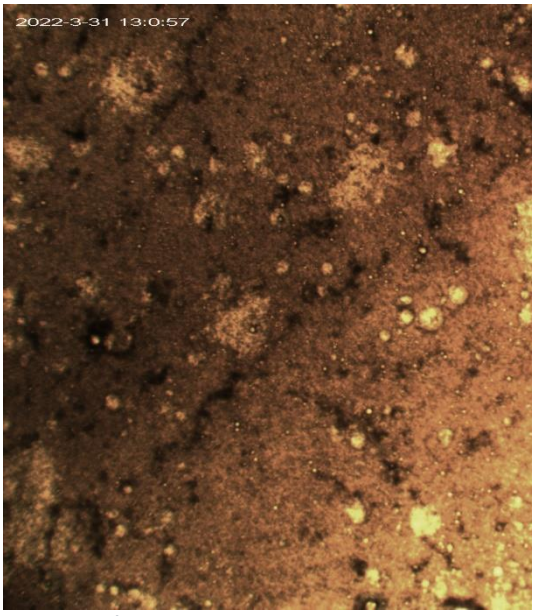


Figure 31

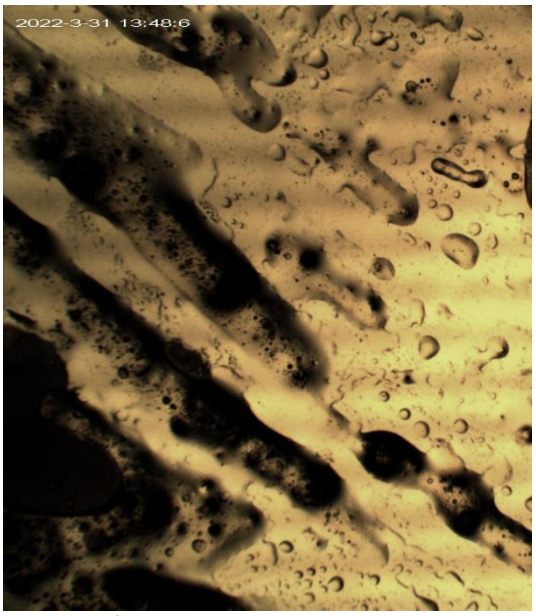
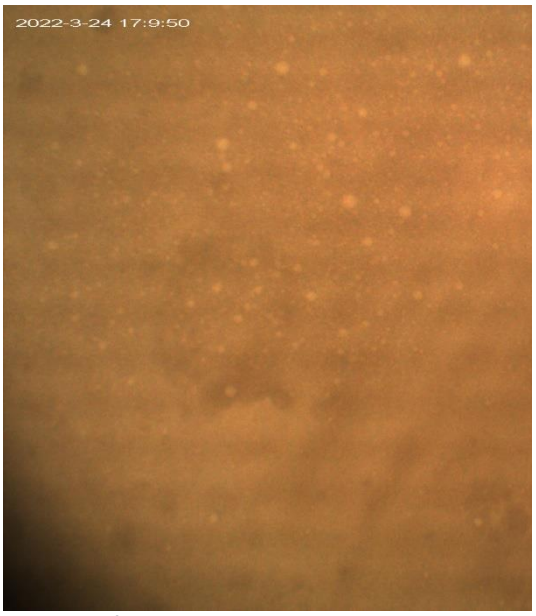
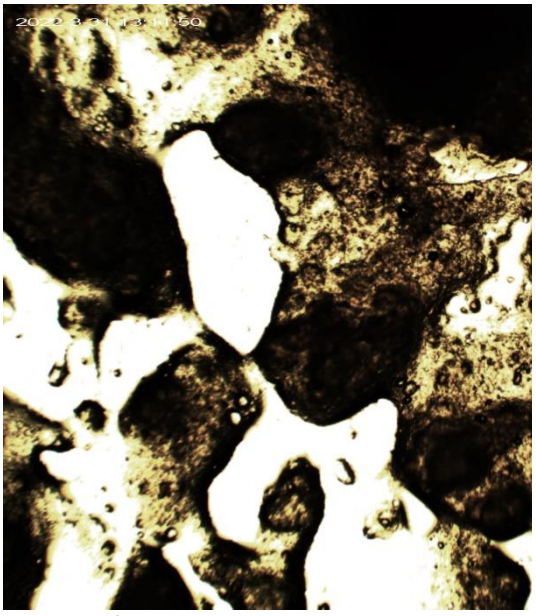
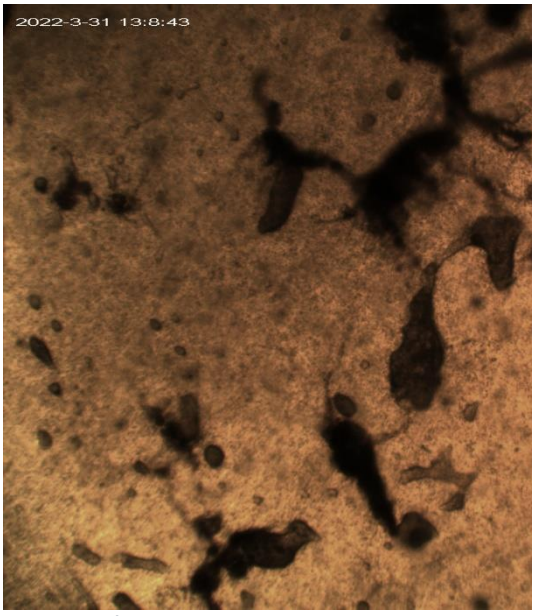
Figures 28-31. Microscopic images for coconut oil emulsion.

Moreover, inside the emulsion (Figure 29) there is a high number of visible droplets dispersed in the liquid, they are larger in size and higher in concentration than before.

The samples with BC look similar to canola oil emulsion images, therefore, it is hard to make a proper conclusion regarding the differences in stability.

4.2.4 Pork Lard

The final sample is presented in Figures 32-35. The droplets of oil inside the bulk of emulsion without BC (Figure 33) look smaller than in coconut oil emulsion (Figure 29) but larger than in olive oil and canola oil emulsions (Figures 25 and 17). At the edge (Figure 32) elongated stains can be seen. They correspond to solidified oil which has been spread over the plate.

	Edge between two phases	**Inside the emulsion**
Without BC	 *Figure 32*	 *Figure 33*
With BC	 *Figure 34*	 *Figure 35*

Figures 32-35. Microscopic images for pork lard emulsion.

For the samples with BC, it can be stated that droplets inside the emulsion are not visible, while at the edge it is hard to distinguish the phases which are mixed with bacterial cellulose branches.

Based on the pictures of all the samples, it can be concluded that the most stable emulsion corresponds to the canola oil sample, while the least stable emulsion is the coconut oil emulsion.

4.3 ESI

The measurements of corresponding heights were carried out with the emulsion stored in plastic tubes. In Figure 36 not-centrifuged samples are presented. It can be observed that samples with BC (E5-E8) have a smaller layer of oil, compared to the samples E1-E4 and a larger water layer.

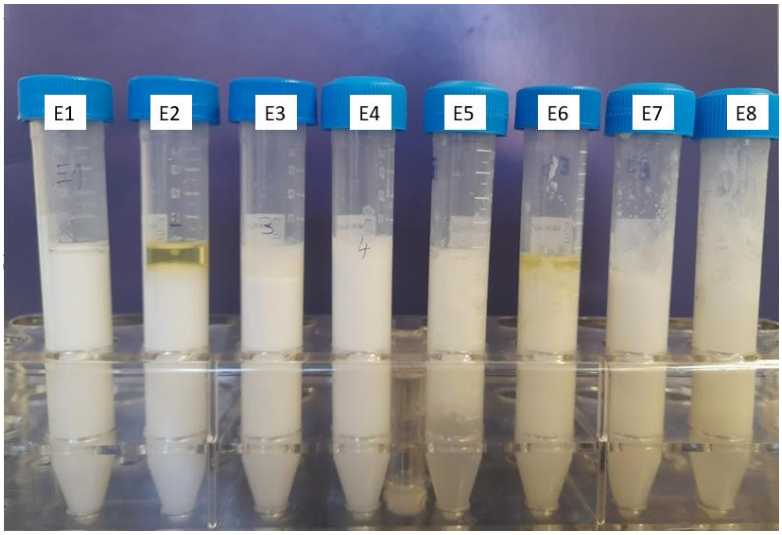


Figure 36. Not-centrifuged samples of emulsion at the moment of measuring.

After centrifuge, the emulsion with bacterial cellulose is completely broken into two phases separated by a layer of the polymer, as shown in the following Figure 37.

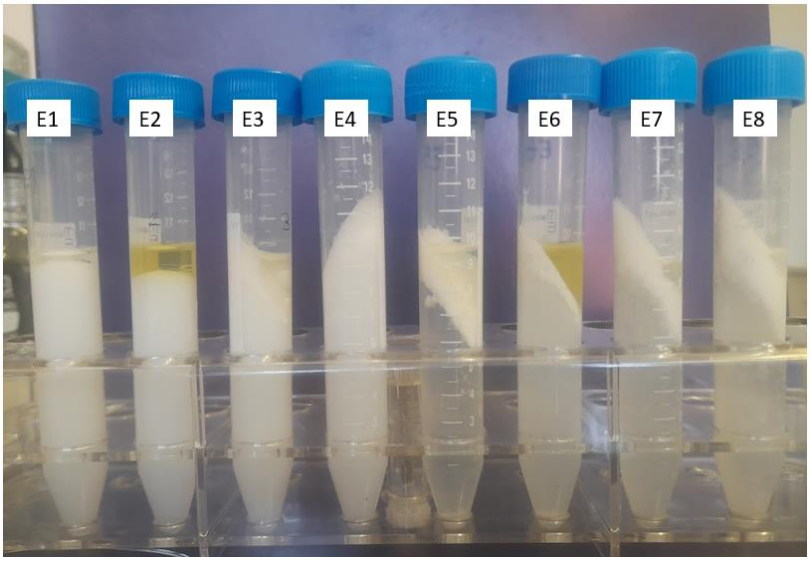


Figure 37. Centrifuged samples of emulsion at the moment of measuring.

However, the samples E1 and E2 (canola oil and olive oil emulsions with no BC) do not seem significantly affected by centrifuge, which indicates that those emulsions are harder to break, therefore they are more stable by centrifugal force. Samples E3 and E4 do not have distinct separation of phases but their shape changed due to the solidification of the oil.

Since two repetitions of each emulsion have been prepared, the average ESI is calculated based on the measurements of two sets of samples. The results are shown in Table 9.

Table 9. ESI results for emulsion samples.

Sample		ESI % before centrifuging	ESI % after centrifuging
E1	Canola oil	85.4 ± 3.33	78.85 ± 6.5
E2	Olive oil	80.78 ± 2.64	70.57 ± 8.93
E3	Coconut oil	83.52 ± 0.5	72.27 ± 6.95
E4	Pork lard	84.12 ± 5.12	66.07 ± 6.15
E5	Canola oil + BC	65.88 ± 5.15	0.93 ± 0.24
E6	Olive oil + BC	89.78 ± 2.89	3.21 ± 2.36
E7	Coconut oil +BC	81.64 ± 10.28	6.74 ± 4.34
E8	Pork lard + BC	69.37 ± 1.28	0.76 ± 0.35

For better visualization of the results, a diagram is shown in Figure 38.

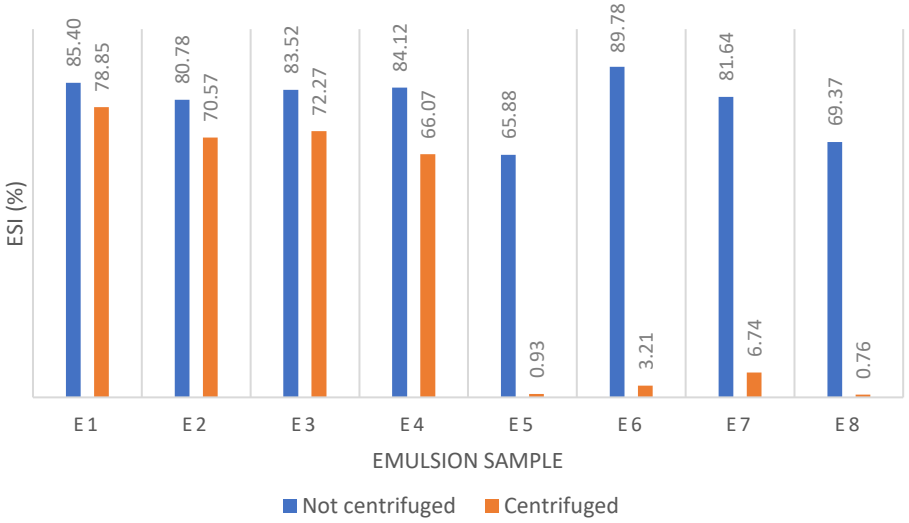


Figure 38. Graphic representation of ESI results.

According to the diagram, the highest ESI corresponds to not-centrifuged olive oil emulsion stabilized with BC (E6), while the emulsions without BC (not-centrifuged either) have practically the same ESI.

As for the effect of centrifuge, it can be said that emulsions of olive oil and pork lard without BC (E2 and E4) are more affected, therefore the emulsion is easier to break compared the canola oil and coconut oil emulsions (E1 and E3). Within the centrifuged samples without BC canola oil emulsion (E1) has the highest ESI.

Based on the results of this assessment, it can be said that within samples without BC, canola oil emulsion is the most stable sample. This conclusion is also backed up by the observations from the Optical Microscopy section.

Regardless of the conclusions, it should be mentioned that this assessment is not particularly accurate because the shape of the tube in which the height is measured is not uniform, since it is conical at the bottom. Moreover, the measurement is taken manually, and this way of measuring is known to bring a lot of errors to the data.

4.4 Fat Phase Oxidative Stability

The results from statistical analysis are presented in Table 10 and correspond to averages of E1% from two replications ± standard deviation. Different superscript letters (a–c) within the same row indicate significant differences due to the type of emusion ($P < 0.05$). Data were evaluated by analysis of variance (one-way procedure) using SigmaPlot 11.0 (Softonic International S.L). Differences between the means were determined by Tukey test ($P < 0.05$).

Table 10. ANOVA results for fat oxidation test.

	Fresh oil	Emulsion	Emulsion with BC
Canola oil	$35,7 \pm 0,8^{c}$	$1,7 \pm 1,3^{a}$	$7,1 \pm 2,6^{b}$
Olive oil	$4,4 \pm 0,1^{a}$	$4,9 \pm 1,5^{ab}$	$7,4 \pm 1,1^{b}$
Coconut oil	$3,8 \pm 0,4^{a}$	$4,4 \pm 1,5^{a}$	$4,9 \pm 1,9^{a}$
Pork lard	$5,4 \pm 0,9^{b}$	$12,8 \pm 2,4^{c}$	$3,5 \pm 2,0^{a}$

As it can be noted from the table, emulsions of liquid oils (canola and olive) experiment higher oxidation when BC is added, because the E1% index of emulsion with BC is significantly higher than that of the simple emulsion. However, in the case of solid fats (coconut oil and pork lard), BC seems to prevent the fat phase from oxidation.

Nevertheless, it should be mentioned that this final test does not contribute considerably to the conclusions, mainly due to the little number of repetitions and data, so the ANOVA test may not be precise enough to consider these results valid.

5. DISCUSSION

Clearly, emulsions of solid fats perform worse than emulsions of liquid oils when it comes to stability. Coconut oil is formed entirely by saturated fatty acids, (see 4.1.3) of a relatively short chain. In emulsions stabilized with BC, these fatty acids do not contribute to stability because the network of microfibrils of BC on the surface is not strong enough to prevent the droplets from coalescence. However, BC has another unexpected effect on coconut oil emulsions which is preventing the oil phase from oxidation during storage.

Moreover, pork lard emulsions are not stable either, although they perform slightly better than coconut oil emulsions. Pork lard is roughly an equal mixture of saturated and unsaturated fatty acids of a larger chain (see 4.1.4). The downside of pork lard is that it is easily solidified at room temperature, which makes it harder for BC microfibrils to form a strong mechanical barrier over solidified droplets of pork lard. To obtain a better comparison of the behavior of solid fats in emulsions, the experiment should be carried out at higher temperatures to maintain the fat liquid.

On the other hand, liquid oil emulsions show higher stability. Olive oil emulsions stabilized with BC have the best performance according to the ESI test. This oil is rich in unsaturated fatty acids (see 4.1.2) that apparently contribute to stability by remaining trapped in the network of BC microfibrils. It happens because unsaturated fatty acids are not straight like their saturated counterparts but are bent at the double bond. This molecular geometry makes easier for them to get entangled in the microfibrils and not collide to from large droplets.

Finally, canola oil, also a liquid oil that is richer in polyunsaturated fatty acids than olive oil (see 4.1.1), has the best performance in emulsions with BC according to the microscopic determination of droplet size. The same logic can be applied in this case. Polyunsaturated fatty acids have more than one double bond, therefore more than one crook on the chain. That is why it is even easier for them to stay attached to the network of BC rather than join other molecules in the formation of a large droplet.

As to the influence of the chain length of the fatty acid on stability of emulsions with BC, it is convenient to reference the Table 8, which shows the average chain and the highest ΣUFA/ΣSFA ratio of the oils. For better visual understanding, a graph based on the aforementioned table is plotted in Figure 39. It can be noticed that canola oil, which, according to the previous observations, is considered the best oil for BC-stabilized emulsions, has the highest parameters among other samples, followed by olive oil and pork lard. Coconut oil shows the lowest chain length among the samples and, as it has been mentioned above, does not contribute to stability of emulsions.

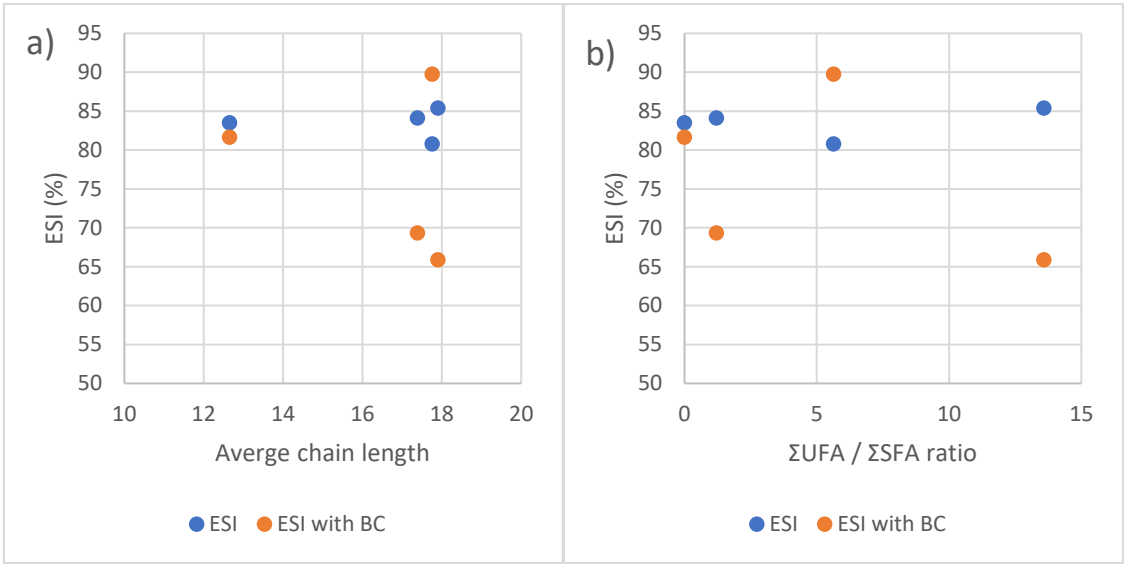


Figure 39. Correlation between the fatty acid composition and the stability of emulsions obtained based on selected oils: a) Average chain length vs. ESI, a) Average chain length vs. ESI, b) ΣUFA / ΣSFA ratio vs. ESI.

Long-chained fatty acids are more favorable to create stability in emulsions with BC. For longer chains there are more possibilities to be trapped in the network of microfibrils created by BC, while shorter chains can move in the liquid with more freedom and eventually collide with each other, resulting in increased coalescence of the droplets and lower stability of the emulsion.

6. CONCLUSIONS

Results obtained show that emulsions made from oils rich in long-chained unsaturated fatty acids definitely show better stability than their saturated counterparts when bacterial cellulose is used as a stabilizer. The major downside of solid fats is their point of solidification, in which bacterial cellulose cannot properly encase the solid droplets of fat in its microfibril network. Moreover, the shorter fatty acid chains significantly decrease the stability, since they move in water with more freedom and easily escape the BC fibril “cage”.

The effect of BC as an emulsion stabilizer has been also confirmed, since the size of oil droplets in stabilized emulsions is significantly reduced in comparison to the non-stabilized emulsions. What is more, it has been discovered that bacterial cellulose prevents solid fat from oxidation during storage, which can become a useful characteristic for many applications of BC (e.g., food, cosmetics).

Finally, more extensive testing and appropriate statistical analysis, such as principal component analysis (PCA), are required to further investigate the effect of fatty acids in triacylglycerols on emulsion stability.

7. TABLE OF FIGURES

8. TABLE OF TABLES

9. BIBLIOGRAPHY

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