Preliminary Evaluation of Different Solvents and Mixture Ratios for Extraction of Lipids: A Case Study for Rapeseed Oil

Lappeenranta–Lahti University of Technology LUT

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Examiner: Postdoctoral Researcher Samuel Emebu

ABSTRACT

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Preliminary Evaluation of Different Solvents and Mixture Ratios for Extraction of Lipids: A Case Study for Rapeseed Oil

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Lipid extraction is useful in the food industry and wastewater management. In the food industry lipids need to be extracted for analysis which is done to provide nutritional information to consumers. Wastewater management is eased by extraction of lipids that otherwise cause blockages and overflow. In addition, lipids extracted from wastewater provide a cost-effective and environmentally friendly feedstock for biodiesel production.

Solvent extraction is the most common method used to extract lipids. Solvent mixtures that contain both polar and nonpolar solvents have been found to be most efficient for lipid extraction. Out of solvents, chloroform has been found as most efficient in lipid extraction. Due to environmental and health issues related to chloroform use, alternative solvents are sought to replace it.

During the experimental part of the thesis three solvents and six solvent mixtures were used to extract a fatty acid mixture containing oleic acid, linoleic acid and palmitic acid. These fatty acids were chosen based on the fatty acid composition of rapeseed oil, a common oil used in food products and biodiesel production. The choice was also affected by the prevalence of the fatty acids in wastewater in addition to the fatty acids representing different fatty acid types.

After solvent extraction, fatty acid yields were analysed using chromatography. A dichloromethane/butanol -mixture with the ratio of 3:2 outperformed other mixtures and solvents. Butanol is relatively rarely used but more environmentally friendly option for lipid extraction. More studies are needed to find environmentally friendlier nonpolar solvents for lipid extraction.

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Liuottimien ja niiden seossuhteiden alustava arvioiminen lipidien uuttoa varten: rypsiöljyn tapaustutkimus

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Lipidien uuttaminen on hyödyllistä elintarvikealalla sekä jätevesien puhdistuksessa. Elintarvikealalla lipidit uutetaan analyysiä varten, jotta voidaan tarjota kuluttajille tietoa tuotteiden ravintosisällöstä. Jätevesien puhdistusta voidaan helpottaa uuttamalla lipidit, jotka muutoin voivat aiheuttaa tukoksia ja ylivuotoja. Lisäksi jätevedestä uutetut lipidit tarjoavat kustannustehokkaan ja ympäristöystävällisen raaka-aineen biodieselin tuotantoa varten.

Liuottimilla uuttaminen on yleisin tapa erottaa lipidejä. Sekä poolisia että poolittomia liuottimia sisältävät liuotinseokset ovat tehokkaimpia lipidien uutossa. Liuottimista kloroformi on todettu usein tehokkaimmaksi lipidien uuttamisessa. Vaihtoehtoisia liuottimia etsitään korvaamaan kloroformia sen käyttöön liittyvien ympäristö- ja terveyshaittojen vuoksi.

Kokeellisen osan aikana kuutta liuotinseosta ja kolmea liuotinta käytettiin uuttamaan vesiemulsiosta rasvahapposeosta, joka koostuu oleiinihaposta, linolihaposta ja palmitiinihaposta. Rasvahapot valittiin rypsiöljyn rasvahappokoostumuksen perusteella. Rypsiöljy on yleinen öljy, jota käytetään elintarvikkeissa sekä biodieselin tuotannossa. Rasvahappojen valintaan vaikutti myös rasvahappojen esiintyvyys jätevedessä sekä rasvahappojen edustavan erilaisia rasvahappotyyppejä.

Liuottimilla uuton jälkeen rasvahapposaannot analysoitiin käyttäen kaasukromatografia. Dikloorimetaani/butanoli -seos seossuhteella 3:2 suoriutui paremmin kuin muut seokset ja liuottimet. Butanoli on suhteellisen harvoin käytetty, mutta ympäristöystävällisempi vaihtoehto lipidien uuttoon. Lisää tutkimuksia tarvitaan löytämään ympäristöystävällisempiä poolittomia liuottimia lipidien uuttoa varten.

SYMBOLS AND ABBREVIATIONS

BuOH	Butanol
DCM	Dichloromethane
DHA	Docosahexaenoic Acid
EPA	Eicosapentaenoic Acid
FA	Fatty Acid
FAEE	Fatty Acid Ethyl Ester
FAME	Fatty Acid Methyl Ester
FFA	Free Fatty Acid
GC	Gas Chromatography
Hx	Hexane
LCFA	Long-Chain Fatty Acid
LDL	Low-Density Lipoprotein
MeOH	Methanol
MTBE	Methyl Tert-Butyl Ether
MUFA	Monounsaturated Fatty Acid
PUFA	Polyunsaturated Fatty Acid
SFA	Saturated Fatty Acid
TAG	Triacylglycerol
UFA	Unsaturated Fatty Acid

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DECLARATIONS

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The originality of this thesis has been reviewed with the Turnitin similarity checking service.

AI usage

The author of this thesis, Viivi Kuosmanen, did not use any AI tools during the preparation of the thesis**.**

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The author, Viivi Kuosmanen, takes full responsibility for the content of this thesis and has reviewed and edited the content generated by the possible use of AI tools.

1 Introduction

Around 25 to 30 percent of daily calories in a healthy diet should come from fats (Meijaard et al. 2022). Due to many food products containing lipids in addition to lipids being used in industrial practices, lipids make up for around 40% of the organic content of municipal wastewater (Muller, Sheik & Wilmes 2014). Lipid extraction is useful in analysis of food products for health-related reasons (Aggarwal & Upadhyay 2026) and in wastewater to ease waste management (Frkova et al. 2020). In addition to waste management, lipid extraction of wastewaters is useful as wastewaters have been suggested as a potential feedstock for biodiesel production (Frkova et al. 2020).

Amounts of lipids are often measured using analytical equipment, such as gas chromatography or liquid chromatography. In order to get accurate analytical results, lipids often have to be extracted from their original sample (Löfgren, Forsberg & Ståhlman 2016). Solvent extraction is the most commonly used, often most affordable and the simplest method for lipid extraction (Ren et al. 2021). In order to efficiently extract a wide variety of lipids, a solvent mixture containing both polar and nonpolar solvents is needed (Saini et al. 2021). Different solvent extraction methods have been suggested but those usually rely on the use of toxic solvents. As most lipids are nonpolar (Saba, Parini & Owais 2023) it is likely that mixtures with higher ratios of nonpolar solvents are most efficient.

The purpose of this thesis is to examine lipid extraction with different solvents and solvent mixture ratios as well as ways to analyse the efficacy of different solvents and solvent mixture ratios in lipid extraction. During the literature part of the thesis, lipids in food products and wastewaters, methods to analyse lipid content and solvent extraction of lipids will be discussed. In the experimental part, three solvents and two solvent mixtures with three different ratios of polar and nonpolar solvents will be tested to see which solvents and which mixture ratios have the best capabilities to extract common fatty acids. The fatty acid extraction yields will be analysed using gas chromatography.

2 Applicable lipids

Lipids are organic molecules that are either hydrophobic or amphiphilic (Traversier et al. 2018). Lipids can be classified into eight groups based on their structure (Xue et al. 2025). These groups are fatty acyls, glycerolipids, glyceryl phospholipids, sphingolipids, sterol lipids, prenol lipids, saccharolipids and polyketides (Xue et al. 2025).

Fatty acyls consist of a hydrocarbon chain with an attached carboxyl group (Xicoy, Wieringa & Martens 2019). All lipids containing a glycerol-group, such as triacylglycerols (TAGs), are considered glycerolipids (Fahy et al. 2005). Glycerolipids are formed by the esterification of fatty acids (FAs) to a glycerol backbone (Xicoy, Wieringa & Martens 2019). Glycerophospholipids in turn contain a glycerol-group in addition to a phosphate-group (Fahy et al. 2005; Xicoy, Wieringa & Martens 2019). Lipids that contain a sphingoid base are considered sphingolipids (Fahy et al. 2005). Sterol lipids have a structure containing four fused rings (Xicoy, Wieringa & Martens 2019).

Fatty acyls include FAs and derivatives of FAs (Fahy et al. 2005). Some lipids included in sterol lipids are cholesterols, estrogen and vitamin D_2 and D_3 (Fahy et al. 2005). In turn, prenol lipids include vitamin E and vitamin K (Fahy et al. 2005). Saccharolipids have a sugar backbone with attached FAs (Fahy et al. 2005).

2.1 Structure and properties of lipids

When referring to fats and oils, usually TAGs are meant (UC Davis Department of Nutrition 2018). TAGs consist of three FAs connected to a glycerol backbone (Barkley & Wang 2013). Although FAs are often found as a component of other lipids, FAs can also exist freely, or in other words as free fatty acids (FFAs) (Barkley & Wang 2013).

FAs can be divided into three groups based on carbon-chain length: short-chain fatty acids (6 carbons or less), medium-chain fatty acids (C6-C12) and long-chain fatty acids (at least 12 carbons) (Hewavitharana et al. 2020). Long-chain fatty acids (LCFAs) can yet be

classified into very long-chain fatty acids when there are at least 20 carbons in the chain (de Carvalho & Caramujo 2018). Moreover, FAs can be divided into two groups based on saturation: saturated fatty acids (SFAs) and unsaturated fatty acids (UFAs). SFAs do not contain any double bonds between carbon atoms (Mititelu et al. 2024). In contrast, UFAs contain either one double bond between carbon atoms (monounsaturated fatty acids, MUFAs) or multiple double bonds (polyunsaturated fatty acids, PUFAs) (Mititelu et al. 2024).

UFAs are usually liquids in room temperature (Harvard T.H. Chan School of Public Health n.d.) whereas SFAs are solid (Mititelu et al. 2024). To make UFAs partially solid at room temperature, oils with high UFA content are heated with the presence of hydrogen which increases the amount of double bonds between carbon atoms in trans-configuration (Harvard T.H. Chan School of Public Health n.d.; Mititelu et al. 2024). The products of deliberate hydrogenation of UFAs are called industrial trans fatty acids (Harvard T.H. Chan School of Public Health n.d.).

Omega refers to the farthest carbon from the carboxyl group in FAs (Valenzuela Baez & Valenzuela 2013). PUFAs can be divided into omega-3 and omega-6 FAs depending on the location of the first double-bond between carbons (UC Davis Department of Nutrition 2018). Linolenic acid is found with omega-3 isomer called α-linolenic acid and omega-6 isomer called γ-linolenic acid (Kim et al. 2014). α-linolenic acid is a precursor to omega-3 fatty acids docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) (Kim et al. 2014).

Based on their structure lipids can be either polar or neutral/nonpolar (Cascant et al. 2017). Polarity is an important factor when analysing lipid content and extracting lipids, as it affects which substances lipids are soluble in. Lipids with long unbranched carbon chains are often nonpolar, whereas polar lipids usually contain a polar compound, such as a hydroxyl group or sugar (Traversier et al. 2018). Some examples of polar lipids are phospholipids, whilst glycerolipids are usually nonpolar (Cascant et al. 2017). FFAs can be either polar or nonpolar with SFAs and MUFAs often being nonpolar and PUFAs more polar (Marlina et al. 2020).

2.2 Lipids in food products

The World Health Organisation recommends that at least 20% of the daily calories are made up of fat (Liu et al. 2017). Total fat consumption does not have a strict upper since there are no distinct health risks (Liu et al. 2017). However, due to fat containing a high amount of calories, high-fat diets often cause weight gain (Liu et al. 2017). A diet containing a higher amount of lipids may also cause pathogenic microbes to grow in the gut (Tsiantas et al. 2022). In contrast, diets that contain a lower amount of lipids cause nutritional deficiencies as the body does not get enough essential FAs or fat-soluble vitamins (Oliveira, Santos & Garcez 2020; Liu et al. 2017).

Analysing lipid content of food products is important in examining food quality and safety as well as defining nutritional levels of food products (Aggarwal & Upadhyay 2026). The FA analysis of food products is needed for the nutritional information sheet often required by legislation (Hewavitharana et al. 2020). The analysis of FAs in food products can help determine which ingredients are healthiest (Oliveira, Santos & Garcez 2020).

The most common lipids found in animals and humans are TAGs (Saini et al. 2021; Valenzuela Baez & Valenzuela 2013). Also, most of the lipids in food are TAGs (Maldonado-Pereira et al. 2022). TAGs can consist of FAs with different carbon-chain lengths and different amounts of carbon-carbon double bonds. (UC Davis Department of Nutrition 2018).

SFA content in food products is of interest as high SFA content is known to cause a plethora of health issues. The most common SFA found in food products is palmitic acid (Feingold 2024). Palmitic acid is a medium-chain FA (Nantapo et al. 2015). It is also the most common FA found in humans (Carta et al. 2017; Feingold 2024). High amounts of palmitic acid are found in vegetable oils (Frkova et al. 2020), dairy and meat (Feingold 2024; Carta et al. 2017).

A diet high in SFAs causes obesity, cardiovascular diseases (CVDs) and high blood cholesterol levels (Hewavitharana et al. 2020; Nantapo et al. 2015). Especially short-chain saturated FAs are responsible for higher low-density lipoprotein (LDL) cholesterol levels (Nantapo et al. 2015). Some foods high in FAs that cause LDL-cholesterol levels to rise include milk, coconut oil and palm oil (Nantapo et al. 2015).

However, some SFAs do not have the same effect. Stearic acid has been suggested to have beneficial health effects, such as reduced risk of high LDL-cholesterol, high blood pressure and cancer (Senyilmaz-Tiebe et al. 2018). Like stearic acid, UFAs have positive impacts on health. UFAs have been connected to a decreased risk of developing CVDs, diabetes, high blood pressure and arthritis (Kathuria et al. 2025; Nantapo et al. 2015). Out of PUFAs, omega-3- and omega-6- FAs lessen inflammation and are an essential part of diets since cannot be produced by the human body naturally (Mititelu et al. 2024). However, PUFAs are easily oxidized which affects the quality of food products (Münch et al. 2024). Lipid oxidation in food products causes changes in flavour and reduces the vitamin content (Schmid, Guihéneuf & Stengel 2016).

Omega-3 FAs can be found in fish, canola oil and flaxseed oil, whereas omega-6 is found in nuts and corn oil (UC Davis Department of Nutrition 2018). Out of omega-3 FAs, DHAs have been found to aid in neural signalling speed, whereas EPAs have relieving effects on depression (UC Davis Department of Nutrition 2018). One example of omega-6 FAs is linoleic acid which is found in example in breast milk and vegetable oils (Meijaard et al. 2022; Frkova et al. 2020).

Unlike omega-3 and omega-6 FAs, oleic acid can be produced by the human body (Barkley & Wang 2013). Oleic acid is a MUFA that lengthens the shelf life of food products and causes positive health effects, such as reduced risk of CVDs (Barkley & Wang 2013), cancer (Kumar & Deak 2025) and cholesterol levels (Pravst 2014). Oleic acid is found in high concentrations in some vegetable oils, such as olive oil (Pravst 2014).

Trans FAs can be found naturally in some animal products (UC Davis Department of Nutrition 2018). However, most dietary trans FAs are produced industrially due to their long shelf-life, cost-efficiency and stability in high temperatures (Mititelu et al. 2024). Industrial trans FAs are often used in highly processed foods and fast foods (Mititelu et al. 2024). Trans FAs are known to cause CVDs, high LDL-cholesterol levels, high blood pressure and high blood sugar (Mititelu et al. 2024). Industrial trans FAs are banned in some countries, such as Denmark, due to their negative health effects (Liu et al. 2017).

Polar lipids have also been found to have positive effects on health (Jardim, Domingues & Alves 2023; Nantapo et al. 2015). Polar lipids have been found to improve cognitive function and memory (Jardim, Domingues & Alves 2023). Polar lipids are also antioxidants and anti-

inflammatory (Jardim, Domingues & Alves 2023). Antioxidant lipids have positive effects on health as well as the shelf-life of food products (Carré et al. 2025).

2.3 Lipids in wastewater

Municipal and agro-industrial wastewater contain significant amounts of lipids which causes major issues in wastewater management (Frkova et al. 2020). Hundreds of millions of euros go towards fixing piping issues related to high fat, oil and grease content (Frkova et al. 2020). Over 700 million people rely on waste management facilities that do not cleanse wastewaters adequately (Willcock et al. 2021).

Inadequate management of municipal wastewaters can cause the spread of pathogens and contributes to global warming (Alsaedi et al. 2022; Fujihira et al. 2018). Wastewater is unfortunately often released into nature when funding is not sufficient (Willcock et al. 2021). If released into nature, wastewaters with high lipid contents can form a surface on top of soil and natural waters that prevents the access of important factors, such as oxygen and sunlight (El-Emam 2023; Ortellado et al. 2025). The absence of water, oxygen and sunlight in soil causes issues with plant metabolism (Bartholomeus et al 2008; Offord, Meagher & Zimmer 2014), whereas in natural waters it causes issues with aquatic organisms (Ortellado et al. 2025).

Almost half of the lipids found in municipal wastewater come from feces, and the rest from kitchen waste (Frkova et al. 2020). Feces contain mostly oleic acid and palmitic acid (Frkova et al. 2020). Both polar and nonpolar lipids are found in wastewater sludges (Alsaedi et al. 2022). Main lipids found in municipal wastewaters are TAGs (Muller, Sheik & Wilmes 2014). In turn, high concentrations of FFAs were found in wastewaters from facilities that produce cooking oils, out of which long-chain fatty acids were most common (Efimova et al. 2013). The median concentration of LCFAs in municipal wastewaters has been found to be around 19-539 mg/L depending on the country (Frkova et al. 2020). Especially high amounts of palmitic acid, stearic acid, oleic acid and linoleic acid are present in wastewaters (Ibrahim & Hamza 2017).

TAGs and LCFAs induce the growth of bacteria in wastewater which in turn causes foaming and bulking (Efimova et al. 2013). Foaming causes the overflow of wastewater sludge and

the release of pathogens (Cheng et al. 2024). Sludge bulking is the increase of sludge volume which makes it more difficult to remove sludge from water (Zhang et al. 2025). The presence of water causes the hydrolysis of lipids (Ulmer et al. 2020) which frees FAs which in turn causes pipes to clog (Chipasa & Medryzka 2006).

2.3.1 Biodiesel made from wastewater derived lipids

Since every year the amount of wastewater high in lipids increases (Chipasa & Medryzka 2006), an alternative method to process lipid-rich wastewaters is needed. Wastewaters with high lipid content have been suggested as a potential feedstock for biodiesel production (Frkova et al. 2020). Currently mostly first- and second-generation materials are being used to produce biodiesel which does not solve the issues related to carbon dioxide emissions (Matwijczuk et al. 2017; Panchal et al. 2025). First generation feedstock for biodiesel production are edible plants (Ramluckan, Moodley & Bux 2014) which exacerbates issues with the food market (Matwijczuk et al. 2017). Second generation feedstocks are non-edible plants that use up vast amounts of farmable land (Ramluckan, Moodley & Bux 2014). The third-generation feedstock, currently mainly available on a research scale, includes algae (El Marji et al. 2026).

Up to 85% of biodiesel production costs are related to feedstock (Siddiquee & Rohani 2011) which makes using fossil fuels instead more economically feasible (Ibrahim & Hamza 2017). The most common oils used to produce biodiesel are soybean, rapeseed, sunflower and palm oils (Demirbas et al. 2016; Nikolic et al. 2025; Demirbas 2009). Soybean, sunflower, rapeseed and palm oil are first generation feedstock (Ramluckan, Moodley & Bux 2014), whereas jojoba, jatropha and castor oil are examples of second-generation feedstocks (Demirbas 2009). The FA composition of the most common oils used for biodiesel production is shown in Table 1.

Table 1. Estimated fatty acid composition (%) of the most common oils used for biodiesel production (Fabiszewska &. Białecka-Florjańczyk 2015; Annisa & Widayat 2018)

Fatty acid	Soybean oil	Rapeseed oil	Sunflower oil	Palm oil
Palmitic acid, C16:0	6	4	7	42
Stearic acid, C18:0	5	2	3	5

Oleic acid, C18:1	20	64	30	41
Linoleic acid, C18:2	64	18	58	10
Linolenic acid, C18:3	5	8	-	-

Biodiesel is composed of mainly fatty acid methyl esters (FAMEs) (Ibrahim & Hamza 2017) that are derived from LCFAs (Veitía-de-Armas et al. 2024). Some biodiesel is also made from fatty acid ethyl esters (FAEEs) (Mendow et al. 2011). Biodiesel can be made by the transesterification of glycerolipids or the esterification of FFAs (Caetano & Castanheiro 2013; Efimova et al. 2013). The most common FAs found in wastewater have a chain length of 14 to 18 carbons which have the most potential to create high-quality biodiesel (Muller, Sheik & Wilmes 2014). FAMEs are made with methanol-based and FAEEs with ethanol-based transesterification (Ibrahim & Hamza 2017; Mendow et al. 2011). FAEEs have generally higher cetane numbers and lower emissions, though ethanol-based transesterification is more sensitive to changes in reaction conditions compared to methanol-based reactions (Mendow et al. 2011).

Better biodiesel quality is indicated by having a high cetane value since it is connected to quicker autoignition and better combustion (Sharma et al. 2025). Higher concentrations of SFAs and longer unbranched carbon chains lead to a higher cetane value (Zarrinmehr et al. 2022). The higher the number of double bounds between carbons, the smaller the cetane number (Marlina et al. 2020). Especially higher stearic acid and palmitic acid concentrations improve the quality of biodiesel (Zarrinmehr et al. 2022). SFAs also improve the storage stability of biodiesel (Zarrinmehr et al. 2022). The presence of some UFAs, such as oleic acid, improves the cold flow properties of biodiesel due to lower viscosity compared to SFAs (Sharma et al. 2025; Zarrinmehr et al. 2022).

The main challenge with using lipids from wastewaters is the high amount of FFAs which increases the cost of biodiesel production (Demirbas et al. 2016; Nikolic et al. 2025) due to increased formation of soap and hindered transesterification of TAGs (Gupta 2025). Fats and oils in wastewater have usually gone through multiple rounds of heating which increases the amount of FFAs (Matwijczuk et al. 2017). Also, the use of biodiesel is criticised for having higher NO_x emission rates than conventional diesel (Panchal et al. 2025). Still, using wastewaters with high lipid content would ease wastewater management, solve the issues related to using first- and second-generation feedstock in addition to saving money (Matwijczuk et al. 2017)

3 Lipid extraction and analysis

Before analysis, lipids usually need to be extracted from the original sample. Lipid extraction for analytical purposes is done in order to remove substances, such as proteins or carbohydrates, that might affect the results from analysis (Löfgren, Forsberg & Ståhlman 2016). Analytical methods are used to measure lipid content. Commonly used analytical methods used include liquid chromatography and gas chromatography (GC). Some spectroscopic methods used include Raman spectroscopy, infrared spectroscopy and ultraviolet spectroscopy.

Gas chromatography is widely used in the analysis of lipid content due to its selectivity and efficiency even though multiple steps of pretreatment are needed (Hewavitharana et al. 2020). Raman has been found useful in the analysis of lipid content in live cells as it is not as invasive as gas chromatography and liquid chromatography (Zhou et al. 2025). Especially mass spectrometry analysis in gas chromatography and liquid chromatography is useful for the analysis of lipids (Venturi et al. 2025). Although liquid chromatography is criticised for not ideal selectivity and high consumption of solvents (Chiu & Kuo 2020), it is often less expensive to use and better for analysis of smaller amounts of FAs (Saini et al. 2021).

Infrared spectroscopy and ultraviolet spectroscopy are sometimes used for rapid screening of lipid content (Oliveira, Santos & Garcez 2020). UV-spectroscopy is used for rapid screening of lipids due to its low cost compared to more accurate analytical methods (Oliveira, Santos & Garcez 2020). However, UV-spectroscopy is not widely used in the analysis of lipid and fatty acid content due to the low absorption range that lipids and FAs have (usually less than 200 nm) (Czauderna et al. 2002; Traversier et al. 2018).

3.1 Solvent extraction of lipids

The traditional solvent extraction methods of lipids are often most efficient and simple for lipid extraction even though often use large amounts of solvents that pose risks for health and the environment (Efimova et al. 2013). Some methods, such as the Folch method, use around 20 times more solvent compared to the sample volume (Hewavitharana et al. 2020), whereas the Bligh and Dyer method uses only 4 times more solvent compared to amount of sample (Axelsson & Gentili 2014). Solvent extraction methods have been praised for great lipid yield and quality as well as easier scalability to broader use (Ren et al. 2021).

3.1.1 Different solvents recommended for lipid extraction and polarity

The polarity of the lipids and the solvents affect the extraction efficiency (Hewavitharana et al. 2020). Nonpolar solvents usually extract more lipids compared to polar solvents (Zarrinmehr et al. 2022). However, polar solvents are useful not only to extract polar lipids but also to disturb hydrogen bonds and ion-dipole bonds between lipids and proteins (Venturi et al. 2025; Saini et al. 2021).

Polar solvents include methanol (MeOH), butanol (BuOH) and ethanol. In turn, chloroform, hexane (Hx), dichloromethane (DCM) and methyl tert-butyl ether (MTBE) are examples of nonpolar solvents. The polarity of solvents is visualised with the polarity index and relative polarity shown in Table 2. Polarity index values compare the polarity of the solvent to the polarity of water (Wakeel et al. 2019).

Table 2. Polarity index values and relative polarity of solvents (Kleiman, Ryu & Esser-Kahn 2015; Dong et al. 2016; Nti-Gyabaah, Gbewonyo & Chiew 2010).

Relative polarity	Polarity index	Solvent
0.762	5.1	methanol
0.654	5.2	ethanol
0.586	4.0	n-butanol
0.309	3.1	dichloromethane
0.259	4.1	chloroform
0.228	4.4	ethyl acetate
0.009	0.1	n-hexane

Hexane has been found to be the most efficient solvent at the extraction of fatty acids (Reis et al. 2013; Frkova et al. 2020). UFAs are often best extracted with polar solvents, whereas best SFA yields have been gained with nonpolar solvents (Zarrinmehr et al. 2022). Ethanol and methanol have been found to be efficient at extracting FAMEs (Ramluckan, Moodley & Bux 2014; Frkova et al. 2020). Dichloromethane has been found to be better at extracting LCFAs than MTBE, whereas MTBE is more efficient at extracting volatile FAs (Sremački et al. 2025).

Nonpolar solvents are often regarded as the most efficient at lipid removal as most lipids are nonpolar (Amna & Faheem 2025). Chloroform is one of the most used nonpolar solvents to extract lipids due to its ability to remove lipids from a wide polarity range (Venturi et al. 2025). Additionally, hexane is often found to be really efficient at lipid extraction (Frkova et al. 2020; Reis et al. 2013). Hexane has also been widely used in lipid extraction due to its affordable price and neutrality regarding oil (Bakhshabadi et al. 2025; Carré et al. 2025). However, both chloroform and hexane have been criticised for being toxic for health and the environment (Cajka & Fiehn 2014; Cascant et al. 2017).

Chloroform is a known carcinogen (Löfgren, Forsberg & Ståhlman 2016), whereas long term exposure to n-hexane causes muscle weakness and poor motor coordination (Kumar & Deak 2025). Chloroform has effects on the ozone layer which expedites climate change (McCulloch 2003). Hexane has been found to cause air pollution (Amna & Faheem 2025) in addition to issues with aquatic organisms and vegetation (Kumar & Deak 2025).

Dichloromethane has been suggested to replace more toxic chloroform (Cajka & Fiehn 2014). Some more environmentally friendly solvents have been suggested to replace hexane, but with varying results (Zapata-Boada et al. 2022). Ethyl acetate seems to be to most potential environmentally friendly solvent to replace hexane (Cascant et al. 2017; Veitía-de-Armas et al. 2024).

When choosing a solvent for lipid extraction, the intended use of the lipids also needs to be considered. Methanol is a preferred polar solvent in the extraction of lipids for biodiesel production since it is also used in the transesterification of TAGs to FAMEs (Muller, Sheik & Wilmes 2014). In contrast, hexane has been found to slow down transesterification (Carvalho & Malcata 2005). In food production, the choice of solvents is especially important. According to the European Food Safety Authority out of liquid solvents only

ethanol, ethyl acetate and acetone may be used without restriction (Carré et al. 2025). Dichloromethane, hexane and methanol are allowed in food production for some purposes with restrictions in residue levels (Carré et al. 2025).

High water content in samples from wastewater and food products cause issues in lipid extraction since it decreases the interactions between solvent and lipids (Saini et al. 2021). Solvents with medium to high polarity, such as methanol or ethyl acetate, can be useful when extracting lipids from samples containing water due to being at least partly miscible (Saini et al. 2021; Showman et al. 2024). Methanol is often found to be the most useful polar solvent as it can also extract non-polar components (Riyadi et al. 2023). In contrast, nonpolar solvents like n-hexane have difficulties removing lipids from water due to their low solubility (Alsaedi et al. 2022).

3.1.2 Solvent extraction methods and mixture ratios

For lipid extraction, a mixture of nonpolar and polar solvents is recommended (Saini et al. 2021). The conventional methods for lipid extraction are the Folch method and the Bligh and Dyer method. The Folch method uses chloroform and methanol with the ratio of ν: ν (2:1) (Hewavitharana et al. 2020). The Bligh and Dyer method modifies the Folch method by adding water in addition to chloroform and methanol with the ratio of ν: ν: ν (1:2:0,8) (Amna & Faheem 2025; Kehelpannala et al. 2020). The Folch and the Bligh and Dyer method are often modified to fit the needs of the experiment at hand in example by using heat or changing the mixture ratios (Kehelpannala et al. 2020).

Solvent mixtures containing chloroform and methanol have been found to gain the highest total lipid yield (Traversier et al. 2018). The Folch method has had best results at the extraction of TAGs (Reis et al. 2013; Pilecky, Kainz & Wassenaar 2024). The Folch method has also been found to extract more FFAs than the Bligh and Dyer method (Reis et al. 2013). FA profiles have not been affected by the ratio of solvents used in the solvent mixtures (Frkova et al. 2020; Pilecky, Kainz & Wassenaar 2024).

Although the conventional methods are often found to be most efficient, more environmentally friendly methods have been suggested to replace the use of chloroform. The BUME-method uses butanol and methanol with the ratio of ν: ν (3:1) (Chiu & Kuo 2020;

Hewavitharana et al. 2020). The MTBE-method uses methanol and MTBE with the ratio of ν: ν (1.5:5) (Cajka & Fiehn 2014; Hewavitharana et al. 2020).

Some other less used methods include the AOAC method and the Bloor method. The AOAC method uses petroleum ether and diethyl ether to extract lipids (Taha, Metherel & Stark 2012). The Bloor method in turn uses alcohol and ether with the ratio of ν: ν (3:1) (Pilecky, Kainz & Wassenaar 2024). Also, Cequier- Sánchez et al. (2008) have suggested a method that uses a mixture of dichloromethane and methanol with the ratio of (2:1). In Table 3, different solvents and mixtures in addition to the lipid sample are displayed.

Table 3. Lipid extraction performance of different solvents and mixtures from varying lipid samples.

Compared solvent mixtures	Best two solvents/mixtures	Lipid Sample	Reference
Hexane Ethyl acetate Ethyl butyrate Ethyl propionate	Hexane Ethyl propionate	Guava seeds and spent coffee grounds	Veitía-de-Armas et al. 2024
Chloroform Hexane Petroleum ether Ethyl acetate Methanol Chloroform/MeOH 2:1 Hx/MeOH 2:1	Chloroform/ MeOH 2:1 Hx/MeOH 2:1	Algal paste	Mathimani, Uma & Prabaharan 2017
BuOH/MeOH 1:1, 3:1 Chloroform/MeOH 2:1	BuOH/MeOH 1:1 Chloroform/MeOH 2:1	Human plasma	Alshehry et al. 2015
Chloroform/MeOH 1:1 MeOH/Hx 1:1 Ethanol/Hx 1:1 Isopropanol/Hx 1:1 Acetone/Hx 1:1 Acetonitrile/Hx 1:1 THF/Hx 1:1	Chloroform/MeOH 1:1 MeOH/Hx 1:1	Microalgae	Shin et al. 2018
Chloroform/MeOH 2:1 DCM/MeOH 2:1	DCM/MeOH 2:1 Chloroform/MeOH 2:1	Dorsal muscle of European seabass	Cequier-Sánchez et al. 2008
Hexane Toluene Chloroform Dichloromethane Acetone	Methanol Dichloromethane	Sewage sludge	Ekka et al. 2024

Ethanol Methanol			
MTBE Chloroform/MeOH 2:1 Hx/Isopropanol 3:2	Chloroform/MeOH 2:1 MTBE	Egg yolk	Showman et al. 2024

Hx = hexane, MeOH = methanol, DCM = dichloromethane, MTBE = methyl tert-butyl ether, BuOH = butanol, THF = tetrahydrofuran

3.1.3 Solvents chosen for experimental part

The solvents chosen for the experimental part are hexane, dichloromethane, methanol and butanol. The most important factor when choosing solvents is lipid extraction efficiency, though environmental and health risks as well as intended use is also considered. These solvents have performed well in lipid extraction studies as seen on Table 3. The solvent mixtures in the experimental part of the thesis are made up of one polar and one nonpolar solvent. The ratios of mixtures have also been chosen based on previous studies on the subject.

4 Materials and methods

The experimental part consists of preparing solvent and fatty acid mixtures, pretreatment for the mixtures when necessary as well as the analysis of lipid content using gas chromatography.

4.1 Chemicals

Fatty acids used in the fatty acid mixture are high purity oleic acid, linoleic acid and palmitic acid. Solvents used are analytical grade hexane, dichloromethane, methanol and butanol. Other chemicals needed in the experiment are xantham gum, n-dodecane and N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA): trimethylsilylchloride (TMCS) (2:1) - solution.

4.2 Mixture preparation and sample pretreatment

The fatty acid mixtures are made up of oleic acid, linoleic acid and palmitic acid. The ratios of each fatty acid in the mixture are based on the fatty acid composition of rapeseed oil found in Table 4. These fatty acids were specifically chosen based on their amount in rapeseed oil as well as other fatty acid sources, such as wastewater, other vegetable oils and food products, to obtain solvent efficiency results on a wider scale.

The fatty acids were measured using an analytical scale, results of which are also found in Table 4. The mixture is left in a 70 ºC oven with a cap on to attain a homogenous mixture since palmitic acid is solid at room temperature, whereas oleic acid and linoleic acid are liquid. The fatty acid mixture was in the oven for an hour. The fatty acid mixture before homogenization in the oven is shown in Appendix 2, Figure 1. The mixture was measured into 9 samples using an analytical scale.

Table 4. Fatty acid mixtures made for GC-analysis alongside fatty acid composition of rapeseed oil.

Fatty acid	Mass measured to mixture used in GC-analysis, g	Fatty acid mixture composition, %	Fatty acid composition of rapeseed oil, % (Fabiszewska & Białecka-Florjańczyk 2015)
Oleic acid	3.7209	74.4	64
Linoleic acid	1.0454	20.9	18
Palmitic acid	0.2334	4.7	4
Total	4.9997	100	86

Four solvents were used in fatty acid extraction: n-hexane, dichloromethane, methanol and 1-butanol. However, pure methanol was not used in fatty acid extraction due to it being miscible in water. In addition to the pure solvents, 2 mixtures with three different ratios (1:1, 3:2, 4:1) each were used in the extractions. The solvents were measured to capped glass containers using a micropipette.

Table 5. Samples of fatty acid mixture and solvent/solvent mixture used for GC- analysis.

Sample	Solvent/solvent mixture	Mass of added fatty acid, mg
1	Hx	20.6
2	DCM	20.2
3	BuOH	20.6
4	Hx/MeOH, 1:1	20.4
5	Hx/MeOH, 3:2	20.3
6	Hx/MeOH, 4:1	20.6
7	DCM/BuOH, 1:1	20.5
8	DCM/BuOH, 3:2	20.4
9	DCM/BuOH, 4:1	20.3

Hx = hexane, DCM = dichloromethane, BuOH = butanol, MeOH = methanol

The fatty acid mixture samples were made into water emulsion by adding 1 ml of emulsifier solution to each sample. The emulsifier solution is made by adding 0.038 g of xantham gum emulsifier to 15 ml to pure water. Fatty acid and emulsifier water solution was shaken with

a vortex twice for around 15 seconds. After forming fatty acid emulsion 1 ml of solvent/mixture was added.

After adding solvent/mixture each sample is centrifuged for 5 minutes at 2000 rpm. The amount of fatty acid in each mixture is displayed in Table 5. After centrifugation two distinct phases are formed: one with water and emulsifier and one with solvents and extracted fatty acids. Hexane and butanol form a layer on top of water emulsion, whereas dichloromethane layer is below water emulsion layer. Solvent layer which contains extracted fatty acids is transferred with a glass pipette to another capped test tube.

Extracted samples were dried using nitrogen gas to get rid of solvents. Using a micropipette 3 µl of n-dodecane is added to samples. In addition, 250 µl of BSTFA: TMCS (2:1) mixture is added. Samples are then shaken with a vortex for a few seconds and left in a 70 ºC oven for 45 minutes. Samples were then transferred to gas chromatography vials with a glass pipette.

Calibration samples of fatty acid mixture were measured with an analytical scale. Masses of fatty acid sample were 5.1 mg, 10.1 mg, 20.1 mg and 30.1 mg. The calibration samples also had 3 µl of n-dodecane added. The calibration samples went through the same silylation process as samples with solvent, though were not dried under nitrogen gas.

4.3 Analysis with gas chromatography

The gas chromatograph used is Agilent Technologies HP 6890 which is displayed in Appendix 2 Figure 4. Flame ionization detector (FID) was used to analyse the samples. Hydrogen was used as the carrier gas. The column used was the Restek MXT 1 column with the internal diameter of 0.53 mm, film thickness of 0.15 µm and length of 15 m. Fatty acids in order of retention time (lowest to highest) are palmitic acid, linoleic acid and oleic acid due to carbon chain length and number of carbon-carbon double bonds as well as trimethylsilyl derivatization (Nikolova-Damyanova 2019; Wan et al. 2007).

The mass of each extracted fatty acid in samples is calculated using equation (1) where A_{FA} is the area fatty acid peak, q_{FA} is the intersection of the fatty acid calibration curve and s_{FA}

is the slope of the fatty acid calibration curve. The calibration curves with slopes and intersections included are displayed in Appendix 1.

$$m_{FA} = \frac{A_{FA} - q_{FA}}{s_{FA}} \quad (1)$$

where m_{FA} mass of extracted fatty acid, mg

A_{FA} area of extracted fatty acid

q_{FA} the intercept of the fatty acid calibration curve

s_{FA} the slope of the fatty acid calibration curve

The yield of each fatty acid as well as total fatty acids is calculated using equation (2) with mass of recovered fatty acids calculated using equation (1).

$$Y(\%) = \frac{m_{FA}}{m_{FA,0}} \cdot 100\% \quad (2)$$

where m_{FA} mass of extracted fatty acid, mg

$m_{FA,0}$ mass of fatty acid in mixture, mg

5 Results and discussion

The fatty acid yields from solvent extraction and analysis with GC-FID are displayed in Table 6. The best yields are near 90% extraction rate from sample 8, whereas the worst ones from sample 9 do not even reach 40% extraction. There are slight differences in how the different types of fatty acids are extracted from the samples with different solvents/mixtures.

Table 6. Yield of extracted fatty acids from water emulsion according to GC-results.

	Oleic acid yield		Linoleic acid yield		Palmitic acid yield		Total fatty acid yield	
Sample	mg	%	mg	%	mg	%	mg	%
1	0.647	53.6	2.14	50.0	8.22	67.3	11.0	53.4
2	0.531	58.4	2.25	53.2	8.78	56.3	11.6	57.2
3	0.651	72.5	3.04	70.5	11.1	67.7	14.8	71.8
4	0.544	53.8	2.16	50.7	8.17	57.2	10.9	53.3
5	0.669	62.8	2.45	57.6	9.49	70.6	12.6	62.1
6	0.565	53.6	1.86	43.1	8.21	58.8	10.6	51.6
7	0.351	45.0	1.54	36.0	6.86	36.7	8.76	42.7
8	0.857	90.5	3.59	84.1	13.7	90.0	18.2	89.1
9	0.333	41.5	1.54	36.2	6.27	35.1	8.14	40.1

Different types of fatty acids are found in the fatty acid mixture: MUFA (oleic acid), PUFA (linoleic acid) and SFA (palmitic acid). Both oleic and linoleic acids are best extracted with almost the same order of solvents/mixtures. Since the fatty acid mixture is mostly made up of UFAs, the solvents/mixtures that extracted the most UFAs also achieved the highest total fatty extraction. The order of solvents/mixtures when extracting SFAs differs more than with UFAs but not significantly. The solvents/mixtures ranked in order of fatty acid extraction efficiency according to results from Table 6 are displayed in Table 7.

Table 7. Solvents/mixtures used in fatty acid extraction ranked in order from best to worst fatty acid yield.

Palmitic acid extraction	Oleic acid extraction	Linoleic acid extraction	Total fatty acid extraction
DCM/BuOH 3:2	DCM/BuOH 3:2	DCM/BuOH 3:2	DCM/BuOH 3:2
Hx/MeOH 3:2	BuOH	BuOH	BuOH
BuOH	Hx/MeOH 3:2	Hx/MeOH 3:2	Hx/MeOH 3:2
Hx	DCM	DCM	DCM
Hx/MeOH 4:1	Hx/MeOH 1:1	Hx/MeOH 1:1	Hx
Hx/MeOH 1:1	Hx	Hx	Hx/MeOH 1:1
DCM	Hx/MeOH 4:1	Hx/MeOH 4:1	Hx/MeOH 4:1
DCM/BuOH 1:1	DCM/BuOH 1:1	DCM/BuOH 4:1	DCM/BuOH 1:1
DCM/BuOH 4:1	DCM/BuOH 4:1	DCM/BuOH 1:1	DCM/BuOH 4:1

DCM = dichloromethane, BuOH = butanol, Hx = hexane, MeOH = methanol

Out of the pure solvents, methanol excluded, butanol was able to extract the best yield of fatty acids. Dichloromethane extracted more UFAs than hexane and therefore also a higher amount of total fatty acids. However, hexane extracted significantly more SFAs compared to dichloromethane. As a polar solvent butanol has better extraction capabilities from samples containing water and often better UFA yields when compared to less polar solvents (Saini et al. 2021; Showman et al. 2024; Zarrinmehr et al. 2022). Fatty acid extraction yield (%) of each pure solvent is displayed in Figure 1.

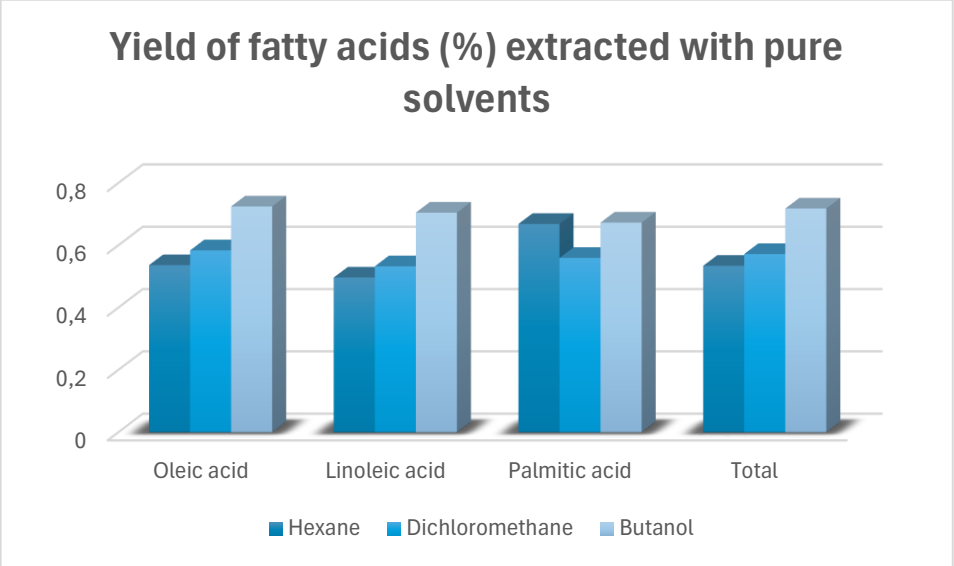

Figure 1. Oleic acid, linoleic acid and palmitic acid yields (%) achieved using pure hexane, dichloromethane and butanol.

Out of hexane/methanol -mixtures, the ratio of 3:2 achieved the best yields in all categories. Hexane/methanol -mixture with the ratio of 1:1 achieved better UFA yields and therefore also better total extraction compared to mixture with the ratio of 4:1. In contrast, SFA extraction was slightly better with the 4:1 ratio. The mixture with the ratio 1:1 has a higher amount of polar solvent (methanol) which generally extracts UFAs better than nonpolar solvents (Zarrinmehr et al. 2022). In contrast, SFAs are generally extracted better with nonpolar solvents (Zarrinmehr et al. 2022) which explains why the mixture with the ratio of 4:1 got better results at only SFA extraction compared to the 1:1 mixture. Fatty acid extraction of each hexane/methanol -mixture is displayed in Figure 2.

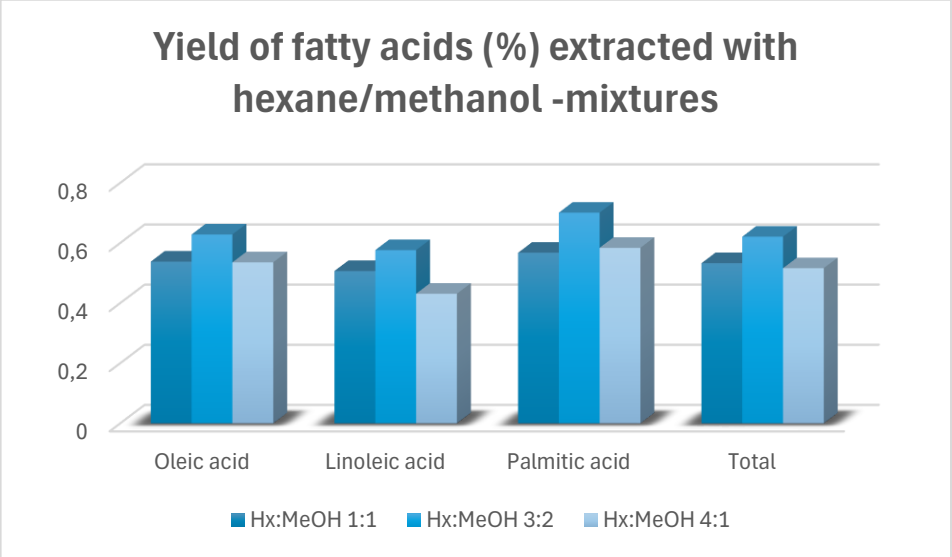

Figure 2. Oleic acid, linoleic acid and palmitic acid yields (%) achieved using hexane/methanol -mixtures with ratios 1:1, 3:2 and 4:1.

The best results with dichloromethane/butanol-mixtures are also achieved with the 3:2 ratio. Better oleic acid, palmitic acid and total fatty acid extractions were achieved with the ratio of 1:1 compared to the 4:1 ratio. The 4:1 mixture ratio extracted a slightly higher amount of linoleic acid compared to 1:1 ratio, though only around 0.2 percentage points (which amounts to 0.07 mg). The difference in extraction between solvent mixture ratio 3:2 compared to other dichloromethane/butanol -mixtures can be at least somewhat blamed on inefficient extraction of solvent/fatty acid -mixture. The inefficient extraction is caused by difficulties in removing the solvent/fatty acid -mixture from below the thick water emulsion since dichloromethane has a higher density than water. Fatty acid extraction of each dichloromethane/butanol -mixture is displayed in Figure 3.

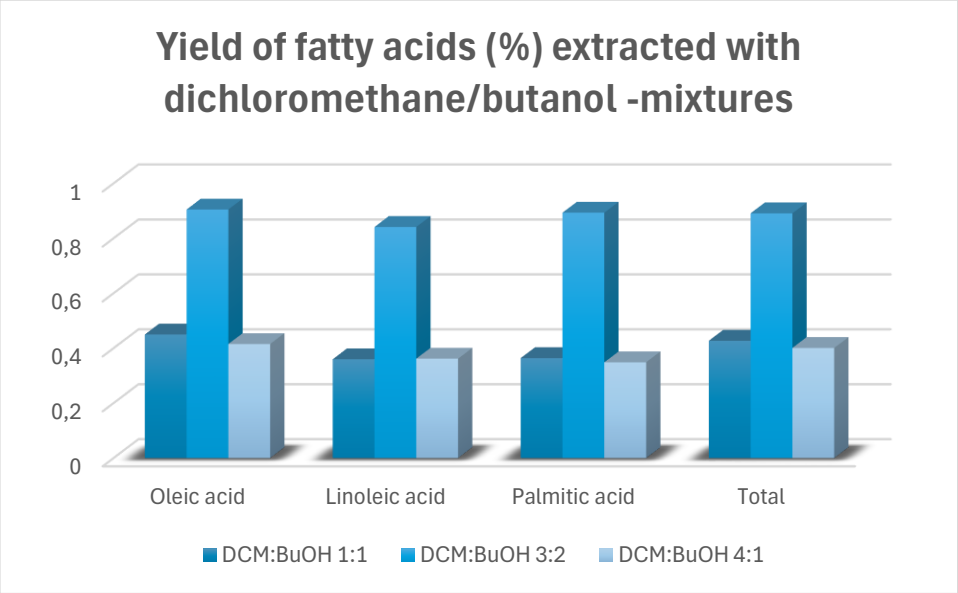

Figure 3. Oleic acid, linoleic acid and palmitic acid yields (%) achieved using dichloromethane/butanol -mixtures with ratios 1:1, 3:2 and 4:1.

6 Conclusions

The purpose of this thesis was to analyse different solvents and mixtures ratios regarding lipid extraction from food products or wastewater. During the literature part of the thesis, lipids and their properties as well as the reasons for lipid extraction were analysed. In addition, different solvents and mixture ratios for lipid extraction were examined.

The experimental portion of the thesis focused on the extraction capabilities of four solvents and six solvent mixtures regarding common fatty acids (oleic acid, linoleic acid and palmitic acid). The fatty acid yields were analysed using gas chromatography. Hexane and dichloromethane as well as methanol and butanol were chosen for the experiment since all were found to be effective in some other studies. The mixture ratios of nonpolar and polar solvents tested were 1:1, 3:2 and 4:1 because of broad range wanted and effectiveness in other studies.

The mixture containing dichloromethane and butanol with the ratio of 3:2 was found to be most effective. Notably, butanol was found to be very effective when extracting fatty acids from the water emulsion when compared to other solvents. Dichloromethane performed better when compared to hexane. The solvent mixture ratio 3:2, where the ratio of nonpolar solvent is higher, was found to be most efficient. In turn, the ratio of 4:1 achieved the worst fatty acid yields.

Through this thesis a more environmentally friendly solvent compared to chloroform, butanol, was suggested. Though dichloromethane also extracted fatty acids efficiently and is more environmentally friendly than chloroform, it is not recommended due to its own environmental issues. The solvent mixture ratio of 3:2 for a mixture with nonpolar and polar solvents was deemed to be ideal in fatty acid extraction. More studies are needed to find more environmentally friendly alternative solvents, especially nonpolar ones.

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Appendix 1. Table of measurements.

Peak areas gained from GC for standard solutions used are displayed in Table 1.

Table 1. Area of fatty acid peaks gained from GC-FID results for standard solutions.

Mass of fatty acid mixture standard, mg	Area of palmitic acid peak	Area of linoleic acid peak	Area of oleic acid peak
5.1	244.8	477.1	7283.7
5.1	254	490.1	6778.4
10.1	376.6	666.8	9719.7
20.1	809.9	1682.1	19517
30.1	1164.9	2579.3	26667

The calibration curve of palmitic acid is displayed in Figure 1.

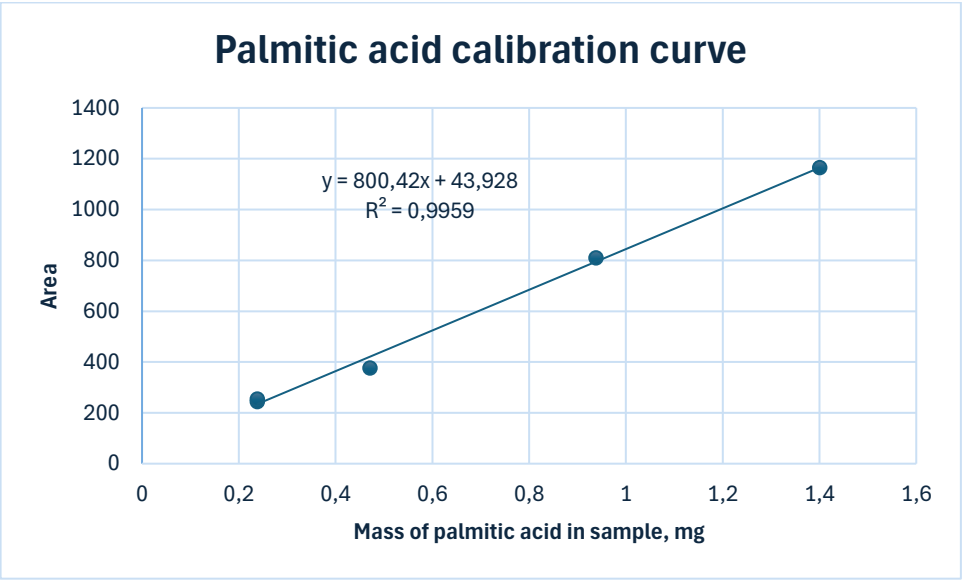

Figure 1. Calibration curve of palmitic acid for GC-analysis.

The calibration curve of linoleic acid is displayed in Figure 2.

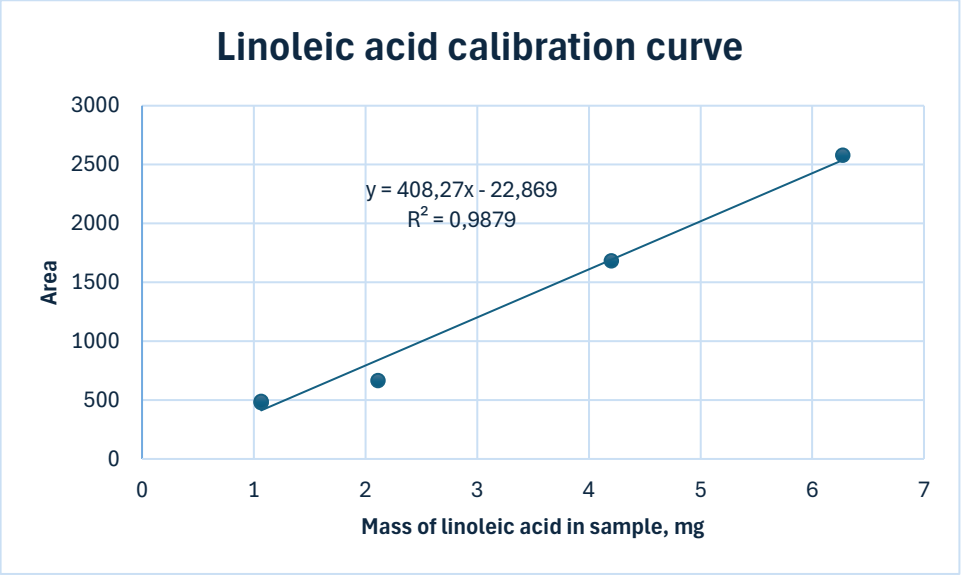

Figure 2. Calibration curve of linoleic acid for GC-analysis.

The calibration curve of linoleic acid is displayed in Figure 2.

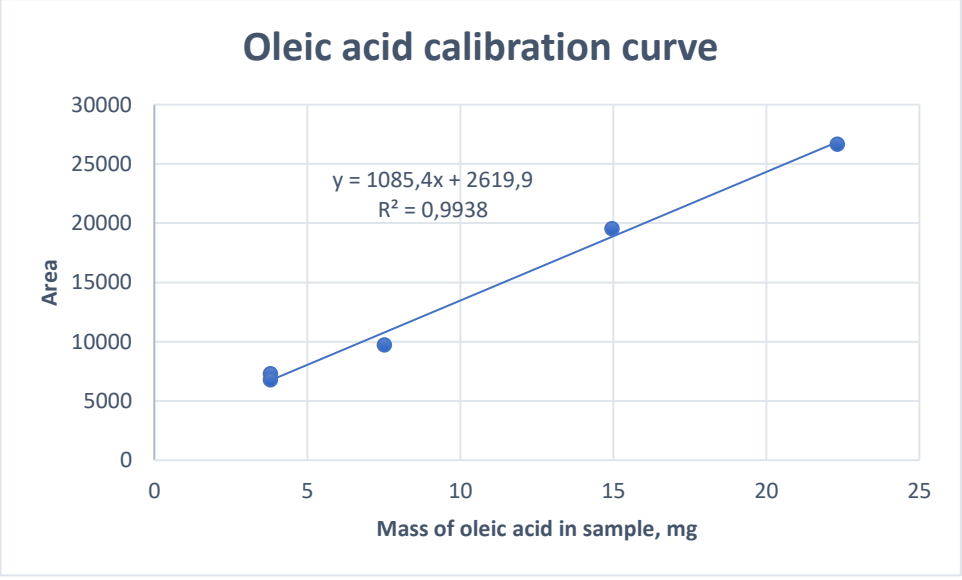

Figure 3. Calibration curve of oleic acid for GC-analysis.

Peak areas gained from GC for samples of fatty acids extracted with different solvents/mixtures are displayed in Table 2.

Table 2. Area of fatty acid peaks gained from GC-FID results for fatty acid samples extracted with different solvents/mixtures.

Fatty acid sample	Palmitic acid peak area	Linoleic acid peak area	Oleic acid peak area
1	562.1	850.3	11543.4
2	469.2	895.3	12144.5
3	565.4	1216.3	14676.4
4	479.6	859.3	11483.9
5	579.2	975.4	12921.1
6	496.4	735.1	11532
7	324.7	606.9	10068.5
8	729.6	1441.9	17527.8
9	310.3	604.2	9427.7

Appendix 2. Pictures from experimental part.

The fatty acid mixture is heated in a 70ºC oven before measuring samples to produce a homogeneous mixture. Palmitic acid is solid at room temperature, whereas oleic acid and linoleic acid are liquids. The non-homogeneous fatty acid mixture is displayed in Figure 1.

Figure 1. Fatty acid mixture containing oleic acid, linoleic acid and palmitic acid with the palmitic acid in a solid phase (white solids at the bottom).

Before silylation fatty acid samples need to be dried from solvents. Samples are dried under nitrogen gas flow. In Figure 2 test tubes with fatty acid samples drying under nitrogen flow are displayed.

Figure 2. Test tubes containing fatty acid samples being dried from solvents under nitrogen flow.

After silylation, fatty acid samples are transferred to gas-chromatography vials. Gas chromatography vials of which capped ones contain samples are displayed in Figure 3. Figure 3 shows four vials on the upper row that contain reference samples and nine vials on the lower row that contain actual samples.

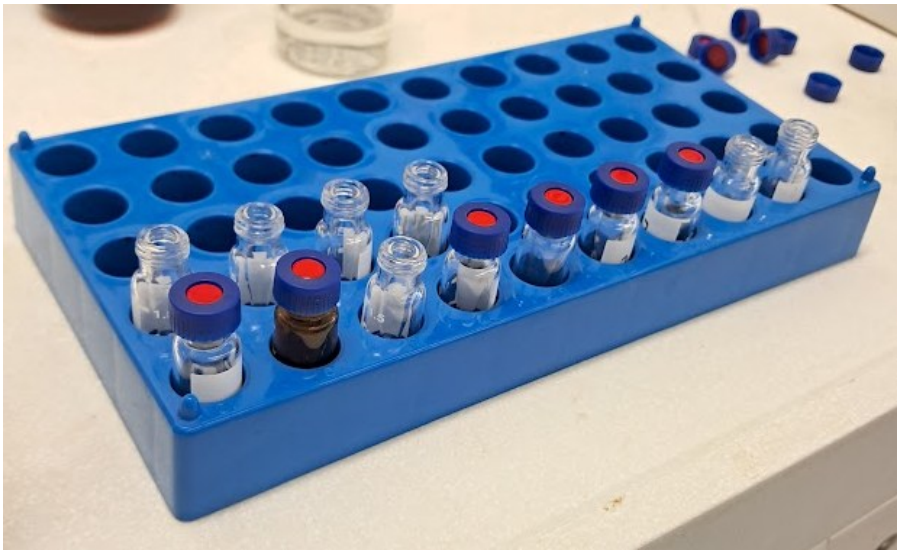

Figure 3. Gas chromatography vials used for analysis of fatty acid samples.

In Figure 4 the gas chromatograph used in analysis is displayed.

Figure 4. Agilent Technologies HP 6890 gas chromatograph used in analysis of fatty acid samples.