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Optimization of Conditions for Catalytic Cleavage of Fatty Acids

Exploring the potential utility of waste oils for a greener
future

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Auðlindadeild
Heilbrigðis-, viðskipta- og raunvísindasvið
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12 eininga lokaverkefni í Auðlindaíftækni
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Ágrip

Birgðir jarðfnaelsneytis í heiminum fara ört minnkandi og slæm áhrifa þeirra á umhverfið verða sífellt skýrari. Sökum þessa fer eftirspurn eftir umhverfisvænum og endurnýjanlegum orkugjöfum vaxandi. Lífefnaeldsneyti, sér í lagi það sem er búið til úr „annarar kynslóðar lífmassa“, er ákjósanlegur valkostur til orkuskipta. Úrgangsolíur hafa verið notaðar í þessum tilgangi, en markmið þessarar rannsóknar er að skoða notagildi myndefna þeirra eftir hvataða klofnun. Notast var við þrjár karboxoxýlsýrur með mismunandi byggingar til að besta hvarfskilyrði til klofnunar. Út frá niðurstöðum voru valin þrjú skilyrði: pH 7 með 0% (v/v) H₂O₂, pH 7 með 3% H₂O₂, og pH 9 með 0% H₂O₂. Þá voru einnig framkvæmdar ýmsar prófanir til að meta þránunarstig olíanna, og samband milli þránunar og myndefna metið. Niðurstöður rannsóknar sýndu fram á það að hægt sé að kljúfa úrgangsolíur niður í minni, óstöðugari sameindir. Meðal sameinda sem mynduðust voru etanól og acetate, en báðar þessar sameindir eru notaðar í margvíslegum iðnaði. Niðurstöður sýndu jafnframt fram á það að nauðsynlegt er að hafa oxara með í hvarfinu ef lokaafurð á að vera oxuð. Lítil fylgni reyndist vera á milli þránunar og myndefna, en það virtist vera samband á milli olía sem voru unnar úr fiski og myndunar á butanóli. Prófanirnar staðfestu að hægt sé að kljúfa þránaðar úrgangsolíur niður í minni, nothæfar sameindir. Vert væri að skoða möguleikann á því að samtvinna ferlið við estrun fitusýra til myndunar á lífefnaeldsneyti.

Lykilorð: Fitusýrur, úrgangsolía, þránun, bestun skilyrða, hvötuð klofnun

Abstract

Imminent shortages in fossil fuels and the negative impact that the burning of these fuels has on the environment increase the demand for eco-friendly renewable alternatives. Biofuels are a viable option in this regard, especially those produced from second-generation biomass such as waste oils. This research aims to optimize conditions for catalytic cleavage of carboxylic acids by using three chemically pure acids of different structures and observing their product formation. Based on the results thereof, three conditions were chosen to cleave waste oils: pH 7 with 0% (v/v) H₂O₂, pH 7 with 3% H₂O₂, and pH 9 with 0% H₂O₂. The rancidity of the waste oils was tested, and the relationship between rancidity and product formation was examined. The results showed that waste oils can be cleaved into smaller volatile carboxylic acids and alcohols, such as acetate and ethanol, that have broad applications in various industries. Furthermore, the results showed that adding an oxidizing agent is crucial for forming oxidized end products. The correlation between rancidity and product formation was weak to moderate, although there seemed to be a relationship between oils of fish origin and butanol production. The findings showed that rancid waste oils can be cleaved into viable products, and the process can potentially be coupled with biofuel production.

Keywords: Fatty acids, waste oil, rancidity, optimizing conditions, catalytic cleavage

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1 Introduction

With record-breaking highs in temperature across the globe and extremes in weather seldom seen before, it is quite clear that the effects of global warming on the environment are devastating. It is thus essential, now more than ever, to develop environmentally friendly waste disposal and recycling methods to reduce the negative impact of human consumption on the planet.

As the earth's supply of fossil fuels rapidly decreases, there is an imminent shortage of these fuels. Consequently, the demand for alternative energy sources is high. People are also becoming more aware of the negative impact that the burning of fossil fuels has on the planet. As a result, biofuels have become a sought-after solution to this problem. Biofuels are seen as a viable option for reducing CO₂ emissions in the transportation sector. However, several obstacles are preventing the biofuel industry from progressing. These limitations may include technological, financial, and political challenges. There is also the issue of whether biomass sources may compete with food supply, which raises concerns regarding food over fuel (Alam & Tanveer, 2020). That is why biomass from sources that do not compete with the food industry may be the best way forward. This includes, for example, the second generation of biomass and waste used to make biofuels.

Waste cooking oils (WCOs) are classified as hazardous waste as they possess some harmful properties and thus, it is important to dispose of them properly. If improperly disposed of they can lead to major ecological issues such as blockages in drains and sewers and contamination of water and soil (Foo et al., 2021). Water and soil contamination can, in turn, negatively impact social, economic, and health in societies, as is the case in many developing countries where WCO is often improperly disposed of. Repurposing WCO to use for biofuel production, namely as biodiesel in the form of fatty acid methyl esters, is environmentally sustainable as it results in renewable energy with lower pollution emissions and positively affects the economy by lowering the cost of waste management and reducing the need for petrochemical oil import, making it a viable eco-friendly option (Degfie et al., 2019). In addition

to biofuel production, WCOs could be chemically treated to form valuable products as oils, and their subunits can be used in various industrial applications.

Mannu et al. (2020) state that more than 190 million metric tons of used vegetable oil is produced yearly, with the European Union contributing about 1 million tons to that amount and according to Hagstofa Íslands, used oil produced in Iceland amounted to 4,561 metric tons in the year 2020. Data from previous years shows an upward trend in used oils produced, as shown in Figure 1, and thus, it is not unlikely that this amount has increased in the years since.

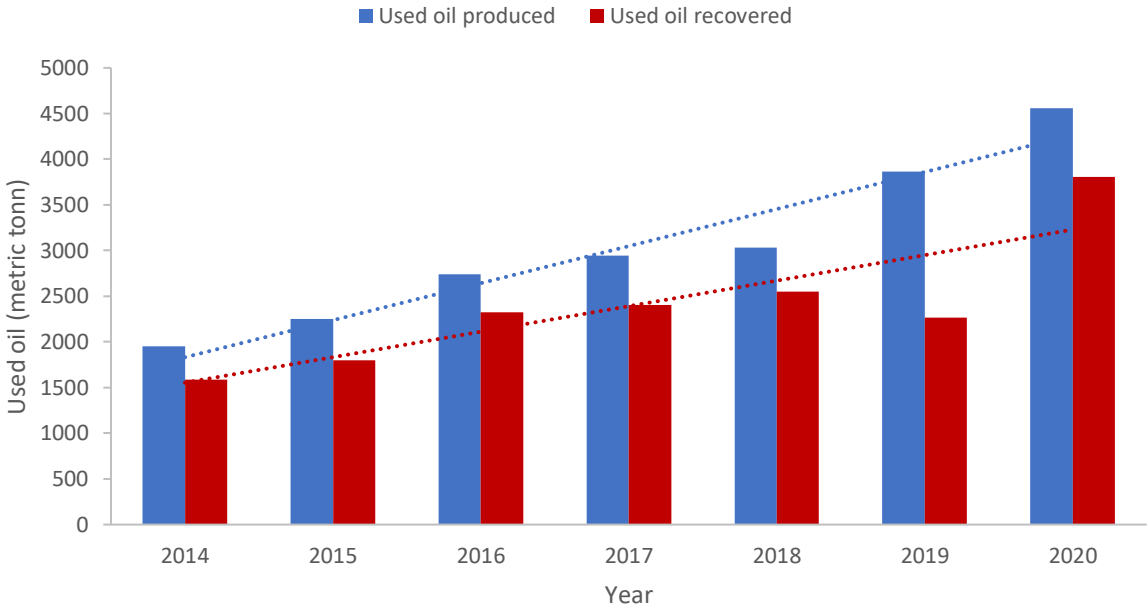


Figure 1: Amount of waste oil produced and recovered in Iceland in the years 2014-2020. Numerical data was obtained from Hagstofa Íslands (Meðhöndlun Úrgangs, n.d.) from data gathered by Umhverfisstofnun. The figure shows data class 01.3 termed "Notaðar olíur".

It is evident that the production of used oil in Iceland has been increasing at a steeper rate than the amount of oil recovered. This highlights the need to implement more advanced and efficient oil recycling and valorization techniques.

1.1 Fats and fatty acids

Lipids are a diverse group of molecules that share common properties, namely being primarily hydrophobic and soluble in organic solvents. Lipids vary in structure from simple, short hydrocarbon chains to more complex molecules such as triacylglycerols, phospholipids, and sterols (Burdge & Calder, 2015). Lipids are chemically diverse, and their biological functions vary accordingly. Fats and fatty acids are essential subgroups of lipids and play a crucial role in many biological processes (Tvrzicka et al., 2011). Not only are they essential nutrients and building blocks for living organisms, but they also have a wide variety of industrial applications. To name a few applications, lipids are used in the oleochemical industry to produce soaps and lubricants and to make polymeric coating, plastics, and paints. Furthermore, lipids are used in the pharmaceutical industry as dermatological delivery agents, in the cosmetics industry to retain moisture, and as pigments (Tao, 2007).

Fatty acids are carboxylic acids with hydrocarbon chains; these chains vary in length from 4 to 36 carbon atoms and can be saturated or unsaturated. Some fatty acids contain three-carbon rings, hydroxyl groups, or methyl-group branches. Unsaturated fatty acids have a double bond somewhere in the hydrocarbon chain, and the degree of unsaturation can vary (Nelson & Cox, 2017). Carboxylic acids are weak acids with pK_a values typically around 4.5 and produce a free hydrogen ion and carboxylate as the corresponding conjugate base in polar protic solvents such as water. A general structure of saturated and monounsaturated fatty acids can be seen in Figure 2.

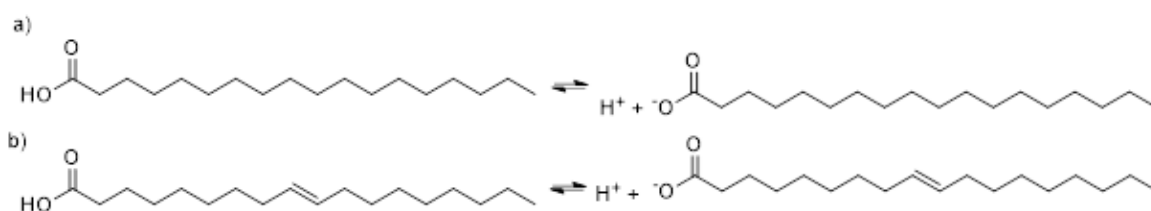


Figure 2: General structure of a fatty acid. Carboxyl group at one end, followed by a hydrocarbon chain that varies in length. **(a)** Structure of a fully saturated fatty acid (stearic acid). **(b)** Structure of a monounsaturated fatty acid (oleic acid).

Unsaturated fatty acids are commonly found to be 14-24 carbons in length. Fatty acids with only one double bond are said to be monounsaturated and are also commonly 14-24 carbons in length. Fatty acids with a higher degree of unsaturation have been found to have longer hydrocarbon chains, with polyunsaturated fatty acids having a common length of 16-22 carbons and 2-6 double bonds and highly unsaturated fatty acids being those with three or more double bonds and chains longer than 20 carbon atoms (Olsen, 2009). Most fatty acids have an even number of carbon atoms as a result of their mode of synthesis, as they are synthesized in the cytoplasm from two-carbon acetate units. A common pattern has been observed in the location of double bonds, which is also a result of the synthesis of these compounds. The double bonds of polyunsaturated fatty acids are rarely conjugated, that is, alternating single and double bonds, but are separated by a methylene group in a system termed the pentadiene system. As a result, the double bonds of most polyunsaturated fatty acids are three carbons apart, as shown in Figure 3.

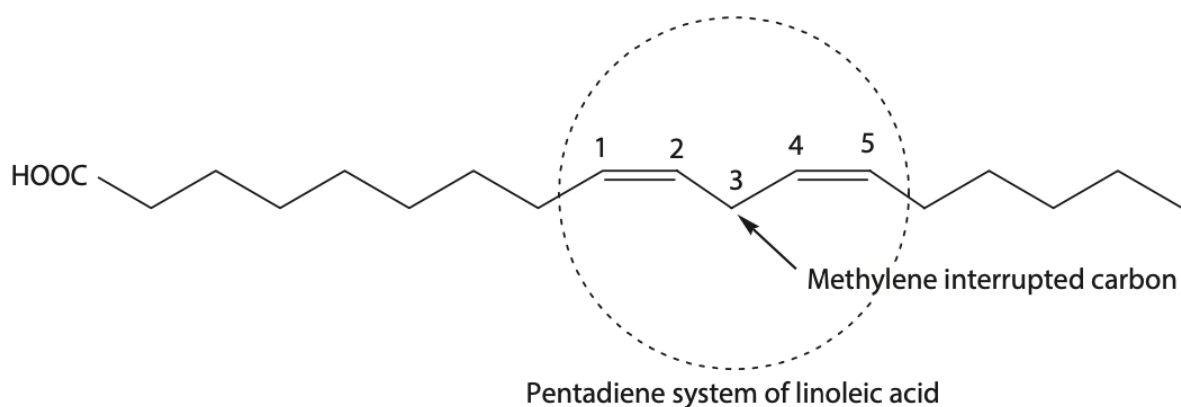


Figure 3: Pentadiene system of linoleic acid. The figure shows the pattern with which double bonds in polyunsaturated fatty acids can be found, due to the presence of methylene-interrupted carbon (adapted from McClements & Decker, 2017).

Because of this pattern, it is possible to predict the location of all double bonds in most naturally forming fatty acids if the location of the first double bond is known. For example, the double bond is located at C9 in most monounsaturated fatty acids. The other double bonds are generally found at C12 and C15 in polyunsaturated fatty acids. The natural configuration of the double bonds is in the *cis* configuration, meaning that the adjacent carbons extend on the same side of the double bond. This will result in a bent configuration (Stoker, 2007). Fatty acids with

a double bond in the *trans* configuration, meaning that the carbons are on opposite sides, are primarily produced chemically by the partial hydrogenation of vegetable oils or by bacterial fermentation in the rumen of ruminants (Dhaka et al., 2011; Lichtenstein, 2003). Structural differences between these configurations can be seen in Figure 4.

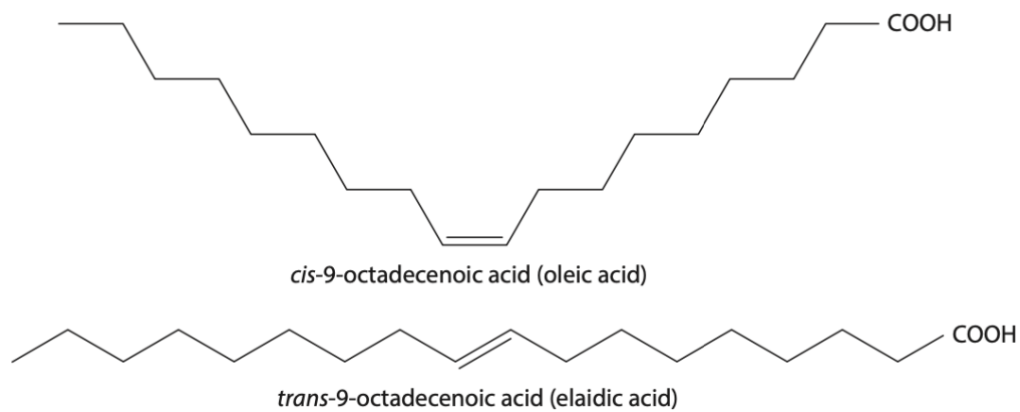


Figure 4: Structural differences between *cis* and *trans* configuration of double bonds in unsaturated fatty acids (adapted from McClement & Decker, 2017)

The physical properties of fatty acids are largely determined by the length of the hydrocarbon chain and the degree of unsaturation. The hydrocarbon chain is nonpolar and insoluble in water, while the carboxyl group is polar and soluble in water. As a result, shorter-chain fatty acids are slightly soluble in water while longer-chain acids are not. The length of the hydrocarbon chain, the number of double bonds, and the configurations of those bonds also affect the melting point. The more bonds there are to break, the higher the melting temperature. Saturated fatty acids have a higher melting point than unsaturated acids due to their linear structure, which results in tighter packing between molecules and stronger van der Waals interactions, requiring more thermal energy to break them apart. Unsaturated fatty acids with double bonds in the *cis* configuration have a bent structure, making it difficult to pack them tightly. However, *trans*-unsaturated fatty acids are more linear, allowing them to pack tighter together, resulting in a higher melting point. For instance, stearic acid, a saturated 18-carbon fatty acid, has a melting point of approximately 70°C, while oleic acid, an 18-carbon monounsaturated fatty acid with the double bond in the *cis*-configuration, has a melting point of 5°C. Elaidic acid, an 18-carbon monounsaturated fatty acid with a double bond in the *trans*-configuration, has a melting point of 44°C (McClements & Decker, 2017).

Living organisms use fats and oils, also known as triacylglycerols, for energy storage. Triacylglycerols consist of three fatty acids linked to a single glycerol through ester linkages. Most naturally occurring triacylglycerols are mixed and contain two or three different fatty acids, while simple triacylglycerols are composed of three identical fatty acids. Natural fats, such as those found in dairy products, animal fats, and vegetable oils, consist of both simple and mixed triacylglycerols, which can contain a complex mixture of fatty acids of varying lengths and degrees of saturation. These differences in fatty acid composition give fats their unique properties. Triacylglycerols are sometimes categorized into two groups, fats and oils, depending on their physical state at room temperature. Fats are triacylglycerols that are solid or semi-solid at room temperature and consist mainly of saturated fatty acids. These are often of animal origin. Oils are those triacylglycerols that are liquid at room temperature, have a higher degree of unsaturated fatty acids compared to fats, and are mainly of plant origin (Stoker, 2007).

Chemical deterioration of lipids can occur either by hydrolytic reactions, resulting in hydrolytic rancidity, or oxidative reactions, resulting in oxidative rancidity. Hydrolytic rancidity results from the formation of free fatty acids from triacylglycerols. This happens when fats interact with water. The water interacts with the carboxyl group on the fatty acid, resulting in that fatty acid being cleaved from the glycerol backbone. The formation of free fatty acids in food reduces oxidative stability, causes problems such as off-flavor, and reduces smoke points. Although free fatty acids are usually formed by interactions between fats and water, they can also be formed by lipases and at extreme pH (McClements & Decker, 2017). Oxidative rancidity occurs when lipids interact with oxygen; for example, when lipid-rich foods are left exposed to oxygen in the air for extended periods, they can spoil and become rancid, giving them an unpleasant taste and odor. This is because the unsaturated fatty acids in the food undergo oxidative cleavage, which creates shorter chain-length aldehydes and carboxylic acids of higher volatility (Nelson & Cox, 2017).

There are several ways to measure the quality and rancidity of oil. One method is to examine the free fatty acid (FFA) content, which can indicate the oil's oxidative stability (Vicentini-Polette et al., 2021). Recent research by Foo et al. (2021) states that WCOs can be divided into two categories based on FFA percentage: yellow grease (FFA < 15%) and brown grease (FFA > 15%). As cooking oils are used multiple times, they tend to become darker in

color and more acidic. Therefore, changes in FFA content and color can be a sign of oil degradation. Other methods aim to monitor lipid oxidation and measure primary or secondary oxidation products.

Methods to measure primary oxidation compounds, peroxides, and hydroperoxides include measuring peroxide value, iodine value, and conjugated dienes. These methods, however, don't necessarily correlate strongly with rancidity since peroxides can deteriorate rapidly and be influenced by other factors that don't necessarily indicate rancidity (Abeyrathne et al., 2021). Peroxide value (PV) measures the amount of peroxides and hydroperoxides produced by the oxidation of hydroxyl groups of unsaturated fats in oils during the initial stages of the oxidation process (Gilbraith et al., 2021). When hydroperoxides are formed from polyunsaturated fatty acid, it often leads to conjugation of the hydrocarbon chain. Conjugated diene content is thus a measure of hydroperoxide formation and is measured via absorbance. It is worth noting that some products formed as a result of the decomposition of hydroperoxides also obtain the conjugated dienes structure and will affect the absorbance. Therefore, peroxide value measurements are more specific (Gordon, 2004). Iodine value is used to determine the degree of unsaturation in oils. Iodine reacts readily with double bonds, making its value proportional to the unsaturation of oils, and it can be used to evaluate oxidative stability (Soares & Rocha, 2018). There is a disadvantage to using primary products to measure oxidation since they aren't necessarily volatile, and more importantly, their decomposition rate exceeds the rate of formation once the latter stages of oxidation start. Thus, It is important to not rely solely on methods targeting primary oxidative products, but couple them with methods aimed to measure secondary lipid oxidation products. Secondary lipid oxidation products result from the breakdown of fatty acid hydroperoxides. These products include aldehydes, ketones, epoxides, alcohols, etc. Measuring techniques such as Thiobarbituric acid reactive substances (TBARS), GC-analysis, and *p*-anisidine value techniques are employed, which often correlate strongly with rancidity (McClements & Decker, 2017). In the TBARS assay, thiobarbituric acid reacts with malonaldehyde, which forms during the breakdown of unsaturated fatty acids, to form a pink color that can be measured with a spectrophotometer (Abeyrathne et al., 2021). *p*-Anisidine value is used to detect the presence of aldehydic compounds in oils. The *p*-anisidine reagent reacts with aldehydes to form a product that absorbs light at 350 nm (Abdelazim et al., 2013). GC-analysis is used to determine the volatile oxidation products of oils. The process

involves dissolving the components of a sample in a solvent, which is then vaporized. This separation technique allows the analytes to be separated by distributing the sample between two phases, a stationary phase, and a mobile phase. The sample mixture is vaporized and carried through a column by a carrier gas. Analytes move from the mobile phase to the stationary phase and are separated based on their physical and chemical properties as they interact with the stationary phase. The output of a GC analysis is a chromatogram, which plots the detector response as a function of retention time. The retention time is the time it takes for a compound to travel through the column and reach the detector (Özek & Demirci, 2012).

Although lipid oxidation is usually undesirable in food, oxidative cleavage methods have been employed in the chemical industry to produce short-chain fatty acids. Short-chain carboxylic acids are precursors for other chemicals and derivatives such as ketones, alcohols, esters, and aldehydes. Short-chain fatty acids, which contain six or fewer carbons, such as acetic, propionic, and butyric acid, are immensely useful and have a broad range of applications. (Tomás-Pejó et al., 2023). Acetic acid is one of the simplest carboxylic acids, with the chemical formula $C_2H_4O_2$ and a molar mass of 60.052 g/mol. It is also the most broadly used of all carboxylic acids, as it is used in the manufacturing of various products, including plastics, textiles, dyes, and pharmaceuticals. It is also used as a food preservative and flavoring agent, as well as in the production of various types of vinegar. Acetic acid can be produced by various methods, including the oxidation of ethanol, the bacterial fermentation of sugars, and the catalytic oxidation of hydrocarbons (Pravasi, 2014; PubChem, 2024). Propionic acid is a simple carboxylic acid with the chemical formula $C_3H_6O_2$ and a molecular mass of 74.08 g/mol. It is soluble in water, ethanol, and ether. The acid is used to produce a wide range of chemicals, including cellulose acetate propionate, herbicides, pharmaceuticals, and food additives. Propionic acid is produced naturally during the fermentation of certain types of bacteria, including *Propionibacterium freudenreichii*, which is commonly found in dairy products like cheese and milk. Propionic acid is produced on an industrial scale by chemical synthesis from petroleum feedstock. The processes are mainly carried out by the following three reactions: direct oxidation of hydrocarbons, the carboxylation of ethylene with carbon monoxide and water, and the oxidation of ethylene. In recent years, propionic acid has gained attention as a potential biofuel due to its ability to be converted to propylene, a fundamental building block for the polymer industry (Goldberg & Rokem, 2009; Ranaei et al., 2020). Butyric acid, also

known as n-butanoic acid, is a four-carbon carboxylic acid with the chemical formula $C_4H_8O_2$ and a molar mass of 88.11 g/mol. It is a colorless liquid with a pungent odor and is found in many dairy products and animal fats in the form of esters. Butyric acid is soluble in water and organic solvents such as ethanol and ether. Butyric acid is used to produce butyrate esters that are used as additives in food, perfumes, and flavorings due to their pleasant taste and aromas. Furthermore, butyric acid is used in the production of plastics, surfactants, and disinfectants. Butyric acid can be synthesized from acetyl CoA, from pyruvic acid and from the degradation of fatty acids (Goldberg & Rokem, 2009).

Oxidative cleavage of fatty acids can be performed with relatively mild oxidizing agents, such as hydrogen peroxide, by adding a catalyst. Zeolites have been used for this purpose in oil refining processes. Zeolites are a type of crystalline, porous aluminosilicates that have shown great potential as catalysts in various chemical reactions, such as the cracking of hydrocarbons to produce gasoline, the conversion of methanol to olefins, and the production of fine chemicals. These materials have a unique structure consisting of a three-dimensional network of interconnected channels and pores. The channels and pores of zeolites are precisely sized and arranged, allowing them to selectively catalyse certain reactions while leaving others unaffected. Zeolites are also highly stable and can be easily synthesized in various sizes and shapes. Zeolites work by providing a surface for reactant molecules to interact and undergo chemical reactions. The pores and channels of the zeolite structure can hold the reactant molecules close to one another, increasing the likelihood of reaction and allowing for the formation of intermediate species. Additionally, the aluminium and silicon atoms in the zeolite structure can act as Lewis acid or base sites, respectively, facilitating the transfer of electrons between reactants and promoting the formation of new chemical bonds. This can increase reaction rates, selectivity, and yield (Primo & Garcia, 2014).

1.2 Selected Carboxylic Acids

Three carboxylic acids were chosen to determine optimal conditions for catalytic cleavage. The acids are commercially available and vary in hydrocarbon chain length and structure regarding double bond configuration. The acids were chosen to determine whether clear trends could be seen in product formation under different chemical conditions despite structural differences.

1.2.1 Crotonic acid

Crotonic acid is a short-chain monounsaturated fatty acid with the chemical formula $C_4H_6O_2$. The acid occurs naturally in crude wood distillate and sponges and is a component of the defensive secretions of various beetles. It is produced on an industrial scale, and its primary use is in the synthesis of copolymers with a range of comonomers. These copolymers have diverse applications such as in paints and coatings, adhesives, ceramics, agrochemicals, and more.

Crotonic acid is the *trans* isomer of 2-butenoic acid, containing one double bond between C2 and C3 in the *trans*-configuration, as seen in Figure 5.

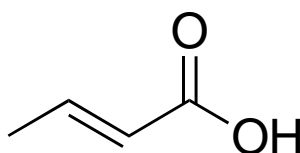


Figure 5: Chemical structure of crotonic acid

At room temperature, crotonic acid dissolves in water with a solubility of 9.4% (w/v) to form a weak acid solution. Crotonic acid can undergo hydrogenation, which yields crotyl alcohol and butyric acid as products. It can also be oxidized resulting in the formation of the peracid (Schulz et al., 2000).

1.2.2 Maleic acid

Maleic acid is a dicarboxylic acid with the chemical formula $C_4H_4O_4$. The acid is not naturally occurring but was first synthesized by a French chemist named Théophile-Jules Pelouze in 1834 by heating malic acid. The acid became industrially important in 1919 and commercially available nine years later (Lohbeck et al., 2000). According to Felthouse et al. (2001) maleic acid, along with its intermediates, maleic anhydride and fumaric acid, can be found in applications in nearly every field of industrial chemistry and is thus immensely important. Applications for maleic acid include the production of polyester resins and alkyd resins and it is also used as an agricultural chemical.

Maleic acid is the *cis* isomer of butenedioic acid. It contains two carboxylic acid groups in the *cis* configuration and a double bond between C2 and C3, as shown in Figure 6.

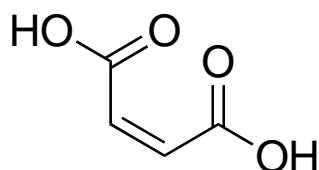


Figure 6: Chemical structure of maleic acid

The double bond and carboxyl groups of maleic acid are highly reactive. Maleic acid dissolves in water, with a solubility of 78.9 g per 100 g of water at room temperature, and its solubility increases at higher temperatures. When heated above 100°C, water is eliminated, and maleic anhydride is formed. Upon further heating, preferably with the addition of catalysts, it undergoes decarboxylation to form acrylic acid. Maleic acid reacts similarly to other carboxylic acids, forming esters and amides, but it does not form an acid chloride. Several chemical reactions involving the double bond have been observed, such as the addition of water to produce malic acid under high temperature and pressure, ozone reaction to create glyoxylic acid, halogens addition to form dichlorosuccinic acid, and catalytic hydrogenation leading to succinic acid (Lohbeck et al., 2000).

1.2.3 Oleic acid

Oleic acid is a monounsaturated fatty acid with the chemical formula $C_{18}H_{34}O_2$. The acid is naturally occurring and is the most commonly found monounsaturated fatty acid, with high quantities found in oils such as rapeseed, sunflower, and olive oil. Oleic acid was first synthesized in 1934 (Kenar et al., 2017; List et al., 2017), and according to the National Center for Biotechnology Information (2024), the acid is widely used in various industries. These include pharmaceutical-, cosmetic-, textile- and food industries where oleic acid serves, among other things, as an emulsifier, surfactant, and lubricant.

The structure of oleic acid was first correctly described in 1898. It has an 18-carbon-long hydrocarbon chain and is classified as an omega-9 fatty acid as it has a double bond at C9

counted from the carbon farthest from the carboxyl group (referred to as the omega carbon). The double bond is in the *cis* configuration, as seen in Figure 7.

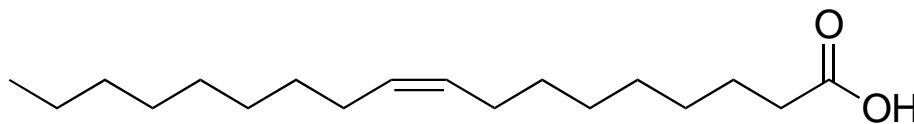


Figure 7: Chemical structure of oleic acid

Oleic acid is insoluble in water but soluble in organic solvents such as ethanol, chloroform, and ether. Because oleic acid is unsaturated, it is susceptible to oxidation of the double bond, resulting in the formation of peroxides and other reactive compounds. Oleic acid can also form salts with metals, known as oleates, that have many important industrial applications such as those described previously (Kenar & List, 2007).

1.3 Research objectives

In this research, conditions for catalytic cleavage of fatty acids were optimized. Three selected carboxylic acids were used to evaluate what combination of pH and H₂O₂ concentration results in the most desirable product formation. Three conditions were then chosen to cleave waste cooking oils and product formation was observed via GC. Furthermore, the rancidity of the oil samples was tested to evaluate the relationship between rancidity and product formation.

2 Methodology

Materials were obtained from Sigma-Aldrich unless otherwise stated. Unless otherwise stated, microtiter plates were read using a CLARIOstarPlus plate reader (BMG Labtech, Germany). Waste oils were collected from private residences and fast-food restaurants in Reykjavík and Akureyri. A few oils were obtained from the lab, two of which were extracted from char waste material by Albersdóttir (2023). Analysis was also conducted on cooking oils that were “fit to use” for comparison. All samples were analyzed in triplicates and stored at room temperature.

2.1 Determining optimal pH and H₂O₂ concentration for fatty acid cleavage

2.1.1 Preparing samples for reaction

Three fatty acids were used to determine optimal pH and H₂O₂ concentration: crotonic acid, maleic acid, and oleic acid. Crotonic acid and maleic acid were diluted to 50 mM concentration while the oleic acid was used undiluted. 200 mM PO₄ buffer solutions with pH ranging from four through ten were created by measuring 200 mL of 1 M PO₄ buffer and lowering the pH with 6 M HCl or raising it with 6 M NaOH as needed. The buffer was then transferred to a 1 L volumetric flask, and the flask was filled with distilled water to get the desired molarity. Five concentrations of hydrogen peroxide (H₂O₂ % v/v) were also tested: 0, 0.5, 1, 2.5, and 5%. A 35% hydrogen peroxide solution was diluted in distilled water to get the desired concentration. Stock solutions were created for all combinations of pH and H₂O₂ concentrations. The PO₄ buffer and hydrogen peroxide solutions were mixed in a 1:1 ratio, resulting in a 100 mM PO₄/2X H₂O₂ stock solution. The stock solutions were prepared in the same manner for all pH variations following the procedure shown in Table 1.

Table 1: 100 mM PO₄/2X H₂O₂ stock solution preparation.

H ₂ O ₂ (%)	35% H ₂ O ₂ (μL)	dH ₂ O (μL)	PO ₄ buffer (μL)
0	0	50	50
0.5	2.857	47.143	50
1.0	5.714	44.286	50
2.5	14.286	35.714	50
5.0	28.571	21.429	50

Molecular Sieve UOP Type 3Å was used as a catalyst for the reaction. The catalyst was ground into fine powder and 200 mg of the Sieve weighed into 16 x 150mm test tubes. 2 mL of 100 mM PO₄/ 2X H₂O₂ stock solution was added to the tubes. Controls were prepared by substituting dH₂O for the stock solution. Controls were tested with and without the catalyst. Test tubes were then autoclaved for 1 hour at 121°C for the reaction to take place.

After the samples had been autoclaved, 1 mL of the aqueous phase was transferred to Eppendorf tubes. 10 μL of 10% w/v aqueous FeCl₃ was added to the tubes to remove any excess H₂O₂. The Eppendorf tubes were stored at room temperature for 20 minutes to allow the FeCl to react and then centrifuged at 13,000 rounds per minute (rpm) for 3 minutes.

2.1.2 Preparing samples for Gas Chromatography

GC master mix was prepared by mixing 600 mL of dH₂O, 100 mL of 25% v/v formic acid, and 100 mL of 0.1% w/v crotonic acid. Crotonate served as the internal standard. For those samples containing crotonic acid, a separate GC master mix was prepared where dH₂O was used in place of crotonic acid and samples run without an internal standard. 200 μL of sample was

transferred from the Eppendorf tubes to 2 mL Crimp Vials. 800 μL of GC master mix was added to the vials and they were tightly sealed.

2.2 Cleavage of waste oils

The same procedure was used for cleavage of the waste oils as was described in chapters 2.1.1 and 2.1.2. However, only three variations of 100 mM $\text{PO}_4/2\text{X H}_2\text{O}_2$ stock solutions were used, that is PO_4 buffer with a pH of 7 with 0% H_2O_2 and 3% H_2O_2 , and PO_4 buffer with a pH of 9 with 0% H_2O_2 . Crotonate was used as the internal standard in GC analysis for all samples.

2.3 Analytical methods

2.3.1 Oil density

The oil density of the waste oils was determined by weighing a set volume of 10 mL on an analytical scale with a readability of 0.1 mg. The resulting weight was then divided by 10 to acquire the density in g/mL. The density of oleic acid was obtained from the bottle.

2.3.2 Colorimetric analysis of carboxylic acids

175 μL of oil sample was added to a well in a 96-well microplate. 25 μL of 1% w/v aqueous FeCl_3 , 25 μL of 1% w/v ethanolic AlCl_3 and 25 μL of 0.05% w/v ethanolic 2,7-dichlorofluorescein were added to the plate, in that order.

Hexanoic acid with concentrations ranging from 0.1 to 10 mM was used to prepare a standard curve. Standards were treated in the same manner as the oil samples in the microplate. The plate was shaken at 200 rpm for 30 seconds and the absorbance read at 510 nm.

2.3.3 Determination of thiobarbituric acid-reactive substance content (TBARS)

300 μL of oil sample and 300 μL of 10% w/v trichloroacetic acid were pipetted into an Eppendorf tube. The samples were mixed thoroughly before being centrifuged at 13,000 rpm for 5 minutes. In that order, 100 μL of the aqueous layer and 100 μL of 0.7% w/v 2-thiobarbituric acid were added to a microtiter plate. The plate was heated in an oven at 90°C for 20 minutes and then allowed to cool to room temperature. A standard curve was prepared using solutions of 1,1,3,3-tetraethoxypropane (TEP) with concentrations from 0-10 μM . A 10 μM TEP solution was prepared by diluting a 1 mM TEP solution (0.22 g TEP/L of dH₂O) in a 1:100 ratio with dH₂O. The procedure for standard preparation is shown in Table 2.

Table 2: TEP concentrations, corresponding MDA concentrations, and standard dilutions

TEP concentration (μM)	MDA concentration (μM)	10 μM TEP (mL)	dH ₂ O (mL)
0	0	0	1
2	2	0.2	0.8
4	4	0.4	0.6
6	6	0.6	0.4
8	8	0.8	0.2
10	10	1	0

Standards were treated in the same manner as the samples. The absorbance was read at 540 nm. Samples with absorbance readings outside the range of the standard curve were diluted in 10% trichloroacetic acid. 1:2, 1:5, 1:10, 1:20, and 1:40 dilutions were prepared, and the procedure was repeated. Results were expressed as μmol malonaldehyde (μM MDA)/100 g of sample.

2.3.4 Determination of free fatty acids

Approximately 2.0 g of oil dissolved in 10 mL of hexane was added to a 50 mL Erlenmeyer flask. Two drops of 1% w/v phenolphthalein indicator in ethanol were added to the flask and the solution was titrated to a light-pink coloured endpoint with a 0.1 M NaOH solution in deionized water. The free fatty acid content of the oil samples was calculated according to Equation (1),

$$FFA (\%) = \frac{V \cdot M \cdot 28,2}{m} * 100 \quad (1)$$

where FFA % is the percentage of free fatty acids, V is the volume of hexane, M is the molarity of the NaOH solution used and m is the mass of the oil sample.

2.3.5 Iodine value

A 1.2 mM triiodide stock solution was prepared by dissolving 30 mg of iodine in 20 mL of absolute ethanol and 3.4869 g of potassium iodide. The solution was then brought to a final volume of 100 mL by topping up with dH₂O. 600 µL of oil sample and the same volume of triiodide solution were added to an Eppendorf tube and the tube was shaken for 5 minutes at 200 rpm using an IKA KS250 shaker. After shaking, the tubes were centrifuged at 13000 rpm for 3 minutes. 300 µL of the aqueous phase was transferred to a 96-well plate using a syringe and the absorption read at 450 nm. The procedure was repeated with the oils diluted 1:10 in hexane. Blanks were also created using undiluted oil samples but dH₂O in place of the iodide solution. A standard curve was prepared using the 1.2 mM triiodide stock solution diluted with dH₂O as seen in Table 3.

Table 3: Preparation of triiodide standards

Triiodide concentration (mM)	Triiodide stock (μL)	dH ₂ O (μL)
0.1	83	917
0.2	167	833
0.4	333	667
0.6	500	500
0.8	667	333
1.0	833	167
1.2	1000	0

The results for iodine value were expressed as g I₂ per 100 g of sample.

2.3.6 Anisidine value

10 mg of oil sample was weighed into 13 x 100mm test tubes and the oil dissolved in 2,5 mL of 2,2,4-trimethylpentane. 250 μL of that solution was pipetted into a microtiter plate and the absorbance read at 350 nm. 0.5% w/v p-anisidine in glacial acetic acid was prepared by mixing 44,6 μL of 4-methoxybenzaldehyde with 10 mL of Acetic acid. 50 μL of the solution was then pipetted into the microtiter plate and absorbance was read again at 350 nm. P-anisidine value was calculated using equation (2) where A_1 is the absorption value before adding the p-anisidine reagent, A_2 is the absorption value after adding the reagent and W is the weight of the sample in grams.

$$p - anisidine\ value = 2.5 * \frac{(1.2 * A_2) - A_1}{W} \quad (2)$$

2.3.7 Peroxide value

50 μL of samples was mixed with 2.35 mL of 75% v/v ethanol, 50 μL of 30% ammonium thiocyanate (w/v) and 50 μL of 20 mM ferrous chloride dissolved in 12 M HCl. The solutions were mixed briefly, pipetted into a microtiter plate and the absorbance read at 500 nm. A blank was prepared using dH_2O in place of the ferrous chloride solution.

A standard curve was made using cumene-hydrogen peroxide with concentrations ranging from 0.08 mM to 8.2 mM. Samples were handled in the same manner as described above and the absorbance read at 500 nm. Absorbance for blanks was subtracted from absorbance values for all samples and peroxide value was expressed as mg cumene-hydrogen peroxide per kg of sample.

2.3.8 Measurement of conjugated dienes

50 mg of oil was weighed into sample tubes and 2 mL of 2,2,4-trimethylpentane was added to the tubes. The oil sample was allowed time to dissolve and then 1 mL of the samples was pipetted with a pasture pipette into a glass cuvette. Absorbance was read at 234 nm using a UV spectrophotometer. The cuvette was cleaned with 2,2,4-trimethylpentane between sample readings. The procedure was repeated for 100 mg of oil sample. Conjugated dienes were expressed as differences in absorbance per 50 mg of oil.

2.3.9 Oil absorbance

Values obtained from „blanks“ in the Iodine value test were used to calculate oil absorbance. The absorbance of dH_2O was subtracted from the absorbance values of blanks to obtain oil absorbance at 450 nm. Oil absorbance was also read at 510 nm.

2.3.10 Gas chromatography analysis

Volatile carboxylic acids and alcohols formed during the reaction were detected using a gas chromatograph (Perkin-Elmer Clarus 580) equipped with an autosampler and a flame ion

detector (FID) and a Nukol capillary column (30 m x 0.32 mm x 0.25 μm , Supelco) for the separation. The method was modified from Örlygsson & Baldursson (2007).

3 Results

Results are expressed as the mean of a triplicate data set unless otherwise stated.

3.1 Optimal conditions for cleavage

Observing product formation via GC determined optimal pH and H_2O_2 concentration for fatty acid cleavage. The results for maleic acid can be seen in Figure 8, crotonic acid in Figure 9, and oleic acid in Figure 10.

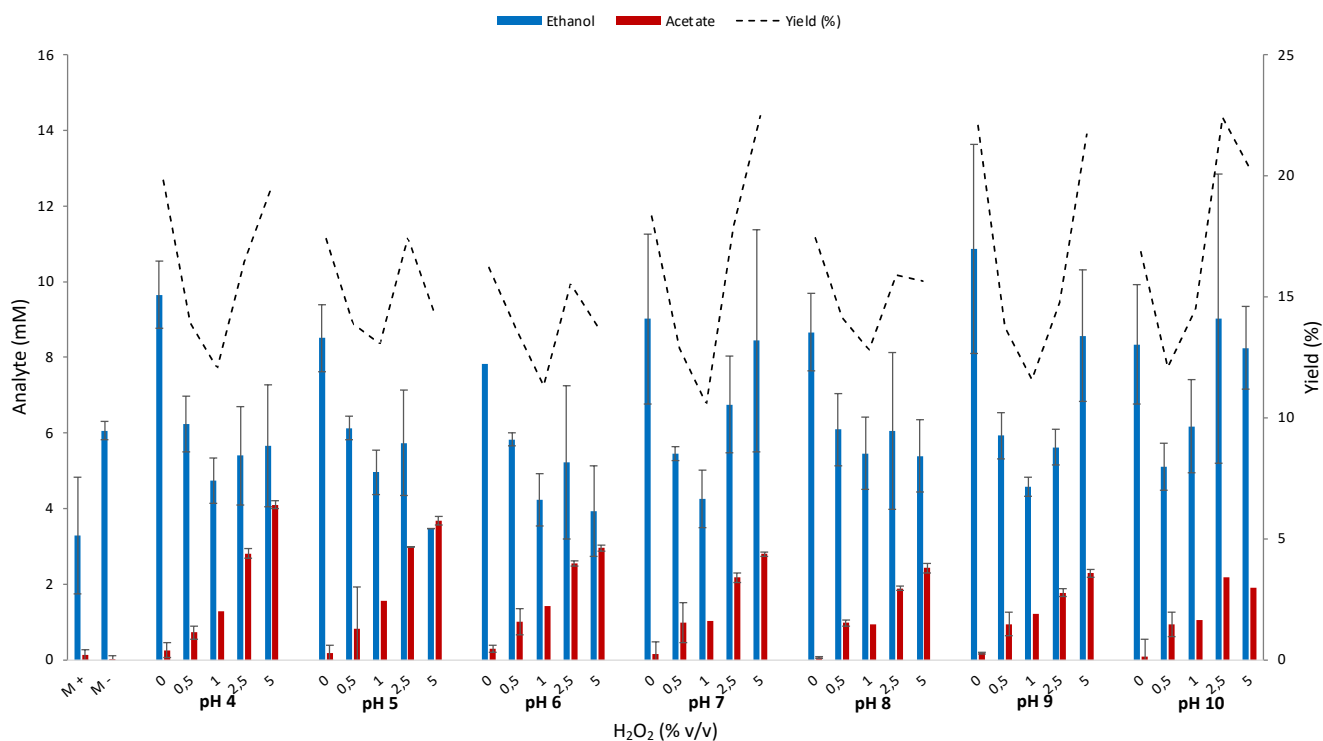


Figure 8: Product formation for catalytic cleavage of maleic acid. The figure shows concentrations of analyte; ethanol and acetate, as well as yields, formed under different chemical conditions. Control samples are represented by M+ for controls containing a catalyst, and M- for controls without a catalyst.

In Figure 8, it can be observed that the production of acetate increases with higher H_2O_2 concentration, as is the case for all pH values. Furthermore, the overall production of acetate

seems to be higher at lower pH levels. The highest concentration of acetate, which is 4.10 mM, was produced at pH 4 with 5% H₂O₂. The lowest excluding the control, 0.09 mM, was produced at pH 10 with 0% H₂O₂. The trends in ethanol formation are not as clear, although ethanol formation does seem to be highest at 0% H₂O₂. The highest concentration of ethanol was 10.87 mM produced at pH 9 with 0% H₂O₂. The least amount of ethanol was formed at pH 5 with 5% H₂O₂ which resulted in an ethanol concentration of 3.46 mM. Furthermore, ethanol is produced in control samples, with a greater concentration found in the control without a catalyst. Since ethanol is overall produced in greater quantities than acetate, it has a greater influence on the overall yield of the reaction. Therefore, it is not surprising that the yield curve appears to be correlated with ethanol production.

Cleavage of crotonic acid on the other hand, resulted in much greater concentrations of acetate being formed as seen in Figure 9.

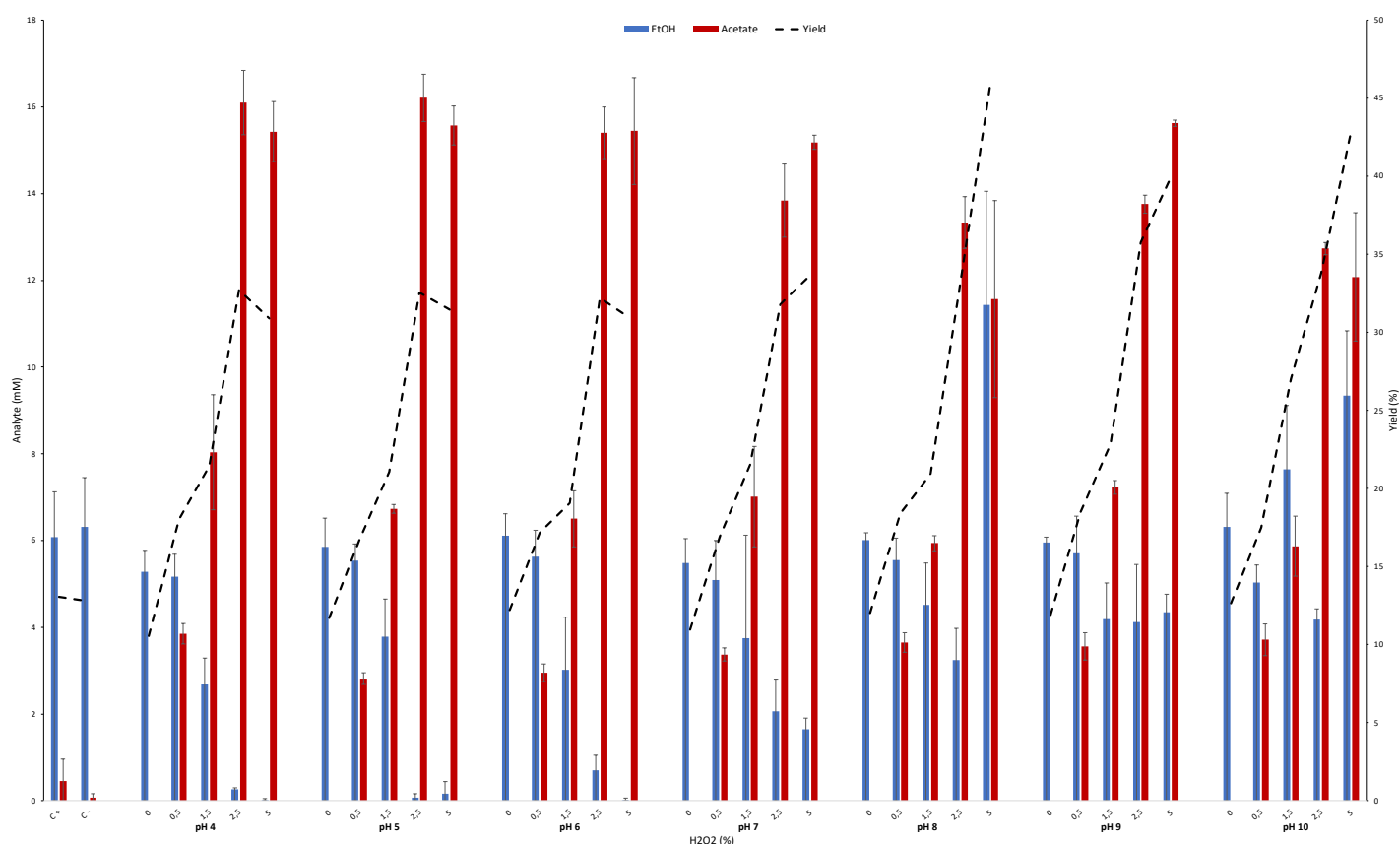


Figure 9: Product formation for catalytic cleavage of crotonic acid. The figure shows concentrations of analyte; ethanol and acetate, as well as yields, formed under different chemical conditions. Controls are represented as C+ for controls containing a catalyst and C- for those without a catalyst.

Figure 9 shows that overall yield is correlated with the amount of acetate being formed. Trends are however similar to that of maleic acid, that is acetate formation increases with increased H_2O_2 concentrations and ethanol is produced in greater quantities at lower concentrations of H_2O_2 . An exception to this occurs at pH 8 and pH 10, where ethanol concentrations seem to be the highest at 5% (v/v) H_2O_2 , with a concentration of 11.4 mM and 9.34 mM respectively. Furthermore, ethanol production in controls with and without a catalyst amounts to similar concentrations as samples containing PO_4 buffer/ 2X H_2O_2 stock solutions. Trace amounts of propionic-, butyric-, and valeric acid were also formed in some samples.

Cleavage of oleic acid showed a similar trend in ethanol and acetate formation, compared to the other selected carboxylic acids. Product formation can be seen in Figure 10.

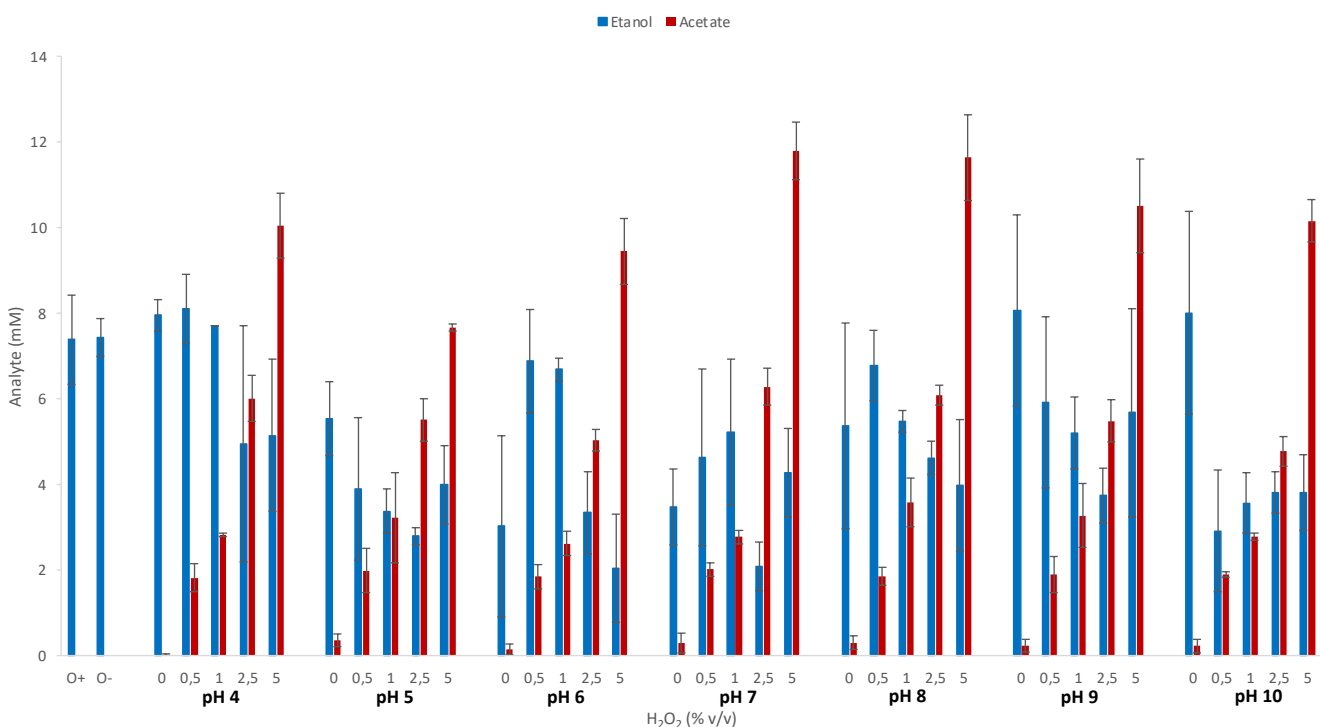


Figure 10: Product formation for catalytic cleavage of oleic acid. The figure shows concentrations of two analyte; ethanol and acetate, formed under different chemical conditions. Controls are presented as O+ for controls containing a catalyst and O- for those without a catalyst.

As seen in the figure, the highest concentration of ethanol was formed at pH 4 with 0.5% H_2O_2 , and the lowest concentration was formed at pH 6 with 5% H_2O_2 . Ethanol concentrations ranged from 2.04 mM to 8.11 mM. The highest concentration of acetate, 11.8 mM, was formed at pH

7 with 5% H₂O₂, and the lowest excluding the control, 0.03 mM, was formed at pH 4 with 0% H₂O₂. Heptanoic and octanoic acids were also formed in the reaction at relatively low concentrations. Concentrations for both acids were similar under all conditions. Heptanoic acid concentrations ranged from 0.407 mM to 0.562 mM. Octanoic acid concentrations ranged from 0.539 mM to 0.657 mM. Longer-chain acids were also formed, presumably at low concentrations. Qualitative results for them can be seen in Table 4.

Table 4: Qualitative summary of larger-chain carboxylic acids formed from the cleavage of oleic acid. The table shows in what samples nonanoic acid (retention time of 15.3 min) and decanoic acid (retention time of 17.3 min) are present, as well as an unknown compound with a retention time of 14.3 min.

pH	Unknown acid (14.3 min)					Nonanoic acid (15.3 min)					Decanoic acid (17.3 min)					
	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	
pH 4	t	t	t	++	+++	+++	++	+	t	t	t	t	t	t	t	t
pH 5	+++	+++	+++	+++	+++	t	t	t	t	t	-	-	-	-	-	-
pH 6	+++	+++	+++	+++	+++	t	t	t	t	t	-	-	-	-	-	-
pH 7	+++	+++	+++	+++	+++	t	-	t	t	t	t	t	-	-	t	t
pH 8	+++	+++	+++	+++	+++	t	-	-	-	t	t	-	t	-	t	t
pH 9	+++	+++	+++	+++	+++	t	-	t	t	t	t	-	-	t	t	t
pH 10	+++	+++	+++	+++	+++	t	-	t	t	t	t	-	-	-	-	t

Results are a mean of peak area on chromatograph and are represented as follows: non-detected (-), < 1000 (t, trace amounts), 1000 - 1500 (+), > 2000 (+++).

H₂O₂ concentrations are represented as A (0%), B (0.5%), C (1.5%), D (2.5%), and E (5%).

3.2 Rancidity testing of waste oils

Numerous rancidity tests were performed on the waste oils to evaluate rancidity and a summary of the results can be seen in Table 5. It is worth noting that all iodine values exceeded the standard curve range and will be reported as greater than 1.2 mM triiodide per 600 μ L of sample and not included in the table. Likewise, values for colorimetric analysis of carboxylic acid are excluded from the table since the values for the standard curve were abnormal, resulting in a horizontal linear curve, and the data was thus unusable.

The density of the oils was very similar between samples, with a range from 0.884 g/ml for sample number 12, to 0.970 g/ml for sample number 4. Similarly, the increase in absorbance per 50 mg oil for conjugated dienes measurement was around 50% for all samples except for sample number 2, which had a 37.01% increase. Free fatty acid content of the oils ranged from 0.183% for oil number 10, to 13.904% for oil number 5. Oil nr.5 had the highest FFA % content by far, with oil nr. 8 following behind with a FFA value of 3.34 %. *p*-Anisidine value was by far the highest for oil nr.3 with a value of 103.67 AnV. The other oils were similar in aldehyde content, with values ranging from 20.84 AnV to 44.64 AnV. Results for peroxide value showed high variability, not only between sample, but also within a triplicate data set. Values ranged from 0 mg CHP/ kg sample to 41548 mg CHP/ kg sample. There was also a high variance in TBARS, with the lowest value being 153.3 μ M MDA / 100 g sample, and the highest 6167.18 μ M MDA / 100 g sample.

Absorbance was read at two wavelengths, 450 nm and 510 nm. Absorbance readings for 450 nm showed little to no absorbance at that wavelength. The samples absorbed more light at 510 nm, with absorbance ranging from 0.45 abs to 2.91 abs.

Table 5: Properties of oil samples. Values are expressed with \pm standard deviation. PRCO = private residence used cooking oil.

Oil nr.	Source	Density (g/mL)	Absorbance at 450 nm	Absorbance at 510 nm	FFA (%)	<i>p</i> -Anisidine (AnV)	PV (mg CHP/ kg sample)	Conjugated dienes ^x	TBARS (μ M MDA/100 g)
1	PRCO #1	0.9003	0.11 \pm 0.03	0.45 \pm 0.01	0.43 \pm 0.29	20.84 \pm 1.72	0.00* \pm 0.00	51.79%	470.21 \pm 84.76
2	PRCO #2	0.9303	0.01 \pm 0.01	1.21 \pm 0.27	0.40 \pm 0.14	20.56 \pm 1.78	6639.69* \pm 4394.94	37.01%	878.65 \pm 68.35
3	PRCO #3	0.9277	0.02 \pm 0.01	1.24 \pm 0.71	2.04 \pm 0.16	103.67* \pm 13.95	7431.00 \pm 2159.63	46.12%	664.77 \pm 75.71
4	Cut off oil	0.9703	0.00 \pm 0.01	0.92 \pm 0.50	0.90 \pm 0.61	30.61* \pm 0.86	1384.17 \pm 953.51	46.02%	2938.24 \pm 211.18
5	Gut oil	0.8915	0.12 \pm 0.07	2.91 \pm 0.45	13.90 \pm 1.58	44.64 \pm 12.95	41548.31 \pm 2542.79	48.90%	4097.28 \pm 214.73
6	Isio4 Veg oil	0.8913	0.07 \pm 0.06	0.56 \pm 0.15	0.44 \pm 0.16	31.43 \pm 2.61	2934.01* \pm 330.28	51.68%	2857.69 \pm 83.12
7	PRCO #4	0.8851	0.01 \pm 0.01	1.32 \pm 0.12	1.02 \pm 0.87	42.86* \pm 15.76	1830.94* \pm 1108.66	51.11%	966.93 \pm 45.12
8	Leirunesti	0.8852	0.02 \pm 0.03	1.69* \pm 0.23	3.34 \pm 0.39	25.01* \pm 7.89	5566.92* \pm 849.85	50.09%	664.91 \pm 109.21
9	Tasty	0.8823	0.03 \pm 0.02	0.71 \pm 0.02	2.36 \pm 0.44	40.88 \pm 15.81	3252.55* \pm 1038.11	49.61%	1037.81 \pm 271.60
10	Þorskalýsi ¹	0.8910	0.00 \pm 0.00	0.73 \pm 0.28	0.18 \pm 0.09	33.66 \pm 11.25	17549.40 \pm 3919.59	51.10%	6167.18 \pm 1010.21
11	Þorskalýsi ²	0.9175	0.01 \pm 0.02	0.60 \pm 0.11	0.44 \pm 0.30	24.13* \pm 1.72	4362.97* \pm 1319.12	48.98%	1352.09 \pm 22.99
12	Þorskalýsi	0.8836	0.01 \pm 0.00	0.79 \pm 0.11	0.22 \pm 0.15	32.46 \pm 11.59	0.00 \pm 0.00	45.31%	2994.18 \pm 145.24
13	Olive oil	0.9037	0.01 \pm 0.00	0.52 \pm 0.15	0.42 \pm 0.15	34.65 \pm 8.77	0.00 \pm 0.00	49.38%	153.30 \pm 97.89
14	Rapeseed oil	0.9338	0.00 \pm 0.00	0.62 \pm 0.05	0.27 \pm 0.003	26.99 \pm 4.38	0.00 \pm 0.00	51.62%	263.20* \pm 7.99
15	Oleic acid	0.89	N/A	1.28 \pm 0.16	N/A	N/A	N/A	N/A	210.97 \pm 31.36

¹, expired 06.2006; ², expired 01.2007; ^x increase in abs at 234 nm per 50 mg of oil; * average of two samples

3.3 Cleavage of waste oils

As mentioned in section 2.2, three combinations of pH and H₂O₂ % were used for catalytic cleavage of waste oils. pH 7 with 0% H₂O₂ will be referred to as condition A, pH 7 with 3% H₂O₂ will be referred to as condition B, and pH 9 with 0% H₂O₂ will be referred to as condition C. Reactions were also performed with and without a catalyst, and those without a catalyst will be marked with (-). Volatile compounds formed during the reaction were analysed with GC.

Concentrations of ethanol and acetate produced for all samples are shown in Figure A 1 in Appendix A. Ethanol was produced in all samples, with production in sample 4 by far the highest. Excluding sample 4, ethanol concentrations were relatively low, ranging from 0.44 mM to 2.22 mM. Acetate was also most readily produced from oil nr.4, but oil nr. 5 followed closely behind. Analyte concentrations for the two oils can be seen in Figure 11.

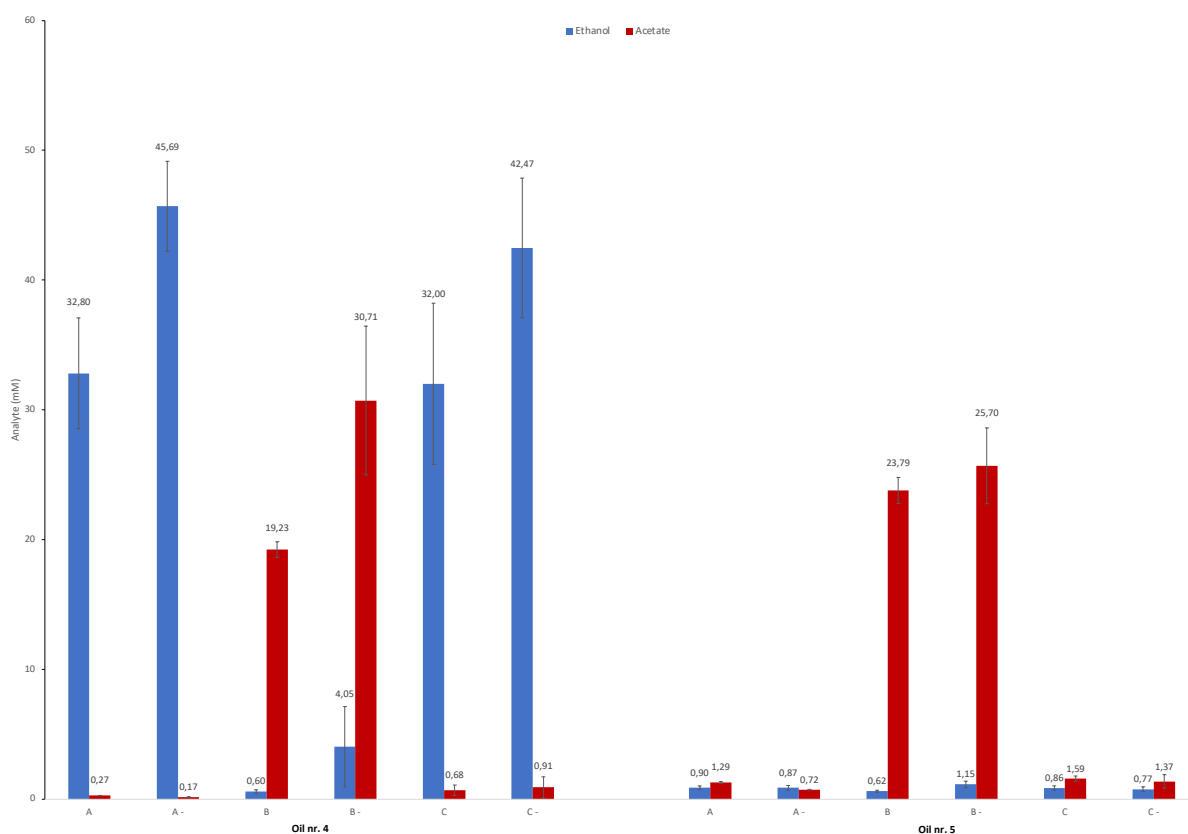


Figure 11: Analyte produced from catalytic cleavage of oil nr.4 and 5 under different chemical conditions.

Oils nr. 4 and 5 also produced other analytes at low but detectable concentrations. Butanol was produced with concentrations < 0.20 mM, heptanoic acid < 0.43 mM, and octanoic acid < 0.56 mM. Some short-chain fatty acids, such as propionic-, butyric-, and valeric acids, were also present in some samples, as can be seen in Figure 12 and Figure 13, but since they were found at very low concentrations and not in all samples within the same triplicate, they were regarded as negligible.

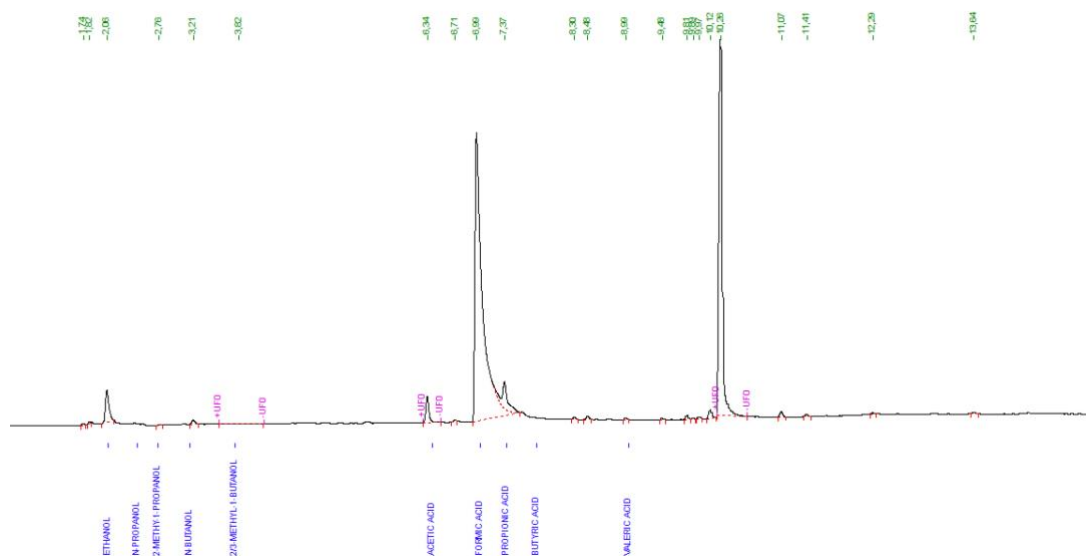


Figure 12: Chromatograph from GC analysis of oil nr.5 under condition A with a catalyst.

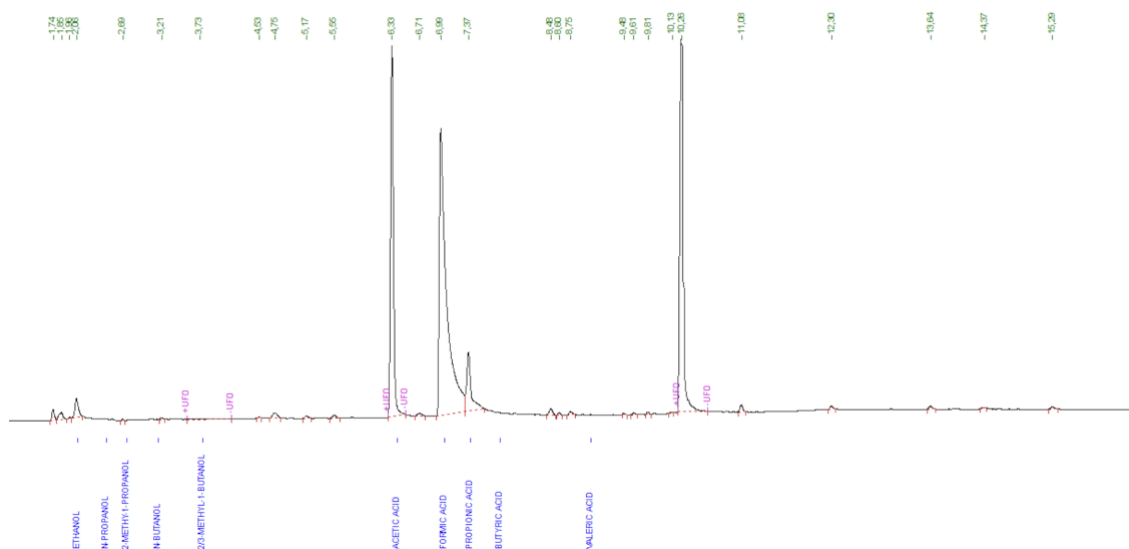


Figure 13: Chromatograph from GC analysis of oil nr.4 under condition C with a catalyst.

Condition B, containing 3% H₂O₂, resulted in the most formation of acetate for all waste oils, although acetate was found at low concentrations in some samples under the other conditions. The concentration of acetate in the samples ranged from 0.00 mM to 30.71 mM. In addition to oil nr.4 and 5., acetate concentrations were high in oil nr.10 and 11. These samples were both from expired Þorskalýsi. Sample nr.12 was also Þorskalýsi, but unexpired. A comparison of analyte formation for these three samples can be seen in Figure 14.

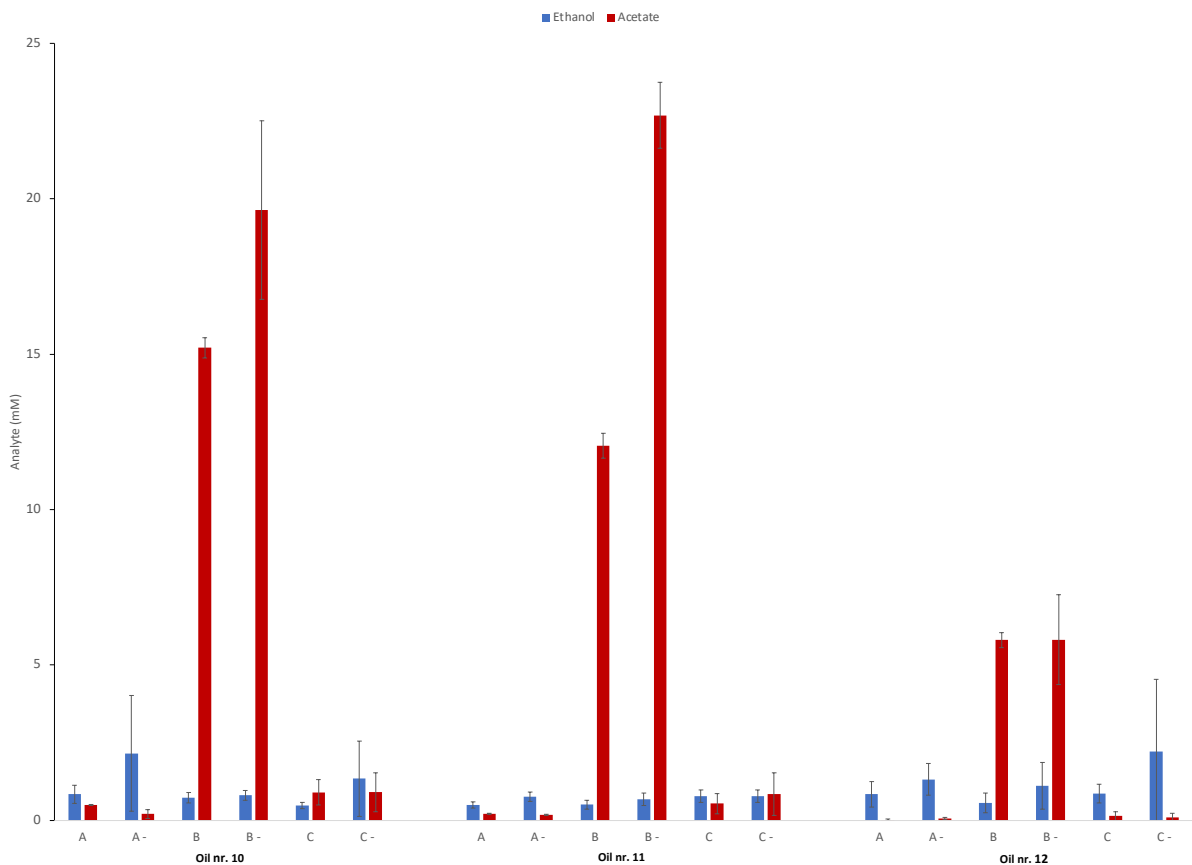


Figure 14: Analyte produced from Þorskalýsi samples. Oil nr.10 and 11 were expired, while oil nr.12 was "fit to use" and was used for comparison.

As evident from the figure, acetate was produced in greater quantities in the expired oils compared to the „fit for use“ oil. Differences in ethanol production between the oils were minuscule, although ethanol seems to be more readily produced in the absence of a catalyst.

Correlation between acetate production in the samples and rancidity was evaluated. Correlation between acetate produced in condition B with catalyst and peroxide value in the oils was moderate to high, with an R² value of 0.794. Little correlation was found between

acetate production and TBARS value, nor with p-Anisidine value, as shown in

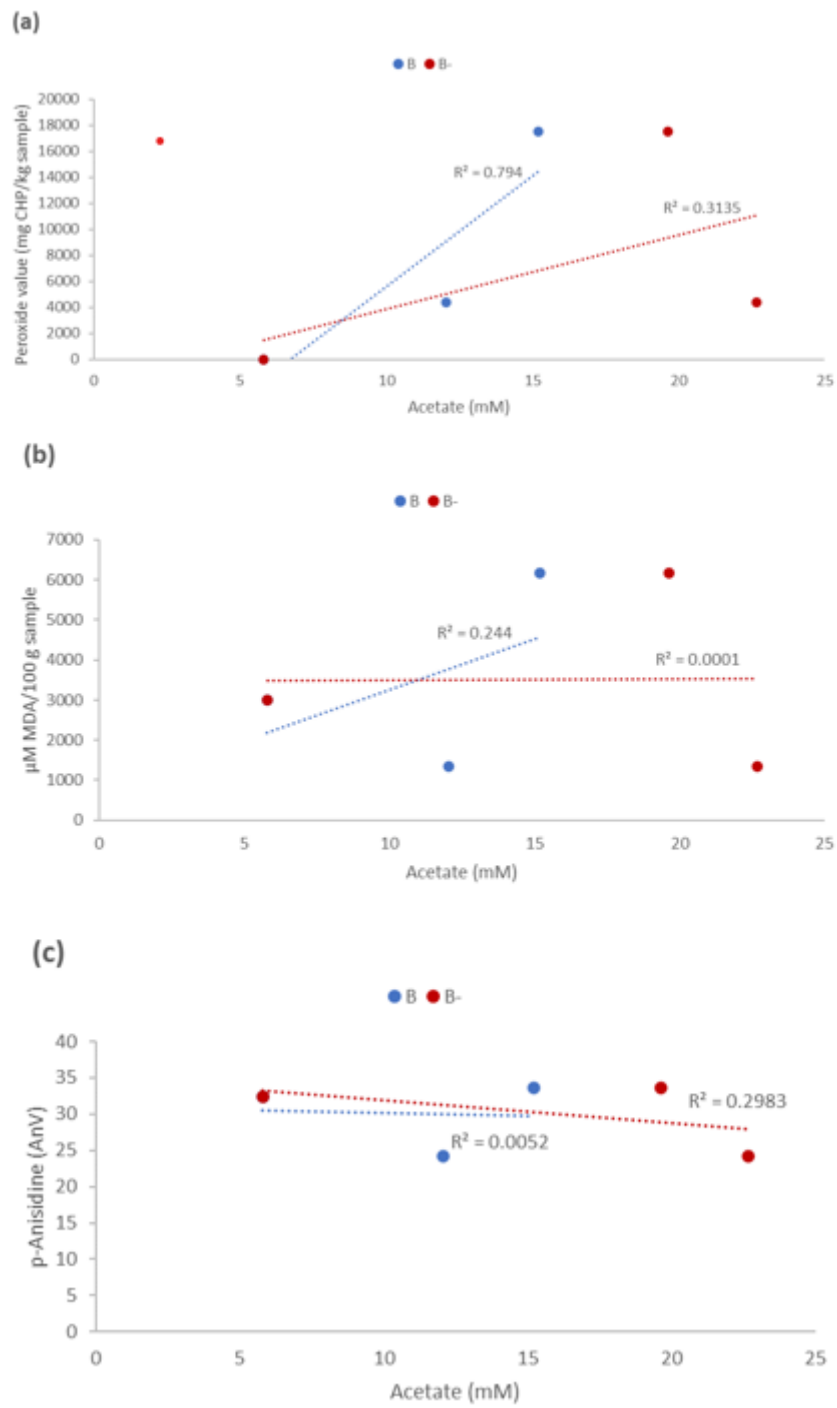


Figure 15.

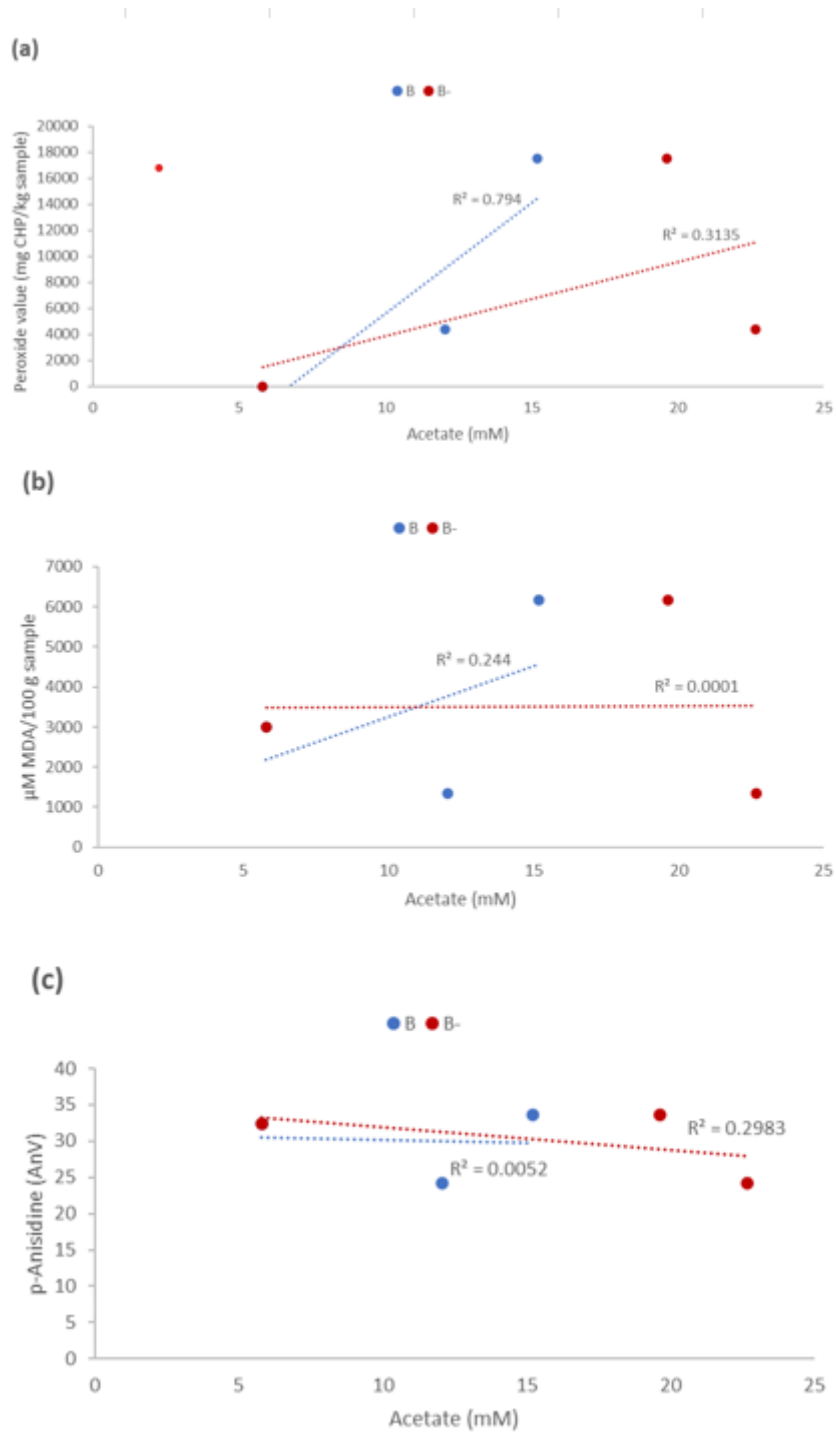


Figure 15: Evaluation of correlation between oil samples of fish origin and acetate production under chemical condition B. (-) refers to the absence of a catalyst. **(a)** Correlation between acetate concentration and peroxide value. **(b)** Correlation between acetate concentration and TBARS value. **(c)** Correlation between acetate production and *p*-Anisidine value.

4 Discussions

4.1 Cleavage of the model carboxylic acids

Results from the cleavage of the selected carboxylic acids were somewhat predictable. Higher concentrations of acetate were formed in samples containing more H_2O_2 . This was expected since higher concentrations of oxidizing agents should result in more oxidized products. Maleic acid and crotonic acid mainly formed ethanol and acetate as volatile products, while oleic acid had a greater variety of products formed. This was to be expected, since maleic acid and crotonic acid are short fatty acids, while oleic acid is longer and thus able to form longer-chained products. Standards for different length carboxylic acids were run to generate a standard curve to be able to quantify the concentration of these acids present in the samples. Unfortunately, only heptanoic and octanoic acid yielded a usable standard curve. For that reason, it was not possible to quantify the concentration of the larger-chain carboxylic acids present.

Interestingly, ethanol was formed in the control samples, with and without the addition of a catalyst, for all selected carboxylic acids. Ethanol production in control samples was on par with production in chemically treated samples for crotonic and oleic acid, and the control without the catalyst yielded a slightly higher concentration of ethanol. This perhaps suggests that the presence of a catalyst is redundant for the formation of ethanol from these acids. Ethanol production was, however, increased in chemically treated samples of maleic acid, but it's hard to state with certainty whether that is due to the presence of a catalyst. More likely it is due to the difference in pH.

4.2 Rancidity and cleavage of waste oils

Looking at Table 5, it appeared there was a correlation between the peroxide value and rancidity of the oils since "fit to use" oils had a peroxide value of 0.0 mg CHP/ kg of sample and the oils that appeared more rancid (darker color, rancid smell, etc.) had a higher peroxide value. However, upon plotting the ethanol/acetate ratio against the peroxide value for the oils, this

did not seem to be the case. TBARS values and *p*-anisidine values were analysed in the same way, with little to no clear correlation, depending on chemical conditions, between rancidity values and the products formed. Correlation was evaluated using the correlation coefficient (R^2). Neupane et al. (2021) state that the coefficient gives the strength of a linear relationship between two variables. R^2 is given as a value between -1 and 1, where zero indicates no correlation and ± 1 indicates that the two variables have a perfect relation. R^2 values for peroxide value ranged from 0.0968 to 0.2786, R^2 values for TBARS ranged from 0.0012 to 0.4891, and for *p*-anisidine the values ranged from 0.0046 to 0.1539. However, upon plotting the total concentration of analyte formed against peroxide value, there seemed to be a moderate correlation between product formation in condition B with a catalyst and the amount of peroxides present in the samples, as seen in Figure 16.

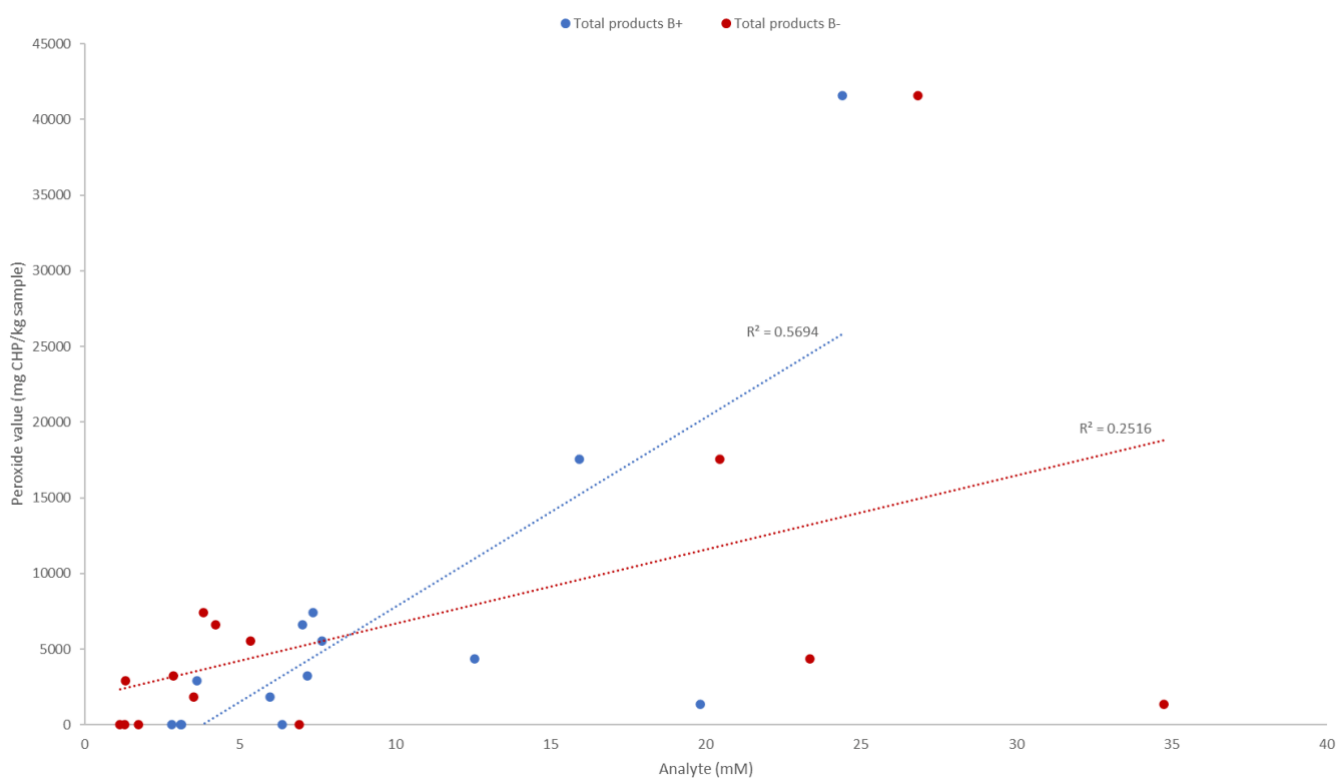


Figure 16: Correlation between analyte produced and peroxide content of waste oils under chemical conditions B (pH 9 and 3% H_2O_2). (+) refers to the presence of a catalyst, while (-) refers to the absence of a catalyst.

As shown in the figure, R^2 value for condition B with a catalyst present was 0.5694. Samples that did not have a catalyst present showed a lower correlation to peroxide value, with $R^2=0.2516$. Acetate was produced in greater concentrations in samples containing a catalyst, and since R^2 aims to evaluate the relationship between acetate production and peroxide, it is not surprising that the correlation is greater in samples with overall higher acetate production.

Oils of fish origin seemed to be the only ones that formed butanol in the reaction, although two exceptions were to this. One sample from oil nr. 7 under condition A showed small concentrations of butanol produced. Sensory analysis of oil nr.7 indicated that it had been used to cook fish since the oil smelled like deep-fried fish, perhaps explaining the presence of butanol in the sample. Likewise, one sample from oil nr. 13 under condition A in the absence of a catalyst resulted in butanol as a product. Since there was only butanol in one sample from oil nr. 13 it could be due to contamination in the GC column. It is assumed that the butanol formation is not a result of the cleavage of these oils since it was only produced in one sample within a triplicate set, under one condition.

Oils nr. 10, 11, and 12 were all Þorskalýsi, with nr.10 being expired the longest and nr.12 being "fit for use". Acetate production was much greater in oils nr.10 and 11, indicating that they have a higher number of free radicals, are more rancid and have less oxidative stability, and can, for that reason, react more readily with the oxidizing agent. Furthermore, as shown in Figure 15, acetate production correlates with the peroxide contents of the oils, indicating a relationship between acetate and primary lipid oxidation products. Analyte produced highlighted the importance of using an oxidizing agent in the reaction if the desired end-product is oxidized.

Interestingly, ethanol was produced in low concentrations in all oils, apart from oil nr.4 and 5, perhaps due to the reactions taking place at neutral and slightly basic conditions. This means that fewer H^+ ions are present in the solution for the carboxyl groups to react with to form alcohols.

4.3 Improving the reaction

Catalytic cleavage of waste oils can be used to form valuable end-products that have a wide range of applications in chemical industries. However, the data clearly show that mainly shorter-chained products were present in the samples analyzed by GC. This was also the case for oils that were supposedly not rancid, those that were „fit to use“. For that reason, it can not be assumed that this was simply because the oils had undergone hydrolytic and/or oxidative reactions during use and storage and had already been cleaved into shorter compounds. Since longer-chain carboxylic acids are insoluble in water, and the analysis was conducted from the aqueous phase of the samples, it is very possible that longer-chain carboxylic acids stayed in the oil phase during the reaction. Methods of forcing the larger carboxylic acids into the aqueous phase, possibly by attaching them to highly polar compounds or those readily soluble in water, before analysis could give a more accurate picture of the composition of the oils. Furthermore, the reaction could be performed at different temperatures since the boiling point depends on the length of the hydrocarbon chain as well as the degree of unsaturation. It is possible that some products evaporated at 121°C and conducting similar experiments over a range of different temperatures might prove beneficial. Since the composition of lipids in the oils differs from one oil to the next, optimization of temperature conditions might prove difficult. Pre-treatment of the samples, where lipids with higher molecular weight are separated from lighter lipids, could be employed to make the composition of oil more uniform for better optimization of the cleavage conditions. Furthermore, the oxidative stability of the lipids could be further reduced prior to conducting the experiment. This could be achieved by microwave treatment of the oils. According to Deng et al. (2022) microwave heating of oils causes them to produce hydroperoxides and secondary oxidation products rather quickly. Furthermore, microwave treatment increases free fatty acid content as well as changes the composition of fatty acids. Upon microwave treatment, if water is present, the unsaturated fatty acid amount decreases, while the amount of saturated and *trans* fatty acids is increased. Employing methods that aim to reduce variability in the samples prior to cleavage could prove useful in optimizing the cleavage conditions, thus decreasing waste. Using a different catalyst in the reaction could also improve yields, especially when the reactants are larger molecules. Molecular Sieve 3Å has a pore diameter of 3 angstroms, hindering molecules with larger critical

diameters from entering the porous matrix. This could potentially be a problem if large carboxylic acids are present in the samples. Using a catalyst with a larger pore diameter might prove beneficial.

With an increased focus on biofuel production as an answer to the imminent shortage of fossil fuels, methods for improving the production of biodiesel are the focus of many scientific studies. Biodiesel is a fatty acid ester, usually produced by the esterification of fatty acids with methanol to form fatty acid methyl esters. However, ethanol can also be used for this process, resulting in fatty acid ethyl esters. Ideally, biodiesel should be made from waste so as not to compete with the food industry for material and land. WCOs could be used for this purpose. Cleavage of the waste oils used in this research resulted mainly in the production of ethanol and acetate. Ethanol can be used directly as bioethanol, but since acetate was produced more readily in the reactions, there is also the possibility of converting acetate to ethanol to use for that same purpose, as well as using ethanol in esterification as mentioned above. Butanol formed in the oils originating from fish could also be used for biofuel production. Producing biobutanol from fish waste oil could be a great way to utilize waste material to produce valuable substances, especially since butanol has a higher energy density and is more effective than other biofuels such as biomethanol and bioethanol (Yeong et al., 2018).

5 Conclusions

Conditions for catalytic cleavage of fatty acids were optimized, with regard to pH and H₂O₂ concentration. Product formation for three structurally different, chemically pure carboxylic acids was analysed via GC and used to determine optimal cleavage conditions. Optimal conditions were determined to be as follows: pH 7 with 0% H₂O₂, pH 7 with 3% H₂O₂ and pH 9 with 0% H₂O₂. Rancidity testing was conducted on waste oil samples, and the optimal conditions were used to cleave said samples. The correlation between rancidity and products formed was found to be minimal to moderate, depending on chemical conditions. There did, however, seem to be a relationship between oils of fish origin and the production of butanol. Cleavage of the waste oils, as well as of the selected carboxylic acids used, resulted mainly in the formation of ethanol and acetate.

The study showed that waste oils can be cleaved into smaller, volatile carboxylic acids and alcohols fairly easily. Products formed in the reaction are valuable since they have broad applications in various industries. The findings of this study suggest that cleaving waste oils can potentially be coupled with biofuel production, thus reducing waste and emissions while producing valuable substances at the same time.

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Appendix

Appendix A: Data for catalytic cleavage of selected carboxylic acids and waste oils.

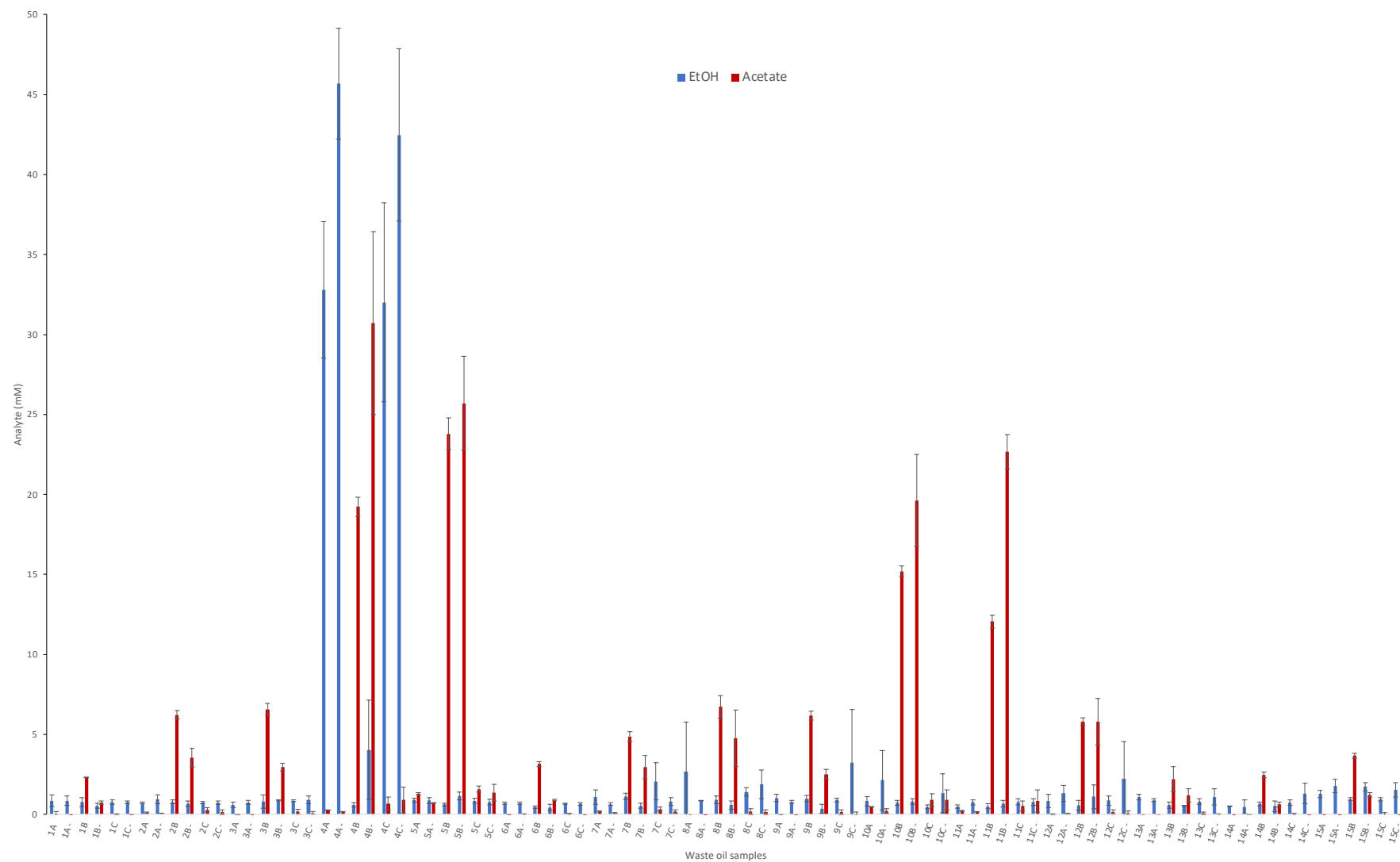


Figure A 1: Concentrations of analyte from cleavage of waste oils. Three chemical conditions were employed; pH 7 with 0% H₂O₂ (A), pH 7 with 3% H₂O₂ (B), and pH 9 with 0% H₂O₂ (C). Minus (-) symbol refers to the absence of a catalyst.

Appendix B: Standard curves for various rancidity testing methods

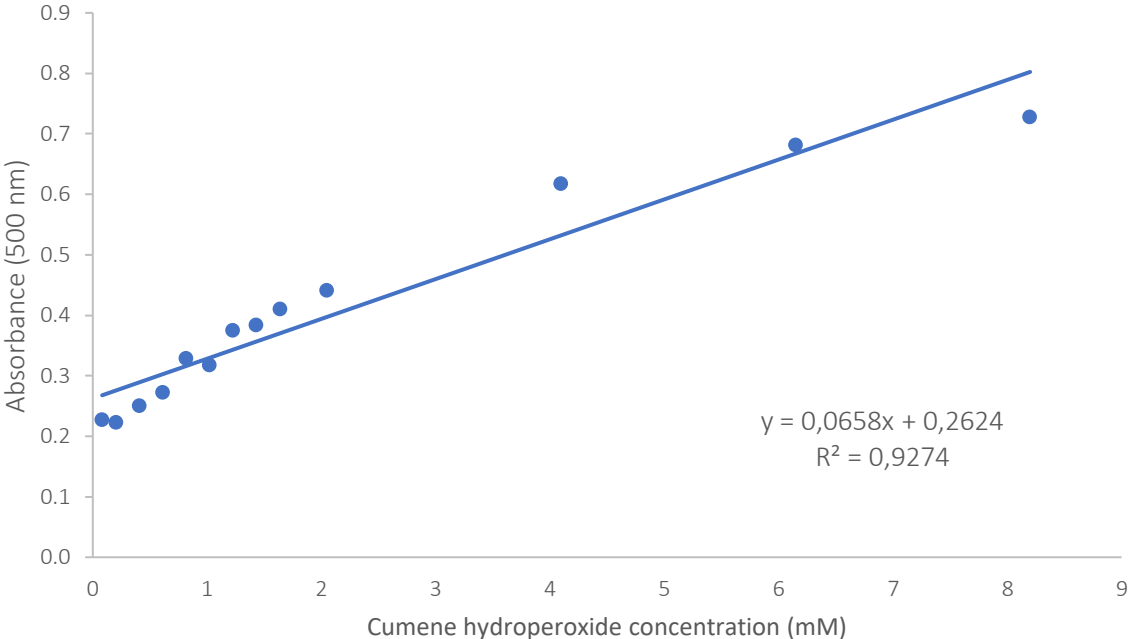


Figure B1: Peroxide value standard curve. The standard curve shows the relationship between absorbance value at 500 nm and concentration of cumen hydroperoxide. The line of best fit was used to calculate peroxide value of waste oil samples.

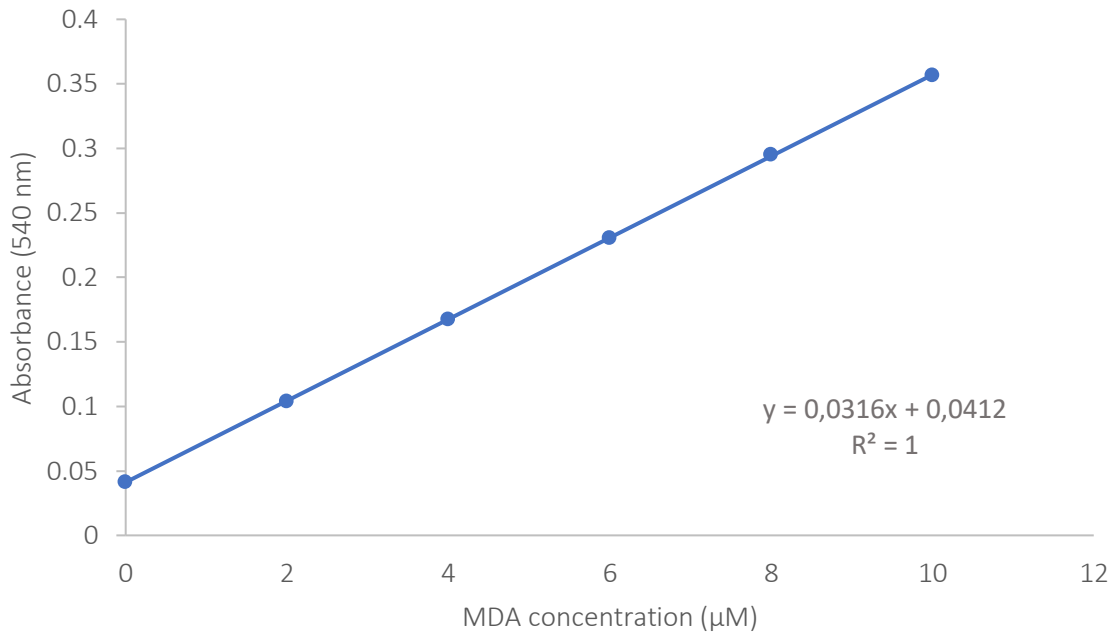


Figure B2: Standard curve for TBARS. The standard curve shows the relationship between MDA concentration and absorbance at 540 nm. The line of best fit was used to calculate TBARS content of the waste oil samples.

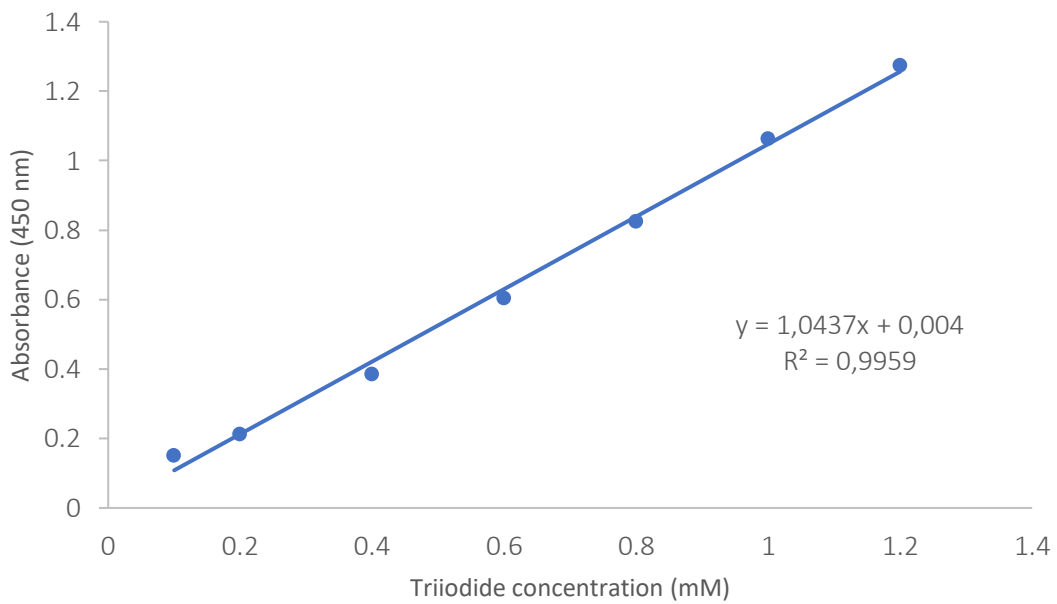


Figure B3: Standard curve for iodine value. The standard curve shows the relationship between Triiodide concentration and absorbance at 450 nm. Equation obtained from the line of best fit was used to calculate iodine value of waste oil samples.