



CHALMERS
UNIVERSITY OF TECHNOLOGY

Development of a method for acceleration of lipid oxidation in pulp

A Master thesis performed at BIM kemi AB and Chalmers University of Technology

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Accelerated oxidation of chemi-thermomechanical pulp to enable a rapid product control of pulp intended for usage in food packages

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Abstract

Usage of cellulosic raw material in applications where plastic is usually preferred, is beneficial in both an environmental and economical perspective. Food packages is an application where plastic is often used because of its many suitable properties. It would, however, be desirable to replace the plastic with cardboard to, at least, some extent. Cardboard can be produced through a variety of processing methods where mechanical pulp is more environmentally viable and cheaper than chemical pulp. Certain challenges are associated with usage of mechanical pulp products due to the sparse use of chemicals in the processing causing lignin and other extractives to be present to a large extent. Chemi-thermomechanical pulp is such a pulp where the extractives present in the resulting cardboard are sensitive to oxidation during which volatile compounds might form. Some of the volatile compounds formed during oxidation are odorous and might transfer through the gas phase from the cardboard package to any foodstuff products packed inside, resulting in a transfer of taint and odour. Not all pulp has this attribute which is why a precise method for quality control of the pulp, executed early in the process, is required. Such product control could determine the suitability of the pulp batch to be used in food packages. The aim of this master thesis is to develop such a method, based on an already existing method. The existing method involves natural oxidation of the extractives for 40 days before product control is possible. The developed method accelerates this process and aims towards creating a method that gives the same results in a shorter time frame.

Linoleic acid is a fatty acid which is present in a large extent in most pulp and its oxidation products are a cause of concern. The focus of the study is to accelerate the oxidation of this compound, which will provide an indication of the oxidation in the whole pulp. Experiments conducted by addition of chemical inducers and accelerators to both linoleic acid in solution and pulp are performed, as well as reference samples without any inducers present. The results show that it is possible to accelerate the oxidation of linoleic acid in solution by addition of iron inducers but that it is harder to control the oxidation of pulp. An azo compound is used as oxidation inducer with varying results. In addition, UV irradiation is examined as a possible oxidation accelerator but with indefinite results. The analysis techniques used are has chromatography with and without headspace solid phase microextraction.

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1

Introduction

1.1 Background

To find and utilise environmentally viable materials and production processes is an important issue which has to be dealt with for the future of all industry. Replacing plastic with cellulose based material is one of many improvements that could make a difference in how we affect the world we live in [1]. Packaging, specifically food packaging, is such an application. Plastic is often the preferred material, and for many good reasons, but the inevitable environmental impact the fossil based material provides is hard to overlook. Using plastics for food packages does, however, provide benefits such as low permeability and good protecting properties that shields the food product from external impact, prolonging their shelf life and thereby preventing unnecessary waste. The importance of protection of the food has to be balanced with issues including energy consumption during processing, raw material costs and environmental impact of chemicals used during processing, the waste generated and the handling and usage of natural resources.

Managing of waste is important when handling products produced from fossil resources, today about 30 % of all municipal waste in the U.S. is packages of different kinds and around 67 % these are food packages [2]. Large amounts of plastic is thrown away every year and being able to use a larger extent of biodegradable cellulosic material would lessen the environmental impact significantly.

Cardboard packages are produced from renewable raw material, it is easily accessible, the production process is less environmentally harmful than the plastic equivalent and the waste generated is degradable. Pulp has a long history of involvement in packaging solutions but in the case of mechanical pulp, where many of the chemicals used in other pulping processes are excluded, it is a bit more complicated [1]. The cardboard produced in these processes has a higher content of lipids present, which are subjective to oxidation processes that might produce odorous volatile compounds. These compounds could affect the quality of food products by gas transfer from package to product [3]. The possible odorous effect is dependent on many factors and not all mechanical pulp affect the food products. Methods for evaluation of pulp and determination of suitability for packaging have become an important issue in such applications. The method ought to be easy, quick and reliable. Today, such product control analysis is based on shelf life simulations where the amount volatile compounds produced is examined during 40 days of storage in climate controlled environments. Usually, when performing such analyses, one traces the production of hexanal which often is the most abundant volatile compound present.

Hexanal is one of the reaction products of linoleic acid which is found in relatively large amounts in most pulp species [4]. The quantitative analyse of hexanal in the headspace of pulp samples provides an indication of the oxidation process in the specific pulp and hence its suitability for usage as food packages. However, 40 days is a substantial delay and it would be preferred to use a less time-consuming method. Acceleration of the lipid oxidation responsible for the formation of volatile compounds would present a significant improvement to the existing method.

1.2 Aim

The current method for product control of pulp intended for packaging applications requires 40 days of natural decomposition through oxidation. The design of an effective method to accelerate the oxidation process and yield hexanal would save resources and time. The aim of this master thesis is to, by substantial literature studies and analytical as well as experimental trials, develop a method to perform such an acceleration in a controlled and effective way.

1.3 Project limitations

Different types of extractives and fatty acids are present in all types of wood but the amount and composition varies with species, geographical location, age etc. Due to these variations, the pulp examined in this study will be from the same batch chemi-thermomechanical pulp, CTMP, stored in cold and dark conditions to prevent decomposition and external influences affecting the quality of the material. The most commonly used trace compound, hexanal, will be used as oxidation indicator and due to it being a oxidation product of linoleic acid, the main focus will be to accelerate oxidation of linoleic acid. Provided the methods designed are successful in accelerating the oxidation of linoleic acid, they will be applied to the CTMP as well. The analytical methods used are limited to gas chromatography and headspace solid-phase microextraction gas chromatography combined with either flame ionisation detector or mass spectroscopy. No more than three methods will be tested due to the time limit of the study. If an accelerating method or inducer is found to be unsustainable, economically or environmentally, this method will not be examined further due to not being profitable when applied in the industry.

1.4 Specification of issues under investigation

Specifically, some issues will be dealt with over the course of the project.

- Is it possible to catalyse or accelerate the oxidation of linoleic acid?
- If so, is the acceleration provided by chemical or physical means?
- Are the found acceleration methods environmentally and economically sustainable?
- How can these methods be designed in a way that is simple and possible to execute with the equipment available?
- What are the most efficient methods to test the accelerators, which temperatures should be used and how are the accelerators mixed with the material?

- Is it possible to control the oxidation with these methods, so that no more or less than the amount “naturally” produced over 40 days is produced in a shorter time frame?

2

Theory

The theoretical background for the thesis is presented in this chapter. The concepts of pulp processing, the raw material of the experimental procedures, the oxidation of lipids and possible acceleration methods are described here.

2.1 Pulp processes and properties

Pulp refers to a suspension of cellulose fibres in water and is the raw material when producing paper and cardboards [1]. Different grades and types of pulp have different properties and characteristics. There are many ways of producing pulp but the main idea is to separate the cellulose fibres from each other and from the lignocellulosic materials. This is done through pulp processing techniques, the three major ones are mechanical, semichemical and chemical. They utilise mechanical or chemical means, or a combination of both, to separate the fibres.

The mechanical pulping has the highest yield, 90 - 98 %, which means that most of the content of the wood are found in the products produced. High yields are essential when designing environmentally conscious processes but it entails that extractives, lignin and other compounds are present [5]. Chemical pulp has a yield of only 45 - 55 % and requires a process including bleaching and usage of a higher amount of chemicals, but at the same time the paper produced offers a higher quality.

Semichemical pulping involves a chemical pretreatment of the wood chips in elevated temperature, washing away around 50 % of the lignin content [1]. This lowers the yield to around 60 - 80 %. The most commonly used process of removal of lignin is executed through washing with cooking liquor that contains sodium sulphate as reagent and sodium carbonate as pH buffer.

Chemical pulping is the dominant production process to produce pulp. About two thirds of all pulp world wide is produced by the chemical pulping process. In chemical pulping the lignin is dissolved and degraded, through the use of chemical processes [1]. Most of lignin is removed but it is not possible to completely eliminate the lignin content. When most of the lignin is removed there is not much left keeping the fibres together, making the separation very easy and without requirement of mechanical treatments, leading to a lower demand of electricity. Due to the low yield, around half of the raw material is lost in the process. Further delignification is achieved by bleaching, and the amount lignin left is essential to the properties of the products. The process most used is Kraft pulping.

The product of mechanical pulping is a paper that has lower quality than the chemical pulping products. Lignin is still present to a high extent, which affects the properties of the paper. The pulp exhibits high light scattering power, a high bulk and high brightness. More electricity is required for the process but it is cheaper [5]. Mechanical pulping uses mechanical force to separate cellulose fibres [1]. Temperatures are kept high and the mechanical energy is partially transferred into heat. Heating of lignin to above glass temperature, is necessary for separation of lignin from the bulk wood matrix. Typically, the grinding processes involves grinding logs against a pulpstone while applying pressure, forcing fibres to be stripped from the surface of the logs. Refining of wood chips is done in a disc refiner. Softening of the wood matrix with either steam or chemicals makes it easier to separate the lignin from the matrix. Chemi-thermomechanical pulp, CTMP, is produced through one of the common refining processes. Both steaming and chemical treatment is performed to soften the lignin. Using chemical treatments enhances properties such as fibre length. Sodium sulfite is the dominating reagent used in chemical treatments. The pulp obtained after refining is screened and bleached and the process consume less energy than other mechanical treatments.

2.2 The challenge of odorous compounds formed in cardboard

Rapid transfer of volatile compounds produced in food packages, either directly through solid-solid interfaces or by gas transfer, to the food products might affect the quality of the product. The compounds formed by oxidation are not harmful but might affect both taste and taint. This has been investigated by Donetzhuber et al. in a study performed at Stora Corporate Research, Sweden [3]. In the case of hexanal, very low amounts have to be present in order to be detectable. If present in very high amounts it might affect the packaged products.

The suitability of a certain cardboard or pulp for usage as food stuff packages depends on a variety of factors and when using CTMP as packaging material an additional challenge presents itself due to the increased amount of extractives present [3]. The amount of odorous compounds and the oxidation rate of the extractives varies which is why a quick and precise method of product control for such pulp is required. If the pulp is not suitable for packages processing corrections has to be conducted in time [3]. It is necessary to keep in mind that a cardboard package consists of a complex system of a large number of compounds, many of them volatile [4]. Chemicals from the processing or molecules produced in microbiological processes within the material might affect the food stuff products as well. The compounds considered here are the ones produced during oxidation of fatty acids.

One significant category of lipids in pulp is fatty acids of various kinds. There are more than 20 different fatty acids in softwood, either in combination with glycerol, an alkyl ester derivative or in the free form [4]. The acids are saturated or mono-, di- or tri-unsaturated and are typically of 16-22 carbons. Linoleic acid is a diunsaturated fatty acid

of 18 carbons and is one of the dominating ones in pulp. One of the oxidation products is hexanal, which is dominating in causing odour over time in the pulp. It is commonly used as an indicator of the extent of oxidation of pulp since it is the most abundant product of linoleic acid [4]. A high value indicates that reaction products from unsaturated fatty acids will be of high amount as well. The natural oxidation of lipids, both bound in other material or in free form, is due to either an auto accelerated free radical process or photo-oxidation [6].

2.3 The oxidation of lipids

The two mechanisms of lipid oxidation are auto-oxidation by radical reactions and photo-oxidation [6, 7]. The auto-oxidation is initiated by formation of free lipid radicals when a hydrogen at an allylic position in the carbon chain of the fatty acid is abstracted. The abstraction of a hydrogen is a high activation energy process but the energy needed varies with position in the carbon chain [8]. The hydrogen adjacent to a double bond or between two double bonds are looser bound and easier to remove than hydrogens at positions further from the double bonds. Still, additional energy or catalysis by transition metals might have to be added in order for the initiation to take place [9]. In the propagation step, the free lipid radicals produced reacts with oxygen present in the environment which causes the formation of peroxy radicals. These react with fatty acids to produce lipid hydroperoxides. In Figure 2.1, the general process of auto-oxidation in lipids is shown [6, 7, 9].

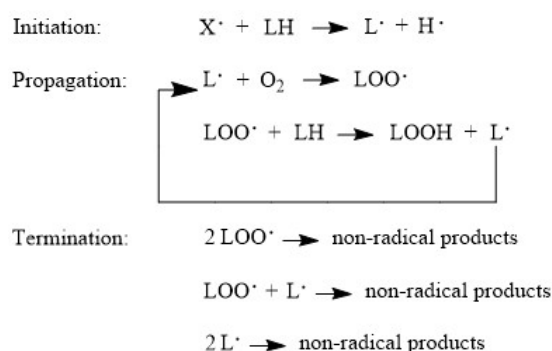


Figure 2.1: *The general schematics for the auto-oxidation process of lipids.*

An example of the auto-oxidation of a lipid, linoleic acid, is shown in Figure 2.2. The radicals formed initially are more stable in the trans configuration, meaning all radicals formed that are of cis configuration transforms into trans configuration and all products are conjugating. The hydroperoxides formed in the auto-oxidation of linoleic acid are relatively stable at room temperature, but in the presence of metals or at high temperatures they decompose to form a variety of products. Generally, lipid hydroperoxides are sensible to a complex process of thermal decomposition [8]. This results in the formation of alkoxy radicals which during a carbon-carbon cleavage process form aldehydes, ketones, alcohols, hydrocarbons, esters, furans and lactones. They might even react again with oxygen to form several secondary products as cyclic peroxides or epoxyhydroperoxides

2. Theory

and ketohydroperoxides. These products can decompose to volatile breakdown products as well.

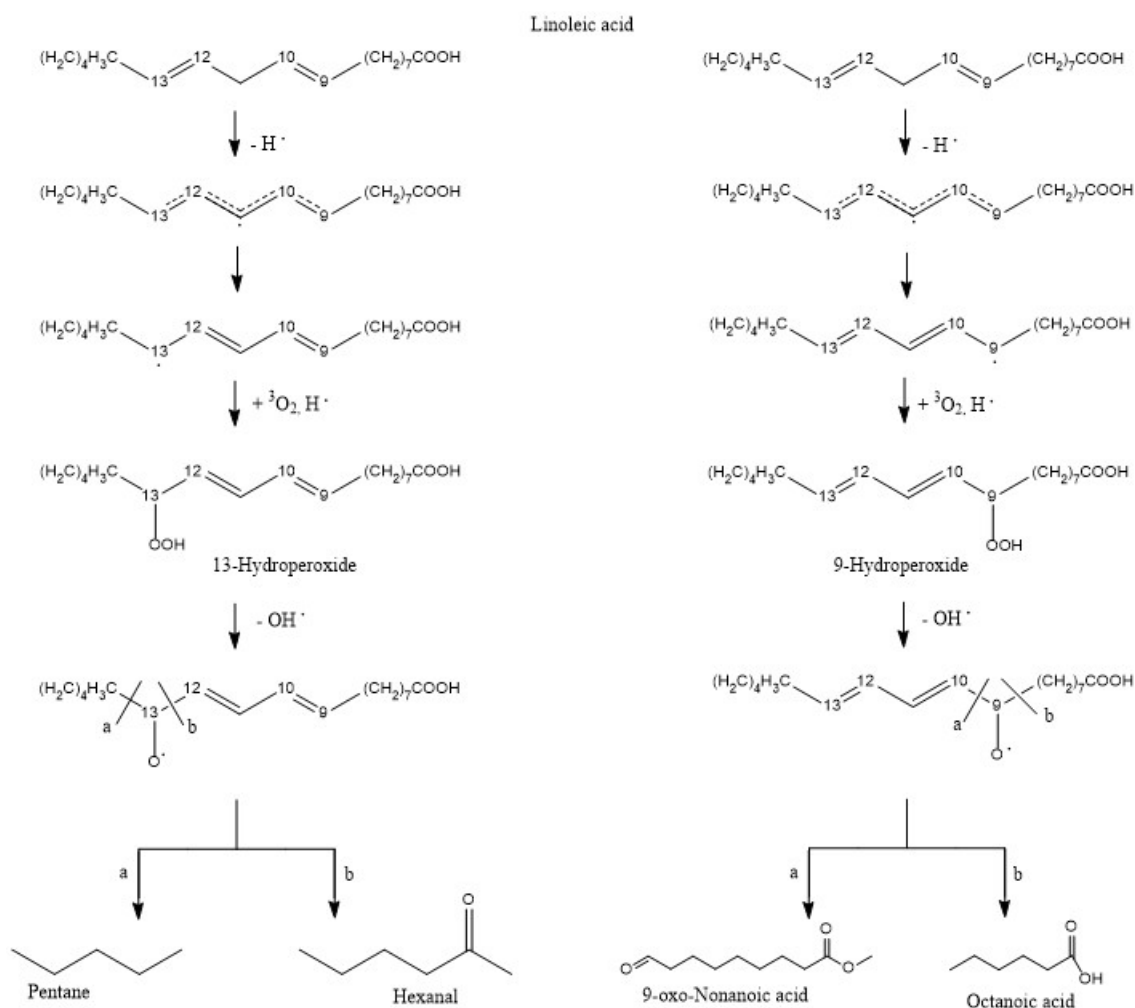


Figure 2.2: Auto-oxidation route of linoleic acid, showing some of the volatile reaction products originating from a peroxyradical. Hexanal is formed from the conjugated fatty acid radical with cleavage at carbon 14.

Due to the lower activation energy of initiation in auto-oxidation of fatty acids with double bonds in the carbon chain, unsaturated fatty acids are more susceptible to oxidation [8]. Radicals will form faster, easier and to a higher extent when the number of double bonds is increased. Linoleic acid is a diunsaturated fatty acid, the oxidation process of which is shown in Figure 2.2. Some of the oxidation products are displayed but a full list of all reaction products formed during oxidation of linoleic acid are displayed in Table 2.1.

The free radical oxidation process of fatty acids in pulp depends on temperature, available oxygen, transition metals present in the raw material and number of unsaturated sites in the carbon chain [10]. The reaction rates can vary significantly between different material and types of wood because of a natural variation in the degree of fatty acid unsaturation and presence of polar components in the bulk. These variations can contribute as much as 30 % respective 20 % to the oxidation process of oils.

Table 2.1: All oxidation products formed during auto-oxidation and photo-oxidation of linoleic acid. In photo-oxidation, two more aldehydes are formed in addition to those formed during auto-oxidation [8]

Auto-oxidation	Aldehydes	Carboxylic acids	Alcohols	Hydrocarbons
	Pentanal	Methyl heptanoate	1-Pentanol	Pentane
	Hexanal	Methyl octanoate	1-Octene-3-ol	
	2-Octenal	Methyl 8-oxooctanoate		
	2-Nonenal	Methyl 9-oxononanoate		
	2.4-Decadienal	Methyl 10-oxodecanoate		
Photo-oxidation	2-Heptanal			
	2-Butenal			

The photo-oxidation of lipids is induced by exposure to light in the presence of a sensitizer such as chlorophyll [6]. This process is not a free radical reaction but involves the production of singlet state oxygen by transfer of energy from the photo-sensitizer. This species is very reactive and the reaction is extremely rapid. If it does not react, it is capable of transferring its high energy to another compound instead [8]. When reacting with unsaturated fatty acids, mostly allyl hydroperoxides are formed. The hydroperoxides, once formed, follow the same path of decomposition and form almost the same products as they would if they were produced by the triplet state oxygen of the auto-oxidation process. The difference in products of linoleic acid is that not all products are conjugated and that 2-heptanal and 2-butenal was formed in addition to those of the auto-oxidation. It has been shown that shorter wavelengths of the light is profitable if accelerated photo-oxidation is desired. The photo-oxidation process is displayed in Figure 2.3.

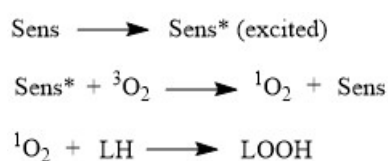


Figure 2.3: The general route for the photo-oxidation of lipids. A sensitizer is needed to induce the reaction [6].

2.4 The role of azo compounds in radical reactions

Azo initiators is a well known group of chemical intermediates. The dissociation of azo compounds results in generation of two radicals, which can be used to induce a variety of radical reactions. They are most known for initiating polymerisation reactions but have been used successfully in other reactions as well [10, 11, 12]. The azo compound shown in Figure 2.4 is 2.2-azobis(2- methylpropionamide), AAPH, which is a hydrophilic

2. Theory

compound that is commonly used in water-borne reactions. AAPH is produced through modification of the very commonly known azo compound azobisisobutyronitrile, AIBN.

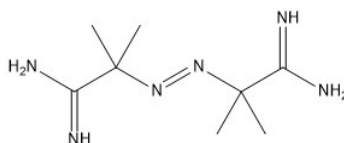
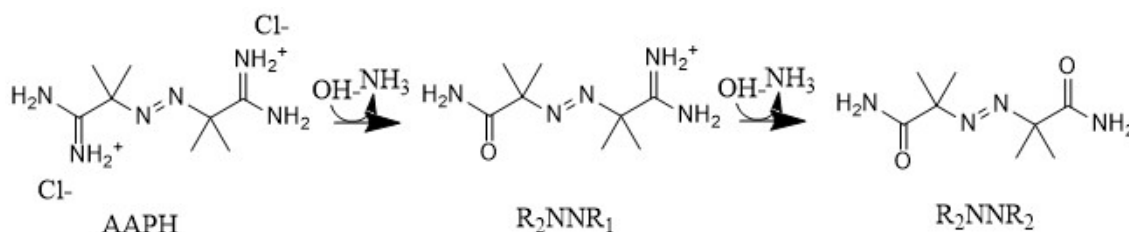


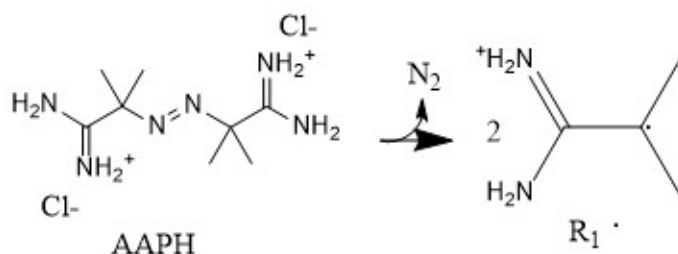
Figure 2.4: The molecular structure of the hydrophilic azo compound AAPH [13].

AAPH has previously been investigated as an accelerator in oxidation processes, Betigeri et al. performed a study where AAPH was used to accelerate the oxidation of biological tissue [14]. The aim of the study was to examine lipid peroxidation where azo compounds were used to initiate the radical reaction. The azo compound form radicals during thermal decomposition and in AAPH, two identical radicals are formed when the nitorgen:nitrogen bond is cleaved. The radicals are relatively stable and risk reconnecting with each other resulting in a non-radical molecule. When in contact with oxygen, the peroxy radicals are highly reactive. It was found that the highest reaction rates were achieved in an acidic environment, with pH around 1.2 [14]. Due to the decomposition being thermally dependent, it is important to keep the temperature at a level where production of radicals is possible. The AAPH is degraded at 40 °C, which is rather typical for azo compounds [15].

The details of the mechanisms of azo compounds are not completely known today, in Figure 2.5 the hydrolysis and thermal decomposition mechanisms are shown.



(a) Hydrolysis of AAPH results in non radical products. The R_2 products are the hydrolysis products of R_1 , which are substitutes in the compound, here the amino group on the AAPH.



(b) Thermal decomposition of AAPH. Peroxy radicals are formed.

Figure 2.5: Two mechanisms of decomposition of AAPH, it is clear that both radical and non radical products can form. Figure (a) shows hydrolysis, while (b) displays the thermal decomposition [15].

As seen in Figure 2.5, thermal decomposition is preferable during initiation since radicals are formed. The thermal decomposition is dominating in acidic conditions at 40 °C, but in basic conditions the hydrolysis paths is dominating [15]. The resulting products of the decomposition are nitrogen gas and two carbon centred radicals, capable of a number of propagation and termination reactions. The peroxy radicals are dominating but alkoxy radicals could form to a negligible extent.

2.5 Iron catalysis and their effect on lipids

Iron is a well known catalysing agent for a number of reactions. It has many benefits, such as availability, environmental viability and low price [16]. Usage of iron has proven successful in catalysing redox reactions as well as being an initiator, capable of initiating many reactions and producing many products. Present in radical reactions, iron ions could react with peroxides and form alkoxy radicals by oxidation of Fe^{2+} to Fe^{3+} . A possible catalytic cycle is seen in Figure 2.6.

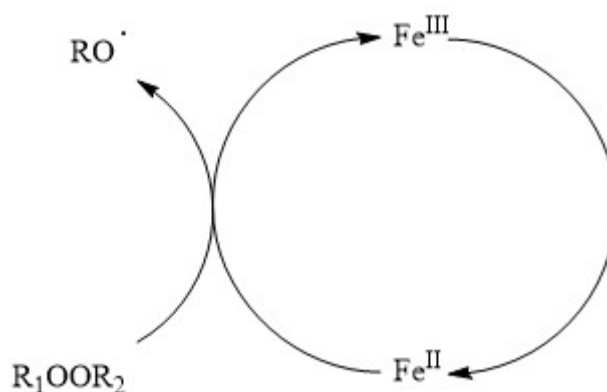


Figure 2.6: The oxidation of Fe^{2+} to Fe^{3+} could produce radicals [16].

The oxidation of Fe^{2+} to Fe^{3+} is necessary for formation of radicals from peroxides [17, 18]. A study performed by Braughler et al. showed that the addition of Fe^{2+} and Fe^{3+} ions in different ratios affected the acceleration of oxidation mechanism in lipids [17]. The ratio 1:3 of Fe^{2+} to Fe^{3+} provided the most promising results.

Iron ions work as initiators in the radical reaction of auto-oxidation in lipids by lowering the activation energy for the abstraction of hydrogen from the lipid during the initiation step [8]. In addition, the iron ions might react directly with lipids resulting in the production of alkyl radicals, as seen in Figure 2.6. Iron could produce reactive species, such as single state oxygen, which would promote an oxidation similar to the photo-oxidation.

2.6 Headspace solid-phase microextraction and gas chromatography

A gas chromatograph is used to identify and quantify organic compounds, both in gas and liquid phase. The results obtained are chromatograms analysed manually or by computer

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software, where the heights and areas of the peaks generated from each compound at a specific retention time is used to acquire the information needed [19, 20]. The technique is based on separation of compounds in an analyte inserted to the column in the machine [20]. Gas chromatography is usually combined with either a mass spectrometer detector or a flame ionisation detector.

One of the useful applications of gas chromatography is the possibility to analyse volatile compounds formed in a material. A sample can be collected from the headspace of a material and information of the composition can be obtained. Solid-phase microextraction, SPME, is an extraction process that is applicable to headspace analysis and a means of collecting the sample before insertion to the column [21]. SPME provides an accurate extraction at ambient conditions of trace compounds at very low concentrations. The general principle of the technique is to expose a coated fibre, compatible with the analyte, to the sample for a specific amount of time. The extraction is complete when equilibrium is reached between the analyte and the fibre coating. In headspace analysis, the fibre is submerged through the vial cap and to the space above the sample.

3

Methods

The project consisted of both extensive literature research and experimental procedures. Some of the experimental descriptions in this chapter are general and specific notes are to be found in the Appendices.

3.1 The design of experimental procedure

The attempted accelerated oxidation could be induced by either physical or chemical means. In this study focus has been on addition of chemical compounds to induce the radical oxidation process of the fatty acids. The possibility to induce photo-oxidation by irradiation of the pulp with UV-light was explored as well.

The chemical oxidation inducers chosen were examined at different concentrations and reaction times, initially at a screening process where the decreasing amount linoleic acid was analysed. The samples showing the most favourable results were analysed with headspace solid phase microextraction gas chromatography, SPME-GC, to obtain quantitative information about the hexanal produced upon addition of inducers in comparison with reference samples. In addition, extraction of pulp was performed to obtain information of the contents of the raw material. The same extraction procedure was used when analysing the impact of UV radiation on the pulp samples. The pulp used was chemi-thermomechanical pulp, CTMP, from Scandinavia. It was stored in a freezer at -20 °C to prevent external influences, ageing and natural decomposition affecting the quality of the material. The dry-content is around 90 %.

Internal standard is used in the quantitative analysis of the peaks and as an indicator of if the experiment or extraction has been successful. Since a known amount internal standard is added one can evaluate if some fraction has gotten lost in the process and one can compare the area of the peak to the concentration added. The internal standard used is heptadeconic acid, an isomer to a naturally occurring fatty acid in the pulp. It has a carbon chain of 17 carbons and is relatively similar to linoleic acid, making it suitable in this application.

The analysis method used in this study was mainly gas chromatography. Two techniques have been used, regular gas chromatography of samples provided from extraction and through the screening process. The production of hexanal over time was examined using a headspace solid-phase microextraction gas chromatography mass spectrometric procedure.

The gas chromatograph used during screening and extraction analysis is a *DANI instruments S.A Master GC* in combination with *DANI automatic samples AS* and *FID*. For the experiments a 15 m non-polar capillary column of diameter 0.25 mm, with (5%-Phenyl)-methylpolysiloxane stationary phase, was used for separation. Injector temperature was set to 300 °C, the oven set on a gradient run from 150 °C to 250 °C with a temperature rise of 25 °C / min. The temperature in the FID was set to 370 °C. The gas chromatograph used for tracing of hexanal was a *Thermo Scientific, the Focus GC* combined with a *ISQ mass spectrometer* and *Autosampler PAL combi-xt*. A 30 m ZB-wax column of 0.25 mm diameter was used for separation. The injector temperature was 250 °C with a split flow of 100 ml/min, the oven was set on a gradient run initially from 35 °C to 75 °C with a temperature rise of 8 °C per minute and then from 75 °C to 200 °C with a rise of 40 °C per minute. The temperature of the transfer line was set to 300 °C. The fibre used in the SPME was a 10 mm polydimethylsiloxane fibre with a thickness of 100 µm.

Reference measurements were performed to obtain an indication of the amount hexanal produced naturally over the chosen time span. It is desired to accelerate the oxidation to the same extent, producing the same amount hexanal from the same amount of pulp, in the presence of inducers.

3.2 Reference measurements of hexanal formation in CTMP

Quantitative measurements of produced hexanal over time in cardboard is a part of the product control at many companies handling pulp. The procedure is performed on the finished cardboard product but in this study the same procedure was applied to the CTMP used. Difference in results are expected since the pulp has not been exposed to the same production process as the cardboard. According to the product control method acquired by a paper and pulp company, the cardboard is to be stored in a climate controlled environment of 23 °C with a humidity of 50 %. Samples of 2 g cardboard are obtained after typically 1, 10 and 40 days. The samples are put in headspace vials and heated at 90 °C for 40 minutes before analysed with headspace SPME in a gas chromatograph. The amounts hexanal measured are usually at a few ppb but are expected to be higher for pulp than cardboard.

In addition, samples of CTMP stored in vials at the same conditions and for the same amount of time are analysed using the same analysis method. The difference between the two sets of samples is that with the first method hexanal is formed over time and driven out of the pulp when applying heat. The other sets of samples are extracted from the headspace of pulp samples stored in gastight vials. The amount hexanal analysed is the amount formed during the days of storage. These samples are intended to be used as references when analysing the amount hexanal formed in the accelerated oxidation pulp samples.

3.3 Extraction procedure of CTMP

Extraction of the CTMP was performed according to a standardised method at BIM kemi AB where microwave extraction is performed frequently, see Appendix B. A sample of CTMP is obtained from the freezer and put into an oven for further drying. The CTMP was divided into two containers containing a weflon button and a magnet. Internal standard is added to both containers.

Acetone was added to the containers and they were heated in a microwave oven. When the samples had cooled down the acetone was transferred into 30 ml vials and then evaporated at 25 °C to 4 ml in a MiVac vacuum evaporator. Of the remaining solvent, a small part is mixed with reagent N.O-bis(trimethylsilyl)trifluoroacetamide, BSTFA, and reacted at 70 °C for 1 h before analysed in the gas chromatograph. Another sample is reacted with trimethylsulfonium hydroxide, TMSH, and analysed instantly. The two different reagents enable analysis of free fatty acids respective total amount fatty acids in the samples.

3.4 Acceleration of oxidation by chemical means

The main focus of the study was to by chemical means initiate or catalyse the oxidation of linoleic acid. The chemical process involved a screening of possible reactions and a headspace tracing of hexanal.

3.4.1 Design of experimental procedure with chemical inducers

The experimental procedures performed are inspired by several articles, among others an article by Fabiano et al. where a variety of azo compounds and other compounds were used to perform an analysis of oxidative stability of different drug compounds in buffer mixtures of different pH [10]. Braugher et al. performed a study where ferric and ferrous iron was added to biological tissue to induce oxidation [17]. Different ratios Fe^{2+}/Fe^{3+} was used and the mol ratio 1:3 was considered the most profitable.

The method used for the experimental procedure was adapted to the time restrictions of the project and the equipment available. The method is the same for both inducers except for variations in pH and reaction times. The conditions are kept close to ambient to enable a method that is as energy efficient as possible. The temperature is 40 °C to induce thermal decomposition of the AAPH. The pH is kept natural in the experiments conducted with iron, but is lowered with acetic acid or hydrochloric acid in the experiments involving AAPH.

The chemicals used were linoleic acid of technical grade (58-74 %), AAPH (2,2-Azobis(2-methylpropionamidine) dihydrochloride) granular 97 % and iron(III) chloride of reagent grade, 97 %, purchased from Sigma Aldrich. The iron(II) sulfate heptahydrate was purchased from Sharlau. Hexanal, 98 %, was purchased from Alfa Aesar. The reagents used was trimethylsulfonium hydroxide (TMSH), 0.2 M in methanol, and N.O-bis(trimethylsilyl)-trifluoroacetamide (BSTFA) > 99 %.

3.4.2 Screening of oxidation inducers

A primary screening of the different accelerating methods was performed with the aim of evaluating if oxidation, or other reaction paths, is achieved by addition of chemicals to linoleic acid. The screening will not provide exact information of reactions or products formed in the sample but is only capable of measuring decrease of concentration of components, specifically linoleic acid. By measuring the decrease in concentration of linoleic acid one can conclude that the component has participated in some reaction changing its chemical structure. This is done by comparing the chromatograms of samples of known concentrations linoleic acid but with or without addition of the oxidation inducers. The non-polar column used in the gas chromatograph is not compatible with solvents containing water, which is why acetone is used as solvent instead.

The screening is performed as such; chromatograms of samples containing linoleic acid and oxidation inducer is compared to chromatograms of reference samples containing a known concentration linoleic acid. Reaction times and concentrations of inducers are varied to find the optimal conditions. The software used to obtain chromatograms was Clarity 7.3. To prepare the reference samples, linoleic acid was dissolved in acetone. A small amount of the solution was transferred into a vial and mixed with reagent TMSH and analysed with the DANI gas chromatograph and FID.

3.4.2.1 Determination of the optimal concentrations of oxidation inducers

A detailed description of the experimental procedure with AAPH and iron is found in Appendix C.1. An acidic stock solution of linoleic acid, internal standard, acetic acid and acetone was prepared. AAPH respective iron ions were added in five different concentrations each, the stock solution was used in the induced samples and a reference sample. An additional sample with neutral pH, by excluding acetic acid, was prepared as well. The stock solution and AAPH were mixed in vials which were transferred to a 40 °C water bath and vigorously stirred. Analysis of the samples were made after 30 and 60 minutes.

The procedure for the iron based oxidation is similar to the one with AAPH. The stock solution was prepared by adding linoleic acid and internal standard to acetone. Five different concentrations were analysed as well as a reference sample. The mol ratio between FeSO_4 and FeCl_3 is 1:3 and the concentration variations are based on differing ratios of FeSO_4 to linoleic acid. The vials were put in water baths of 40 °C during vigorous stirring. Analysis of the samples was performed after 60 minutes.

3.4.2.2 Determination of optimal reaction times

A detailed description of the experimental procedure is found in Appendix C.2. The three concentrations displaying the most satisfactory results in the previous experiment, were analysed at different reaction times. The experimental procedure was the same as in the previous experiment. The samples were analysed after 5, 30, 60, 120 and 200 minutes.

The experiments conducted with iron as oxidation inducer are performed similarly. Three

samples displaying satisfactory results in previous experiments were prepared and analysed again after 5, 10, 20, 30 and 60 minutes. Additional analysis was performed on one of the samples after 3 and 4.5 h.

3.4.3 Tracing of hexanal in the headspace of accelerated samples

Extended analysis was performed on the attempts with promising results in the screening process. The samples were analysed using *Thermo Scientific, the Focus GC* and *ISQ mass spectrometer*. The experiments were conducted on either CTMP in water or linoleic acid dissolved in ethanol and water. The CTMP is mixed with water to a dry-content of 4 % which allows the chemicals to blend with the pulp and a sufficient amount pulp is fitted into the vials. The linoleic acid is to a large extent bound and not so accessible in the pulp and it is predicted that the reactions will proceed differently in the pulp in comparison with in direct contact with the linoleic acid. Water is used as solvent, because it is easily accessible, environmentally viable and cheap. Water is used through the whole pulp process and it is profitable to use water in the laboratory trials as well.

The linoleic acid is dissolved in ethanol before milliQ water is added. The solubility of linoleic acid in water is low, causing suspensions to form if the concentration of linoleic acid in the solution is too high. The initiator used, AAPH, is hydrophilic and soluble in both ethanol and water.

For characterisation and quantitative analysis of the compounds found in SPME GC-MS, the software XCalibur is used. Calibration curves are produced to enable quantitative analysis. They were prepared by dissolving hexanal in milliQ water at six different concentrations in the interval 0.054 mM to 0.81 mM. New calibration curves are prepared when the gas chromatograph has been used for other purposes between experiments.

3.4.3.1 Oxidation of linoleic acid in the presence of oxidation inducers

Several experiments are conducted with the oxidation inducers as initiators. Initially short reaction times are used because the screening process indicated that linoleic acid is quickly reduced in the sample when inducers are added. The reaction times were continuously prolonged over the course of the study. Detailed descriptions of the various experiments are found in Appendix D.1. Generally, linoleic acid is dissolved in ethanol and milliQ water is added while stirring. If AAPH is to be used, hydrochloric acid is added dropwise to $\text{pH} < 1$. The solution is added to vials and the inducer is weighed and added. The two most beneficial concentrations AAPH are the ones of mol ratio 1:1 or 1:2 AAPH to linoleic acid and the most beneficial mol ratios of FeSO_4 to linoleic acid are 2:1 and 1:1, the mol ratio between FeSO_4 and FeCl_3 is 1:3. These are the ratios used in all tracing experiments. The reactions were conducted in vials placed in 40 °C oil baths while stirring and then analysed with headspace SPME-GC-MS. Because acetone was used as solvent in the screening of the inducers, experiments with acetone was conducted in a similar way as well, as described in Appendix D.1.1. The aim was to dismiss the hypothesis that a change of solvent might affect the results of the experiments.

3.4.3.2 Experiments conducted with an increased concentration reactants

The same procedure but with a significantly higher concentration of linoleic acid and inducers was performed. Experiments were performed with ten times the concentration, providing interesting results. Additional experiments of long reaction times; 10 h and 1, 3, 6, 12 and 20 days, were performed with higher concentrations as well. These samples contained 0.1 volume% linoleic acid and the initiators were added in the same ratio as before. By mistake, the ratio iron to linoleic acid was changed from 2:1 to 1:2, FeSO₄:linoleic acid.

3.4.4 Oxidation of CTMP in the presence of oxidation inducers

The detailed descriptions for experiments conducted with AAPH and Fe²⁺/Fe³⁺ are found in Appendix D.3. CTMP is weighed and put into vials and milliQ water is added until the dry content is about 4 %. The amount of CTMP is relatively small, 0.5 g, and is limited by the volume of the vials. The content of linoleic acid will be in μg scale and the previously used ratio of linoleic acid to oxidative inducer is not valid in this case. Larger amounts has to be introduced to the CTMP sample because of the inaccessibility of the linoleic acid in the pulp in comparison with the linoleic acid solution. In order to get a homogenous solution of oxidative inducer and pulp, the amount inducer used is determined to 0.1 % of the weight of the pulp in each vial.

To the vials prepared for AAPH experiments HCl is added dropwise providing a low pH. The oxidative inducers are added to all vials and the samples are stirred vigorously to achieve the highest homogeneity and dissolution of CTMP possible. The vials are then transferred to an oil bath of 40 °C, the same conditions used in previous experiments. All samples are analysed at 10 h and 1, 3, 6, 12 and 20 days.

3.5 UV-radiation as accelerating technique

Attempts to induce photo-oxidation was performed by exposure of CTMP to UV- radiation, described in Appendix E. CTMP was weighed and divided onto two petri dishes. One was placed in the light of a UV lamp of wavelength $\lambda = 254 \text{ nm}$. Both were put in a climate controlled room of 50 % humidity and 23 °C. Samples of about 0.8 g were gathered at 1, 5, 7 and 13 days from both petri dishes and extracted according to Method 3.3. The amount linoleic acid in respective sample is analysed and compared. For results see Appendix E.

4

Results

Results from the pulp analysis, screening process and hexanal tracing are presented here. All results are obtained through gas chromatography.

4.1 Analysis of CTMP

Initially, an extraction of the CTMP, used as raw material in the study, was performed to obtain information of the contents. The chromatogram is displayed in Figure 4.1 and the components are listed in Table 4.1.

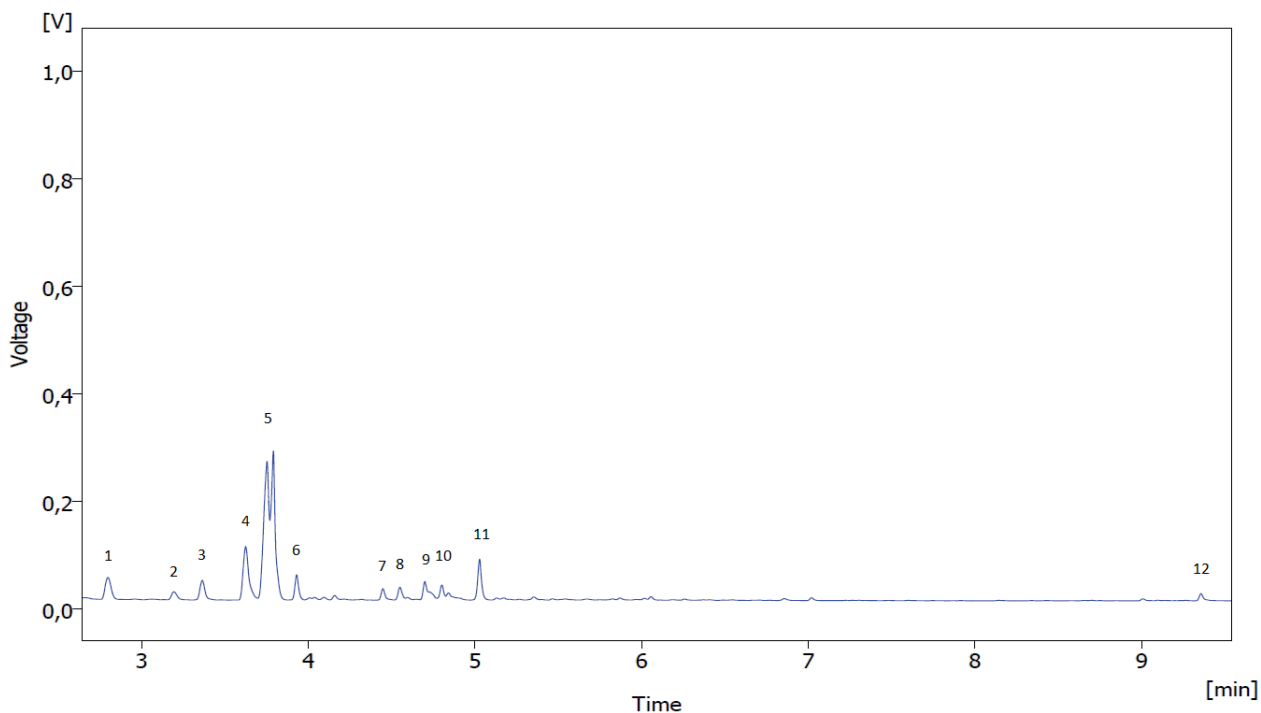


Figure 4.1: Chromatogram obtained during extraction analysis of CTMP.

4. Results

Table 4.1: The contents of the CTMP, used in the study. The reagent TMSH used provides a clear view of the total amount of fatty acids present.

Nr.	Retention time [min]	Compound	Group
1	2.80	Hexadecanoic acid	Saturated fatty acid
2	3.20	Heptadecanoic acid	Saturated fatty acid
3	3.37	Internal standard	Saturated fatty acid
4	3.63	Linolenic acid	Tri-unsaturated fatty acid
5	3.79	Linoleic acid and oleic acid	Di- and mono-unsaturated fatty acids
6	3.93	Stearic acid	Saturated fatty acid
7	4.45	Isopimaric acis	Resin acid
8	4.55	Pimaric acid	Resin acid
9	4.70	Resin acid	Resin acid
10	4.80	Resin acid	Resin acid
11	5.03	Dehydroabietic acid	Resin acid
12	9.36	Stigmastanol	Phytosterol

The reagent used in the gas chromatograph analysis enables a visualisation of all fatty acids in the sample. In Appendix F, a chromatogram presenting both free fatty acids and total fatty acids is displayed. For visualisation of free fatty acids a different reagent is used.

4.2 Quantitative analysis of hexanal formation in CTMP reference samples

Hexanal was traced in the headspace of reference CTMP samples. The samples were analysed according to a standardised method in gastight vials. The values obtained were supposed to give an idea of the amount hexanal produced through natural oxidation in the pulp over a time span of 40 days. The graph shown in Figure 4.2 displays samples of CTMP stored in vials in a climate controlled environment and then analysed with headspace solid-phase microextraction gas chromatography (SPME-GC). The same CTMP but with another, standardised sample preparation method obtained from the pulp industry, is analysed and seen in Figure 4.3. The sample preparation procedure is found in Method 3.2 and Appendix A.

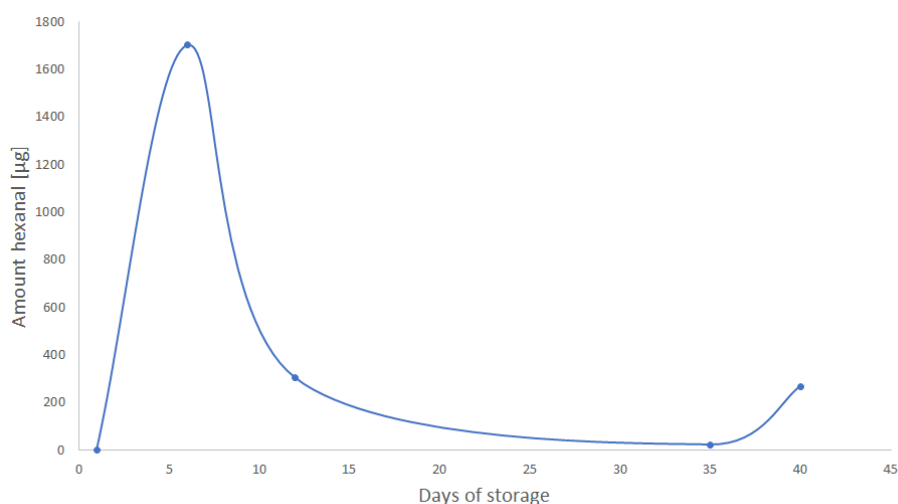


Figure 4.2: Quantitative hexanal analysis. The total amount hexanal present in the headspace of the CTMP sample, stored in vials, is displayed as μg at a specific storage time.

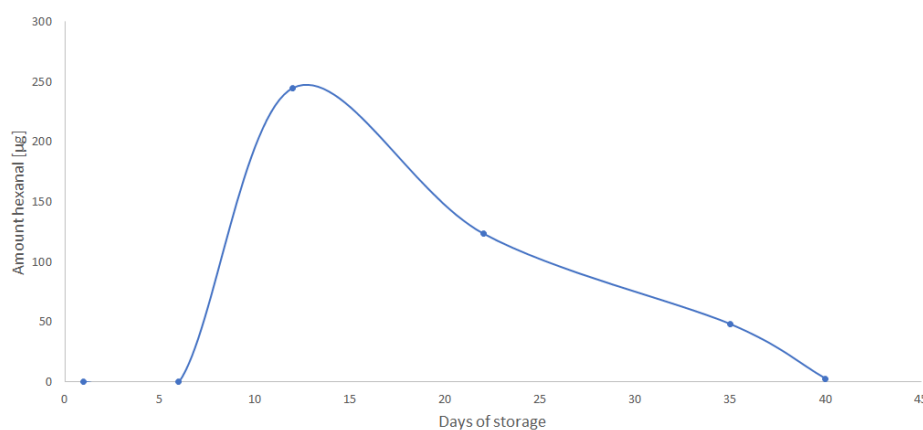


Figure 4.3: Quantitative hexanal analysis of CTMP samples stored openly and heated. The total amount hexanal present in the head space of the CTMP sample is displayed as μg at a specific storage time.

4.3 Results from the screening of oxidation inducers

Initial experiments with oxidation inducers added to linoleic acid in a variation of conditions were performed to find the optimal reaction conditions. The figures in this section displays alterations in reaction time and concentrations. The bar chart in Figure 4.4 visualises an experiment of different concentrations AAPH in relation to linoleic acid. The y-axis is the area below the linoleic acid peaks in the chromatogram obtained through gas chromatography analysis. The x-axis is the different mol ratios of AAPH added to the linoleic acid solutions in relation to the content linoleic acid. The chart provides an indication of if addition of AAPH has any effects the concentration linoleic acid in the sample.

4. Results

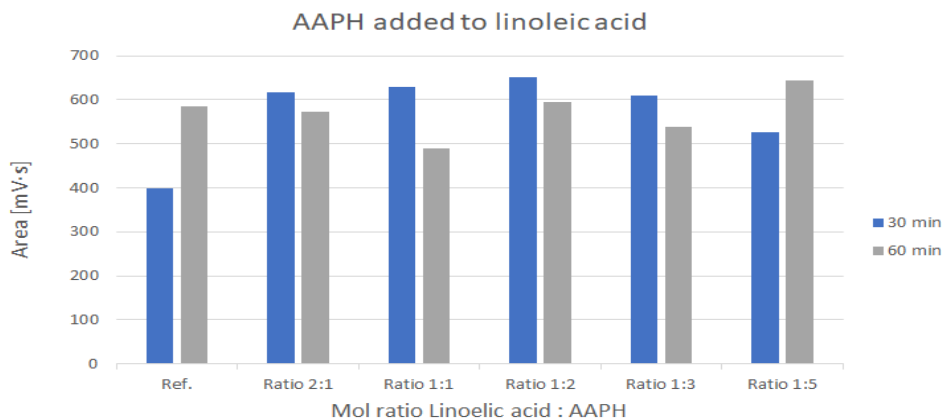


Figure 4.4: Five samples of different mol ratios linoleic acid to AAPH was compared at two different reaction times. The area on the y-axis is the area below the linoleic acid peaks in the chromatograms obtained from the gas chromatography analysis.

The samples with ratios providing the most satisfactory results were analysed again with varying reaction times. In Figure 4.5, three different ratios are displayed with reaction times in the interval 0 - 195 minutes.

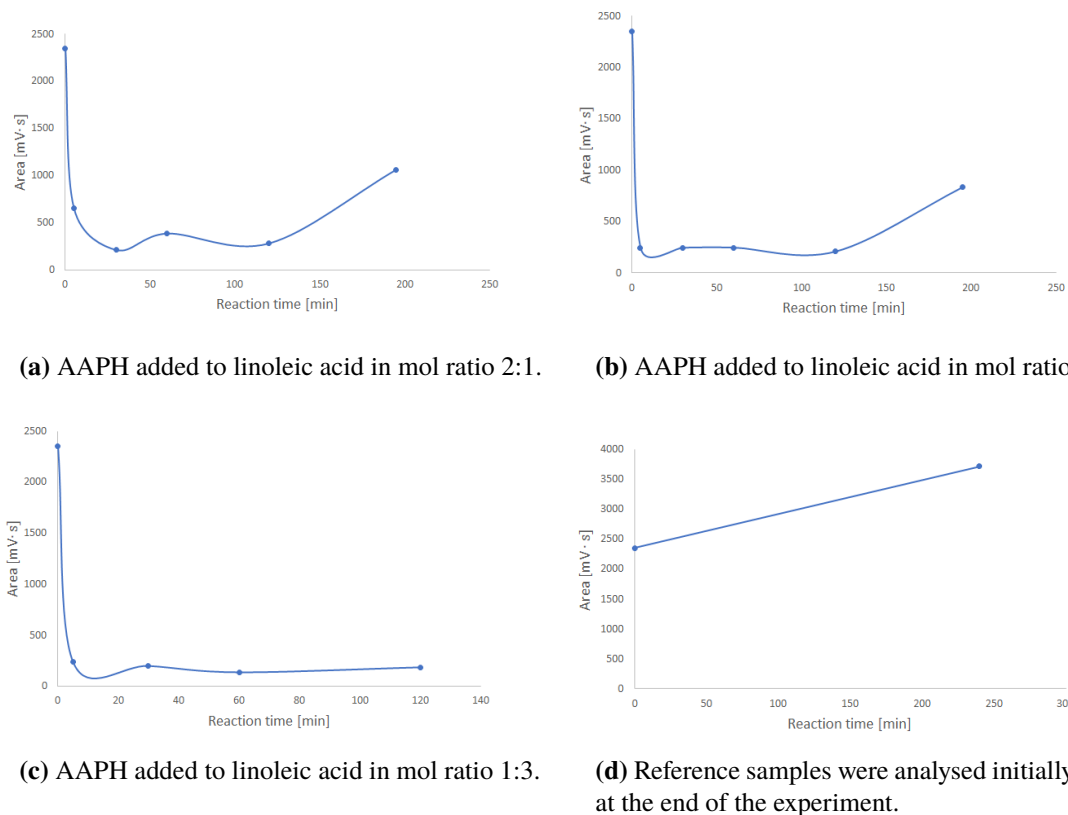


Figure 4.5: The samples displaying interesting results in the concentration analysis is examined again with varying reaction times. The y-axis is the area below the linoleic peaks in the chromatograms and the x-axis is the reaction times in minutes.

The same procedure was performed for the $\text{Fe}^{2+}/\text{Fe}^{3+}$ samples. In Figure 4.6, samples of varying concentrations at a set reaction time is displayed. The x-axis shows samples of different amounts FeSO_4 and FeCl_3 added to linoleic acid. The amounts are calculated based on differing mol ratios Fe^{2+} to linoleic acid in solution, shown in the x-axis. Figure 4.6 b) displays the same samples as Figure 4.6 a), the reference sample is excluded for clarification. The samples displaying the most satisfactory results are analysed again with variations in reaction time. In Figure 4.7, samples of three concentrations are analysed over a time interval of 0 - 180 minutes.

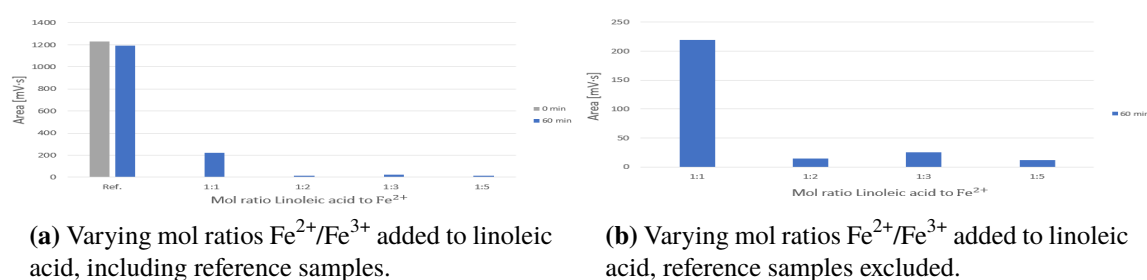


Figure 4.6: Five samples of different mol ratios Fe^{2+} to linoleic acid. In Figure (b), the results are displayed without reference samples.

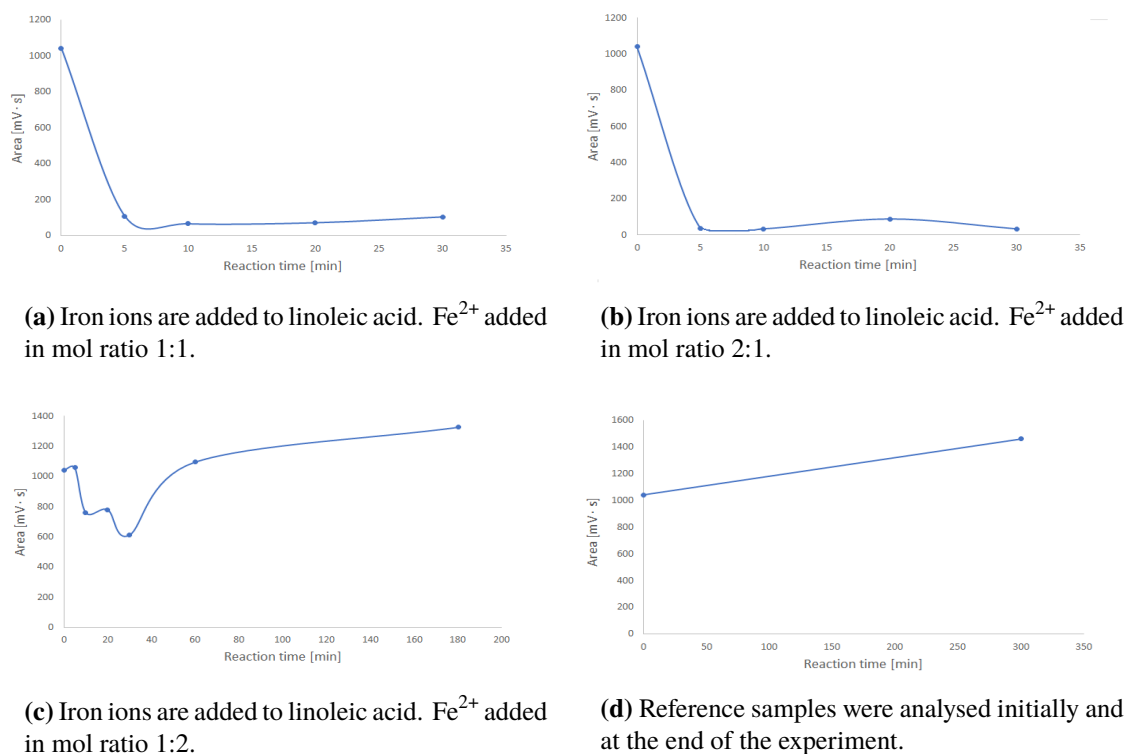


Figure 4.7: Samples displaying interesting results in concentration analysis are analysed again at different reaction times. The y-axis is the area below the linoleic peaks in the chromatograms and the x-axis is the reaction times in minutes.

4.4 Results from quantitative analysis of hexanal formation in accelerated samples

The oxidation inducers were added to both CTMP in water and linoleic acid solutions of the fatty acid dissolved in ethanol and water. In Table 4.2, a compilation of samples of different ratios, concentrations and reaction times of linoleic acid and inducer are displayed. All the samples shown in Table 4.2 are performed on linoleic acid solution and not CTMP.

Table 4.2: Several attempts were performed before adjustments led to formation of hexanal. The composition with a 10 times increase of concentrations were used in further experiments. Here, t_r is reaction time and LA is linoleic acid.

Composition	Ratio	t_r [min]	Outcome	Composition	Ratio	t_r [min]	Outcome			
AAPH : LA	1:2	5	Neg.	Fe^{2+} : LA	1:1	10	Neg.			
		10	Neg.			40	Neg.			
		15	Neg.			180	Neg.			
		30	Neg.			195	Neg.			
		40	Neg.			2:1	10	Neg.		
	1:1	5	Neg.		40		Neg.			
		10	Neg.		180		Neg.			
		15	Neg.		195		Neg.			
		10 x conc.	1:1		45		Neg.	10 x conc.	2:1	45
					120	Pos.	120			Pos.

The samples containing linoleic acid and oxidation inducers in a ten times higher concentration than previous experiments provided production of hexanal at relatively short reaction times. Further analysis with the same method and linoleic acid to inducer ratios are performed on both linoleic acid solution and CTMP. The results of the experiments conducted with iron ions in linoleic acid solution are displayed in Figure 4.8. The quantitative analysis displays the amount hexanal produced and gathered from the headspace of the vials at a specific reaction time in the interval 10 h to 20 days.

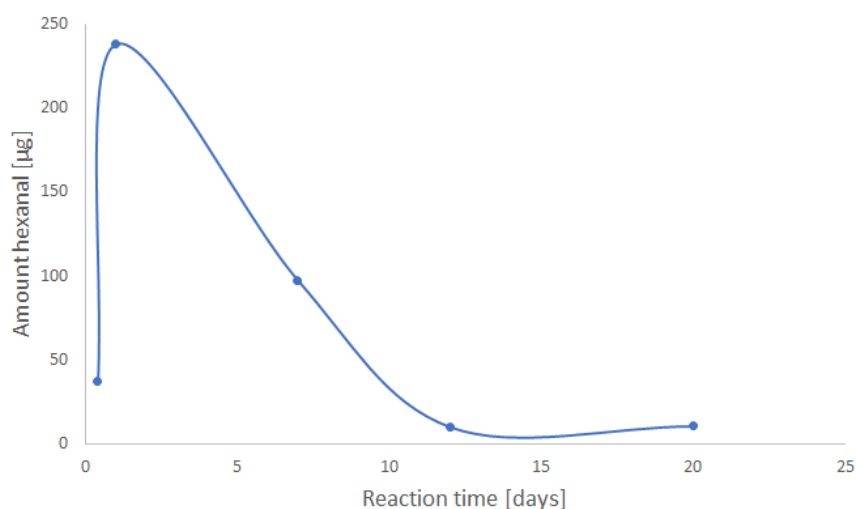


Figure 4.8: Tracing of hexanal produced in linoleic acid upon addition of Fe^{2+} and Fe^{3+}

The reference samples of linoleic acid solution are displayed in Figure 4.9. A clear peak is seen at day 7, either hexanal is produced quickly and decomposed again or some abnormality occurred.

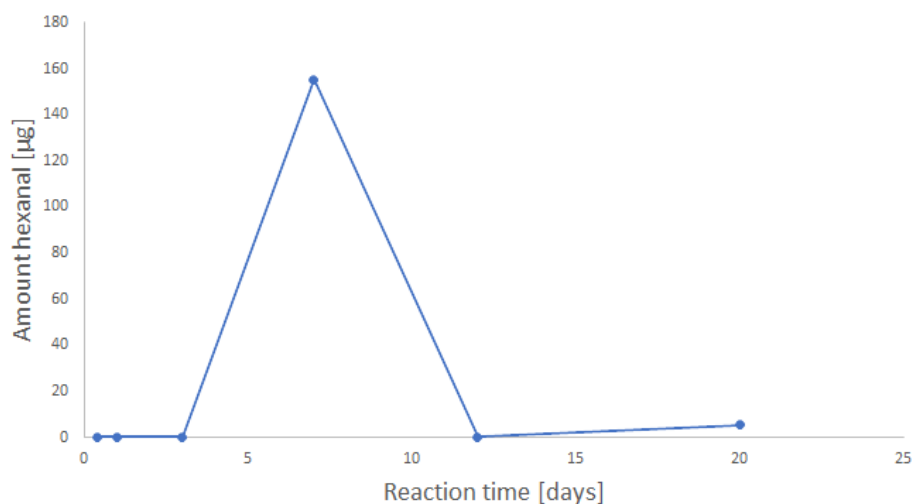


Figure 4.9: Tracing of hexanal produced in the reference sample containing linoleic acid solution.

Experiments performed with AAPH as oxidation inducer did not provide the same interesting results. No hexanal could be traced in the time interval that started at 10 h. It was, however, detected in an earlier experiment executed in the same way but at a reaction time of 2 h as seen in Table 4.2 indicating a short reaction time.

4.4.1 Hexanal calculations

Hexanal was encountered in the reference samples of CTMP stored and exposed to natural oxidation, in iron ion induced linoleic acid solution and in the reference sample of linoleic acid solution, as seen in Figures 4.2, 4.3, 4.8 and 4.9. The measured amount hexanal was compared to the amount linoleic acid respective CTMP in the samples. The amount hexanal produced during oxidation induced by $\text{Fe}^{2+}/\text{Fe}^{3+}$ ions in ppm of mols linoleic acid present in the solution is seen in Table 4.3.

Table 4.3: Amount hexanal (mol) formed upon oxidation of linoleic acid induced by $\text{Fe}^{2+}/\text{Fe}^{3+}$ ions. The hexanal is displayed in mol and in ppm of the linoleic acid (mol) present.

Reaction time	Induced with iron ion		Reference sample	
	Hexanal [mol]	ppm	Hexanal [mol]	ppm
10 h	3.714E-07	1154.899	0	0
1 day	2.376E-06	7387.959	0	0
7 days	9.730E-07	3025.120	1.551E-06	4848.163
12 days	1.003E-07	311.739	0	0
20 days	1.038E-07	322.765	5.415E-08	169.229

The CTMP reference samples are presented in a similar way in Table 4.4. The amount hexanal formed is presented in mg occurring in the headspace and in ppm of the amount (g) CTMP present in the vial. Two methods have been used, both the standardised method and the CTMP stored in gastight vials.

Table 4.4: Amount hexanal (mg) in untreated CTMP samples used as references and stored over an interval of 40 days.

Days	Hexanal vial sample [mg]	ppm	Hexanal standr. sample [mg]	ppm
6	1.702	1702.248	0	0
12	0.303	305.694	0.245	242.999
22			0,123	61.693
35	0.022	10.995	0.048	23.905
40	0.267	135.990	0.0027	1.300

4.5 Alternative products formed through reaction of hexanal

During analysis of the chromatograms acquired in the tracing of hexanal, a suspicion arose that hexanal could have decomposed or reacted over time in the vials. This is a possible explanation to the peaks of amount hexanal in the headspace in the hexanal trace experiments, Figures 4.2, 4.3 and 4.8. In Table 4.5, reaction products of hexanal with molecular weights matching compounds found through analysis of chromatograms are seen. It was clear that the amount of and heights of the peaks increased with time.

Table 4.5: Possible reaction products formed through reaction of hexanal. Matching molar masses was encountered in the chromatograms.

Product	Molar mass [g/mol]
Hexanoic acid	116.16
Hexanol	102.16
Hexylamin	101.19
Dihexyl ketone	198.34
Dihexyl ether	186.33
Acetals	

In Figure 4.10, molecular structures of hexanal and the products listed in Table 4.5, are shown.

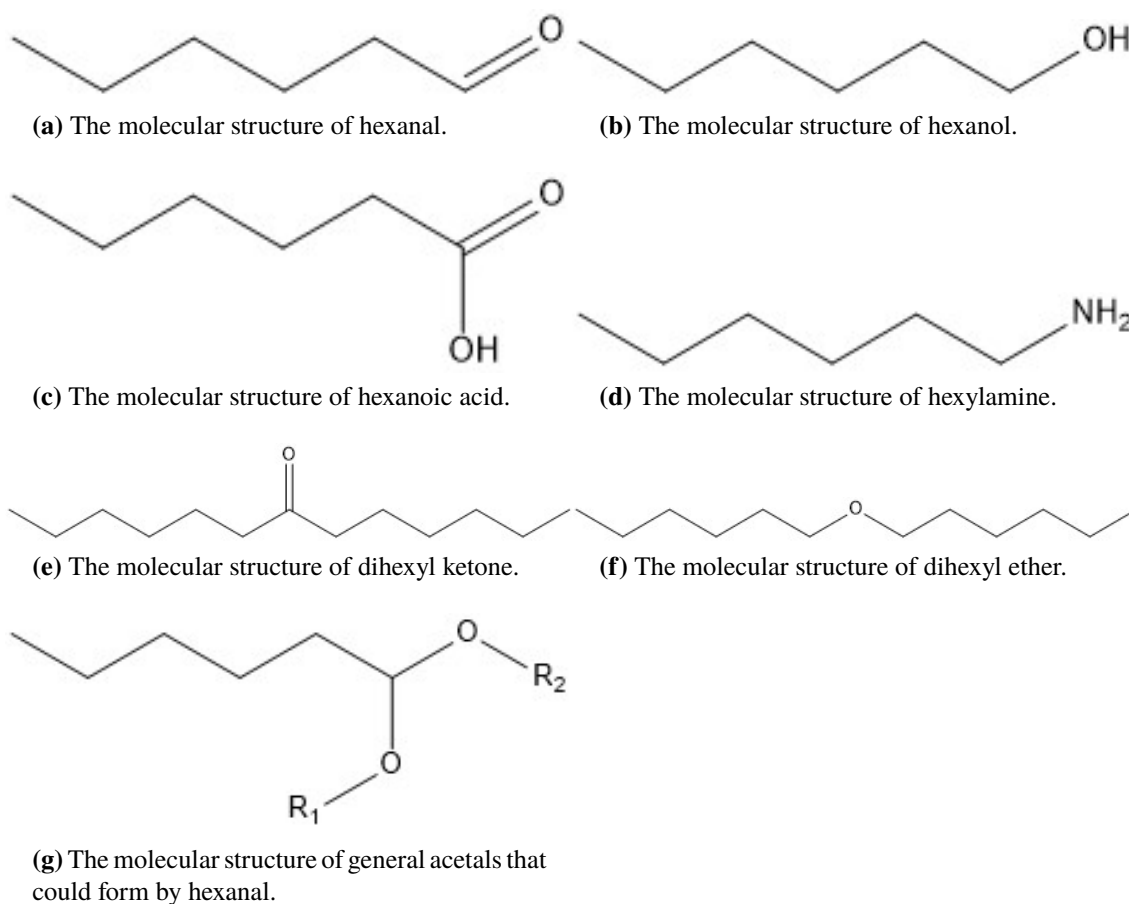


Figure 4.10: Hexanal and its possible alternative reaction products are displayed.

4. Results

5

Discussion

The screening indicated rapid reaction of linoleic acid upon addition of both inducers. Only a few minutes was needed for drastic reduction of linoleic acid in the solutions. It was noticed, however, that the linoleic acid content in the solution seemed to increase again after a certain time. This pattern is seen in all reference samples as well as in some of the accelerated samples, specifically in those involving AAPH. The increase was not so high as to reach the initial value but an increase was measured. This strange behaviour could be explained by a decrease of solvent in the solution, which would cause a higher concentration of other components. The decrease of solvent could be due to evaporation during the reactions and extraction of samples to be analysed. If this theory is correct, the increase of linoleic acid after a certain point in the accelerated samples indicates that the reduction of solvent becomes faster than the rate of the oxidation reaction. Another theory is that the AAPH, somehow forms complexes with the linoleic acid that is dissolved when the sample is taken off the heat. This would not, however, explain the increase of linoleic acid in the reference samples.

When applying the conditions of the screening to hexanal tracing experiments it seemed that the reaction times found in the screening was not valid. At least 45 minutes was needed in the case of $\text{Fe}^{2+} / \text{Fe}^{3+}$ acceleration and 2 h for the AAPH, as seen in Table 4.2. These reaction times are significantly longer than initially assumed leading to several negative outcomes before approaching the reaction times necessary. A change of solvents from acetone to a mix of water and ethanol could have had a big impact on the oxidation and reaction times. However, this was dismissed since experiments with acetone was conducted in the tracing to rule out that factor. Acetone was used in the screening because of the high solubility of linoleic acid and because the non-polar column of the DANI gas chromatograph was not compatible with water, making an alternative solvent necessary. Acetic acid was used in the screening as acid and this too could have affected the results, if it interacted with the inducers or the linoleic acid.

The solubility of FeSO_4 was increased in the tracing experiments, due to the change of solvent, leading to the formation of a more homogeneous solution. In the screening process, FeSO_4 did not dissolve properly in the acetone, which made the reaction dependent on the surface area of the particles in suspension. This is also the case in the samples with significantly higher reactant concentrations in the tracing experiments. Because of the large amount powder, the solution became saturated and this is probably a cause of some variations in the results.

Another pattern noticed is that the amount hexanal measured in the samples peak at a

certain time and then decreases. This is seen in both reference samples and in the sample induced with iron ions. In the standardised method of measuring hexanal in pulp this was expected since hexanal is formed over time and the volatile compounds will transfer to the atmosphere. When heating, the hexanal left in the sample is released and the amount will decrease over time. The reason for the hexanal reduction in samples stored in gastight vials, however, is hard to explain. The strongest theory is that the hexanal produced, being relatively reactive, have formed other products. The production of hexanal seems to be relatively rapid initially and over time, the hexanal reacts with itself or other compounds formed. The more compounds formed in the headspace and on the surface of the pulp and linoleic solution, the faster the reaction of hexanal to an alternative product will be. When passing the peak, the formation of hexanal is slower than the reaction rates of the alternative reactions, causing the amount of hexanal in the headspace to decrease. The same theory is applicable to the AAPH induced linoleic acid solution samples. Hexanal was found at reaction times of 2 h but not at 10 h. The AAPH could have been so effective in producing radicals that the produced hexanal got involved in radical reactions, causing it to completely convert into alternative products over the 8 h between the two measurements. In Table 4.5, in the Results section, possible hexanal products are listed. Some of them were found when analysing the chromatograms with the software, XCalibur. It seemed like these compounds increased over time, both in amount and in number, making the assumption quite credible.

The iron oxidation inducer was able to accelerate the production of hexanal, in comparison to the reference sample, as seen in Figures 4.8 and 4.9. The peak of hexanal produced occurred at 24 h for the sample induced with iron ions and in the reference the peak occurred around 7 days. Giving a substantial increase of rate of oxidation. The value at the peak of the reference sample is close to but not as high as in the accelerated sample. This could be explained by the peak not being at exactly 7 days, but might be higher at 6 or 8 days instead. It could also mean that the inducer oxidises compounds that would not naturally undergo oxidation. Releasing more hexanal to the headspace than would be found in the reference sample.

The reference samples of pulp have not been used as intended because the samples of CTMP induced with oxidation inducers have not been successful. Furthermore, it would be hard to analyse them because of the hexanal peak and decreasing amount over time. The total amount hexanal formed during a time interval of 40 days is, because of this, still unknown. The reason for the failure to accelerate hexanal production in CTMP over the chosen time interval of 20 days might be because the amount hexanal produced was too small for the mass spectrometer to recognise. Only a small amount CTMP was present in each vial, due to limitations in the vials' volume, and this would result in very small concentrations produced hexanal. It is unlikely, but possible, because the machine have potential of tracing very low quantities of trace molecules. CTMP, even without oxidation inducers, should produce some hexanal over the time interval making it hard to believe that no hexanal at all was formed. The factor that might affect the natural oxidation is the water added to obtain as good mixing of inducers. It might be possible that the hexanal did not transfer to the headspace but stayed in the water, further experiments are necessary to establish this. Furthermore, formation of a homogeneous solution of water, CTMP and

inducers is necessary for reliable results, something that was not completely achieved in this study. The amount CTMP in each vial was very small and introduction of more water would mean easier access for the inducers to interact with the fatty acids in the pulp but it would also mean that an even lower amount CTMP would be fitted in each vial. A method for better mixing of inducers to the pulp would be desired.

Much of the fatty acids in the pulp is bound to glycerides and not present in their free form, this makes the reaction more complicated. It has to be examined if the oxidation inducers are valid for pulp and not only linoleic acid solutions. Optimal conditions for such experiments has to be developed. It is important to determine if the accelerating methods are valid for all kinds of pulp and if the kinetics of the reactions are the same in all examples. It is possible that the reactions is affected by and will vary too much with variations in composition of the pulp, that they are not directly applicable to all types. It is desired for the reaction to take place in a similar fashion as the natural oxidation.

In further experiments the AAPH method should be analysed in the interval 2 - 10 h to track the development of hexanal and localise the peak of hexanal produced. Better results in the hexanal tracing when using iron as oxidation inducer would probably have been obtained if the ratio 2:1 Fe^{2+} to linoleic acid had been used. This is the ratio providing the best results in the screening but unfortunately it was not the ratio used in the experiment. A combination of the two oxidation inducers should be examined as well, since they might give positive results. Such experiments have been conducted in studies by Pozdeeva et al. [22]. Ratio of AAPH to iron ions would have to be examined but the same conditions used in this study is expected to work. Further method development is necessary in the case of mixing oxidation inducers with pulp. A homogenous mixture is required to enable access of the inducers to the fatty acids. A adequate amount pulp need to be introduced to the gastight vials in order to measure the hexanal formed. If the reaction would not have to be conducted in gastight vials with analysis of the headspace, the problems with low amounts hexanal in the headspace and the suspected production of alternative reaction products, could be avoided. It might be possible to perform analysis of the reactive oxidation products formed during the oxidation processes instead of analysing a trace compound, in this case hexanal. It would not be possible to focus on the oxidation of one compound but the amount of produced reactive oxygens would indicate the oxidation of all lipids in the pulp. The analysis would be performed on the solution where the reaction takes place and this might make analysis more accurate and easier.

Experiments with UV light irradiated CTMP was performed as well. No results are presented from these experiments, because of their inconsistency, but the chromatograms are to be seen in Appendix E. The chromatograms show the content linoleic acid in UV irradiated samples in comparison with reference samples. No conclusions can be drawn from these experiments, it did however seem like the UV light affected the pulp in some way, because the irradiated part obtained a darker colour. If further experiments were to be conducted a way of exposing all pulp to equal amount UV irradiation need to be found.

Both iron compounds, FeSO_4 and FeCl_3 are relatively cheap and easily accessible [23, 24]. They have a low environmental impact and are relatively harmless. This makes them suit-

5. Discussion

able for usage in industry and compatible with pulp and paper processes. The AAPH is flammable at elevated temperatures and is not as cheap [13]. It is hazardous for aqueous environments and need to be used with caution. In summary, the iron ions are preferable to use in the industry for economical and environmental reasons.

6

Conclusion

To conclude the discussion and results obtained one can state that chemical oxidation inducers can be used to accelerate oxidation of linoleic acid and yield the trace compound hexanal. It can be done with relatively cheap and environmentally viable chemicals and in close to ambient conditions which are applicable to the current pulp manufacturing process. The mechanisms of the acceleration is not completely known in all its details but in general AAPH produces radicals by thermal decomposition which initiates the formation of free lipid radicals. The iron ions either lowers the activation energy for abstraction of hydrogen from the fatty acids which initiate formation of radicals or reacts directly with the fatty acid or by formation of single state oxygen. There might be other alternatives for chemical oxidation inducers, such as a combination of AAPH and iron ions or other transition metals.

In the CTMP reference samples, the results displaying the hexanal formation in the headspace, are interesting to compare to the accelerated CTMP samples because they give an indication of how the curve should look like when hexanal is produced. However, the accelerated CTMP samples did not present any results showing hexanal in the headspace, making the comparison impossible.

Due to further reactions and decomposition of hexanal the total amount produced is hard to calculate. When performing the linoleic acid solution experiments the references displayed a peak of amount hexanal at 7 days and in the accelerated sample the peak was seen after only 24 h. Giving a substantial increase in rate of oxidation in the sample. The AAPH samples do accelerate the oxidation process but it is unclear to what extent. It seems like the process is very rapid in the conditions examined in this study and further experiments are necessary. The hexanal was encountered at 2 h reaction time but not at 10 h. The peaks of amount hexanal in the gastight vials are thought to be because of formation of alternative products through reactions of the produced hexanal. This theory is applicable to all samples where hexanal was found in the headspace.

In general, it can be stated that the method developed need adjustments and further experiments are required. The specific areas that need developing is a method of examining the total amount hexanal formed in the samples, a way of mixing the inducers in pulp and a way of analysis that works even though the amount pulp possible to fit in vials is quite small. Further experiments conducted with AAPH is desired, the method probably works but it is unclear to what extent. If these adjustments are performed the methods would probably be applicable in industry and the chemicals used are appropriate in such applications, both in an environmental and economical perspective.

6. Conclusion

There might be better ways of testing the accelerators. Measurement of reactive oxygen species generated in the material during oxidation, might be possible to measure instead of a trace compound, in this case hexanal. If so, the reactions would not have to be conducted in gastight vials, because analysis would be performed on the solution where the reaction takes place, which would make the whole procedure easier.

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A

CTMP reference samples

Reference samples was performed according to two methods. These are referred to as the standardised method and the closed vial method.

A.1 The standardised method

1. CTMP was taken from the freezer, weighed and transferred to a petri dish.
2. The dish is placed in a climate controlled room. Temperature is 23 °C and humidity is 50 %.
3. Samples of 2 g are obtained at 6 occasions. After 1, 6, 12, 22, 35 and 40 days.
4. At the time of analysis the sample are weighed and transferred to gastight vials.
5. The vials are heated in an oven for 40 minutes at 90 °C.
6. Doublets of each sample are analysed with headspace SPME-GC-MS.

A.2 The closed vial method

1. CTMP was taken from the freezer and weighed.
2. Samples of 1 and 2 g are put in gastight vials and transferred to a climate controlled room. Temperature is °C and humidity is 50 %.
3. Vials are collected after 1, 6, 12, 22, 35 and 40 days.
4. Doublets of each sample is analysed with headspace SPME-GC-MS.

B

Microwave extraction of pulp

Extraction of pulp enables analysis of the lipid content.

1. Pulp is weighed to 0.8 g and dried in an oven at $T = 40\text{ }^{\circ}\text{C}$ for about 4 h.
2. The dried pulp sample is put into a container together with a stir bar and a weflon button.
3. Internal standard, $V = 50\ \mu\text{l}$, is added to the container. The internal standard is chosen depending on the compound analysed, C17 (heptadecanoic acid) for linoleic acid.
4. Add 25 ml acetone to the container.
5. The container is put into the microwave oven and heated to $T = 120\text{ }^{\circ}\text{C}$ for 55 minutes.
6. When the solution is cooled to below $40\text{ }^{\circ}\text{C}$ the extract is transferred to a 30 ml vial and put into a vacuum evaporator, MiVac, to evaporate excess solvent. The program is set to $23\text{ }^{\circ}\text{C}$ for 12 minutes which provides a solution with $V = 3\text{ ml}$.
7. $200\ \mu\text{l}$ of the extract is transferred into a vial and $50\ \mu\text{l}$ of the silica containing reagent BSTFA is added.
8. The vial is heated for 1h at $T = 70\text{ }^{\circ}\text{C}$.
9. $100\ \mu\text{l}$ of the extract is added to another vial and $50\ \mu\text{l}$ of the methylating reagent TMSH is added to the sample. The reaction proceeds instantly without further preparation.
10. The samples are analysed with GC-FID.

C

Screening of oxidation inducers

The screening consisted of experiments with variations in reaction times and concentrations.

C.1 Varying concentrations of oxidation inducer added to linoleic acid solution

1. A stock solution is prepared by mixing internal standard, heptadecanoic acid, 6 mg per sample, with acetone and linoleic acid, 6.667 μl per sample, and in the case of AAPH, about 4 ml acetic acid was added until the pH was around 2.
2. The granular heptadecanoic acid require heating and stirring in order to dissolve.
3. Reference samples were taken from the stock solutions and analysed to get an initial value.
4. 100 μl TMSH is added as methylating agent and is used in all samples.
5. AAPH, FeSO_4 and FeCl_3 is weighed according to table C.1.
6. The samples are put in water baths of 40 °C under vigorous stirring and GC analysis are performed on all AAPH samples at 30 and 60 min and iron ion samples at 60 min.

Table C.1: Ratios and amount of inducers added per sample. LA is linoleic acid.

Sample ratio LA:AAPH	AAPH (g)	Sample ratio LA: FeSO_4	FeSO_4 (g)	FeCl_3 (g)
2:1	0.0018	1:1	0.0195	0.0625
1:1	0.0035	1:2	0.0390	0.1249
1:2	0.0070	1:3	0.0585	0.1875
1:3	0.0105	1:5	0.0975	0.3125
1:5	0.0175	1:8	0.1561	0.5000

C.2 Varying reaction times of oxidation inducer in linoleic acid solution

The concentrations displaying the most satisfactory results were analysed at different reaction times. The iron ions oxidation and AAPH oxidation is conducted in similar ways and described below.

1. Internal standard (heptadecanoic acid) was weighed, 6 mg per sample, and transferred to a beaker.

C. Screening of oxidation inducers

2. Acetone was added to the beaker, 10 ml per sample, making the stock solution.
3. A stir bar was added to the beaker and the heptadeconic acid flings were diluted by rigorous stirring.
4. Linoleic acid, 6.667 μl per sample, was added
5. The AAPH stock solution consisted of 4 ml acetic acid and 10 ml acetone and was prepared in the same way, pH is around 2.
6. An additional sample of AAPH with stock solution without acetic acid is prepared as well.
7. 100 μl of the stock solutions were placed in vials and run as initial reference sample.
8. The different amounts of Fe_2SO_4 , FeCl_3 and AAPH was weighed, see table C.2.
9. Stock solution and inducers are transferred to 30 ml vials containing a stir bar.
10. The vials are placed in water baths at 40 °C and rigorous stirring.
11. Samples were taken at 5, 10, 20, 30 and 60 minutes for iron ions. In vial 3 additional samples were taken after 3 and 4,5 h as well.
12. Samples were taken at 5, 30, 120 and 200 minutes for AAPH
13. Reference samples were taken at the end of the experiment as well, about 5 h.

Table C.2: The amount oxidation inducer added to the different samples. LA is linoleic acid.

Sample ratio LA:AAPH	AAPH (g)	Sample ratio LA: FeSO_4	FeSO_4 (g)	FeCl_3 (g)
2:1	0.0018	1:1	0.0195	0.0625
1:1	0.0035	1:2	0.0390	0.1249
1:2	0.0070	1:3	0.0585	0.1875
1:3	0.0105	1:5	0.0160	0.3125
1:5	0.0175	1:8	0.0256	0.0832
No acetic acid; 1:1	0.0035			

D

Tracing of hexanal

D.1 General description of oxidation inducers added to linoleic acid solution

The experiments conducted are based upon the screening performed. The ratio chosen and reaction times used are the ones showing most promise in earlier attempts.

1. 6.92 μl linoleic acid per sample is added to a solution 5 ml ethanol.
2. When diluted 5 ml milliQ water is added while stirring.
3. If the samples are conducted with AAPH hydrochloric acid is added dropwise til a pH of 0.7.
4. The solution is transferred to vials, each containing a stir bar.
5. Varying amounts AAPH, FeSO_4 or FeCl_3 is added to the vials.
6. The vials were placed in an oil bath for their chosen reaction times.
7. The reactions are slowed down by cooling in ice baths before analysis with headspace SPME-GC-MS.

The different ratios and reaction times tested are displayed in Results, Table 4.2.

D.1.1 Acetone used as solvent

Since acetone was used as solvent in the screening process, a set of experiments were conducted to rule out the possibility that either acetone or the ethanol and milliQ water solution affects the results. The experiments were conducted in the same way as before and with the ratios 1:2 Linoleic Acid : FeSO_4 and 1:1 Linoleic acid : AAPH. The reaction times used were 30 min and 2 h and the solvent did not seem to affect the results.

D.2 Increase of concentrations of reactants

Samples of the same ratios but performed with a 10 times higher concentrations are analysed. The interesting results led to more experiments conducted with even higher concentrations.

1. Fe samples are of ratio 1:2 (Linoleic acid : Fe^{2+}) and AAPH are of ratio 1:1 are performed at a first experiment
2. A stock solution is made in the same way as before, by mixing of linoleic acid, 66.7 μl , ethanol and milliQ water.
3. HCl (aq) is added to the samples indented for AAPH addition, pH is around 1.

4. Oxidation inducers are weighed and added to vials containing the stock solution, $m_{Fe^{2+}}=0.098$ g, $m_{Fe^{3+}}=0.325$ g, $m_{AAPH}=0.0349$ g.
5. All samples are transferred to a oil bath keeping the temperature 40 °C.
6. The reaction is cooled down in an ice bath and analysed with headspace SPME-GC-MS.
7. Reaction times used here are 15, 45 and 120 min.

Additional samples of linoleic acid in 0.1 vol% is performed and executed in the same way but with longer reaction times of 10 h and 1, 3, 6, 12 and 20 days. The stock solution consists of 5 ml ethanol, 5 ml milliQ water and linoleic acid. The volume linoleic acid is calculated with the perception that the linoleic acid has a purity of 60 %. Ratios are 1:1, AAPH : Linoleic acid and 1:2, FeSO₄ : Linoleic acid.

D.3 Accelerated samples of CTMP

The oxidation inducers were added to the CTMP in two sets of experiments, the execution was very similar and the general description is written below. The pulp was solved in water to obtain a dry content of 4 %. The CTMP has a dryness of 90 % initially, when dissolving to a 4 % dryness the solution is thick and it is hard to obtain a homogeneous dispersion of the oxidation inducers in the pulp. Dissolving in more water would make it easier to blend the inducers but a very small amount CTMP would fit into each vial.

1. 0.5 g CTMP is dissolved in 11.02 ml milliQ water in a vial.
2. Usually about 0.1 % of the pulp is linoelic acid and the ratios would be determined according to this. However, here 1 % inducer is added. The higher amount is due to the challenge to obtain a homogeneous mix if very small amount inducers are used.
3. The inducers are weighed and added to the vials, $m_{AAPH} = 0.005$ g, $m_{FeSO_4} = 0.005$ g, $m_{FeCl_3} = 0.016$ g.
4. HCl is added to the samples containing AAPH to obtain pH around 1.7.
5. The vials are stirred with spatulas before transferred to a a heated oil bath of 40 °. Samples are analysed after 1, 3, 6 and 10 h and after 1, 3, 6, 12 and 20 days.

E

Experiments conducted with UV irradiation

The effect of UV irradiation on CTMP was evaluated.

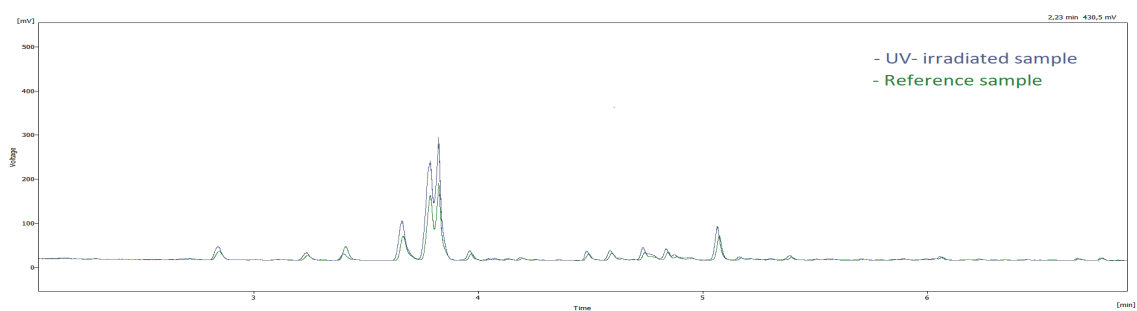
1. CTMP was weighed and spread onto petri dishes.
2. Half of the dishes was placed below a UV- lamp of $\lambda = 254$ nm and the other half out of the UV light.
3. The temperature was 23 °C and humidity 50 %.
4. Extraction was performed on 0.7 g samples from both the UV irradiated and the reference CTMP at 1, 5, 7 and 13 days.
5. The amount linoleic acid still present in the CTMP was evaluated.

In Figures E.1 - E.4 chromatograms obtained through analysis with extraction is shown. The reagents TMSH and BSTFA are used, providing information on the total amount fatty acid in the sample respective the amount free fatty acids. The linoleic acid, which is the compound of interest in this analysis, has its peaks at retention time 4.84 min in samples reacted with BSTFA and 3.79 in TMSH treated samples, this is clarified in Table E.1.

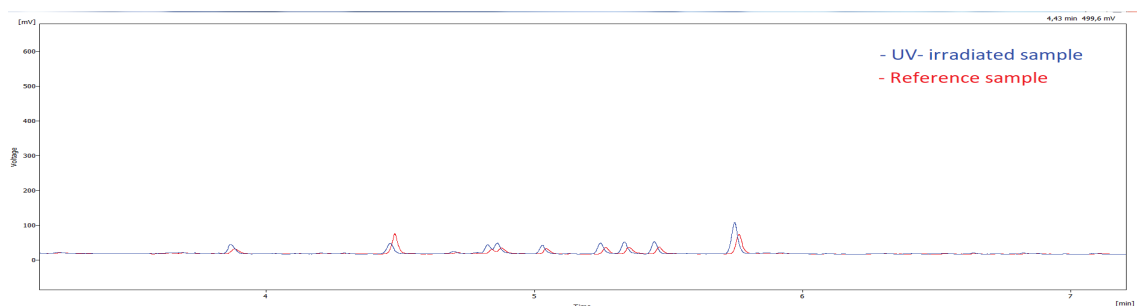
Table E.1: Retention times for linoleic acid.

Reagent	Retention time [min]
TMSH	3.79
BSTFA	4.68

E. Experiments conducted with UV irradiation

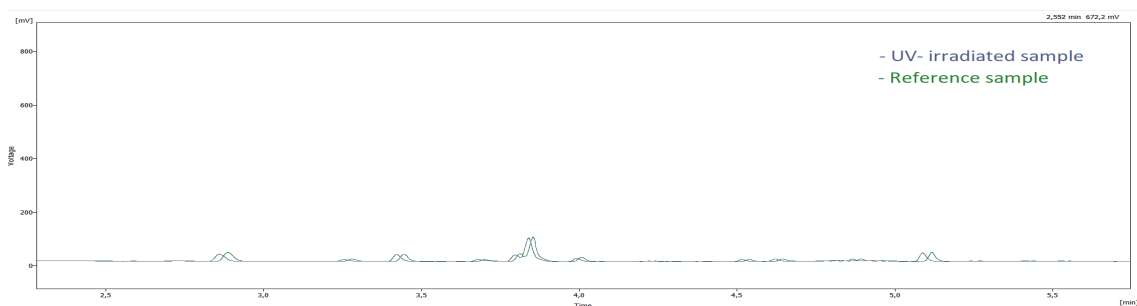


(a) Total amount fatty acids in the samples are analysed using reagent TMSH.

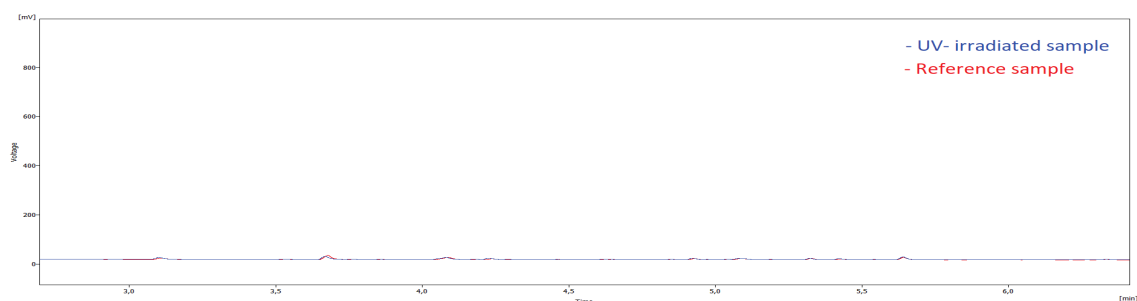


(b) Free fatty acids in the samples are analysed using the reagent BSTFA.

Figure E.1: Chromatogram from analysis of UV- irradiated and reference samples after 5 days.



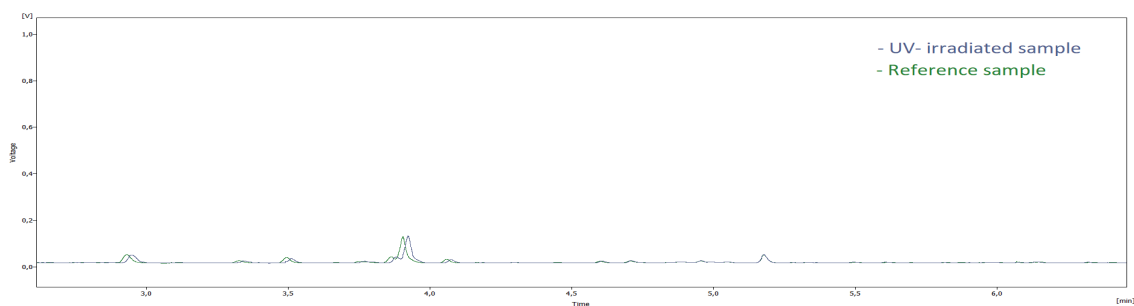
(a) Total amount fatty acids in the samples are analysed using reagent TMSH.



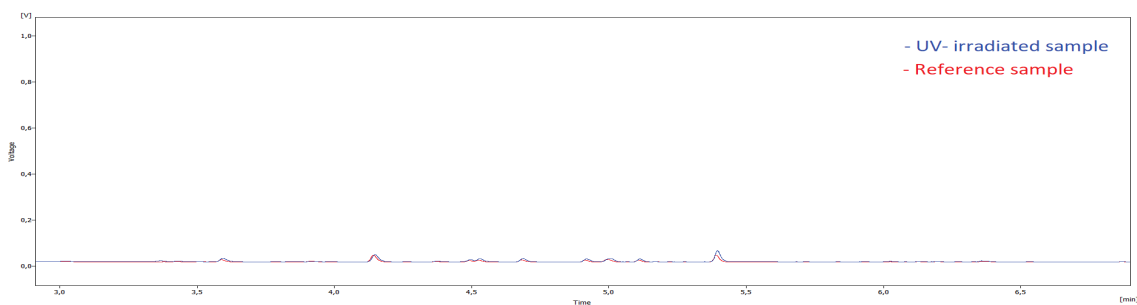
(b) Free fatty acids in the samples are analysed using the reagent BSTFA.

Figure E.2: Chromatogram from analysis of UV- irradiated and reference samples after 5 days.

E. Experiments conducted with UV irradiation

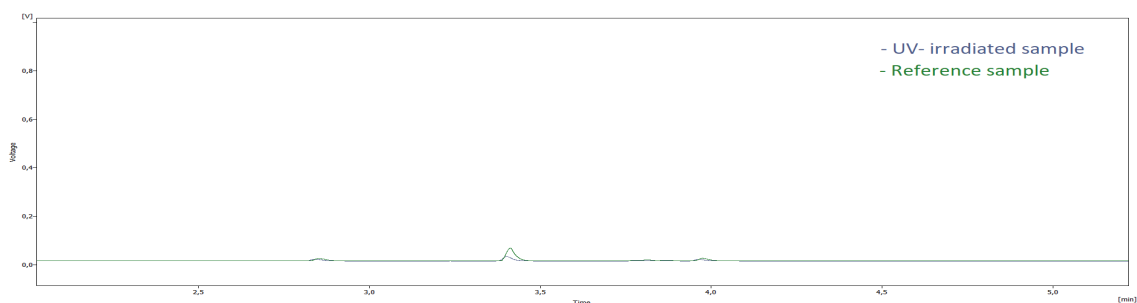


(a) Total amount fatty acids in the samples are analysed using reagent TMSH.

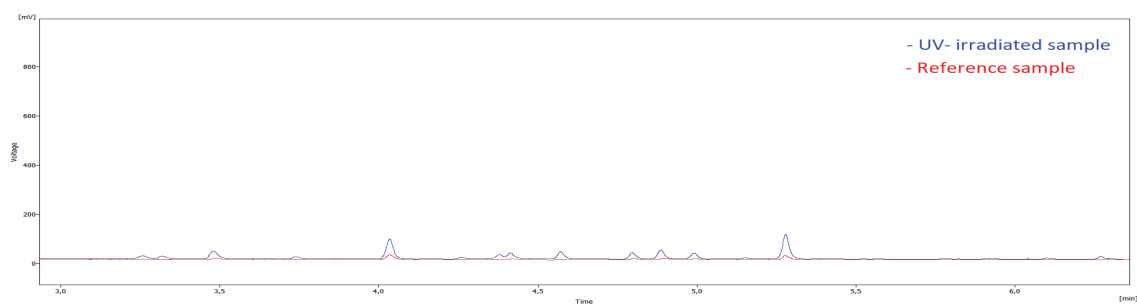


(b) Free fatty acids in the samples are analysed using the reagent BSTFA.

Figure E.3: Chromatogram from analysis of UV- irradiated and reference samples after 7 days.



(a) Total amount fatty acids in the samples are analysed using reagent TMSH.



(b) Free fatty acids in the samples are analysed using the reagent BSTFA.

Figure E.4: Chromatogram from analysis of UV- irradiated and reference samples after 7 days.

F

Chromatograms obtained through extraction CTMP

Extraction and GC- analysis of the CTMP used in the study resulted in the chromatogram shown below. Both methylating (TMSH) and silylating (BSTFA) reagents have been used in the analysis, making all fatty acids respective free fatty acids visible in the chromatogram seen in Figure F.2. In Tables F.1 and F.2 lists of compounds and their retention times are listed for both silylated and methylated samples.

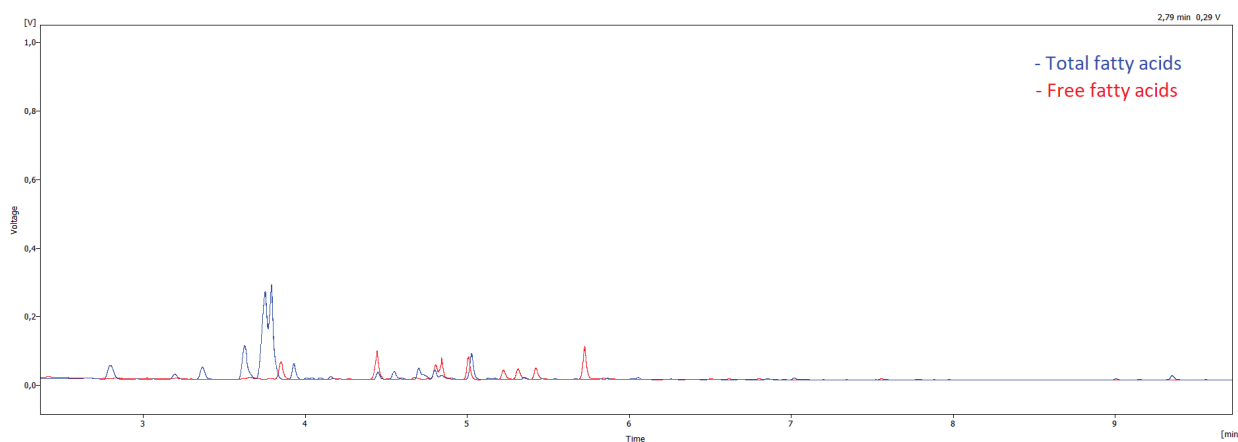


Figure F.1: Chromatogram displaying the extractives of the CTMP used as raw material in the study. Both free fatty acids and total amount fatty acids are displayed using reagents BSTFA respective TMSH.

F. Chromatograms obtained through extraction CTMP

Table F.1: The contents of the CTMP used in the study. The reagent, TMSH, used provides information of the total amount of fatty acids present in the pulp.

Retention time [min]	Compound	Group
2.80	Hexadecanoic acid	Saturated fatty acid
3.20	Heptadecanoic acid	Saturated fatty acid
3.37	Internal standard	Saturated fatty acid
3.63	Linolenic acid	Triunsaturated fatty acid
3.79	Oleic acid	Unsaturated fatty acid
3.79	Linoleic acid	Diunsaturated fatty acid
3.93	Stearic acid	Saturated fatty acid
4.45	Isopimaric acis	Resin acid
4.55	Pimaric acid	Resin acid
4.70	Resin acid	Resin acid
4.80	Resin acid	Resin acid
5.03	Dehydroabietic acid	Resin acid
9.36	Stigmastanol	Phytosterol

Table F.2: The contents of the CTMP used in the study. The reagent BSTFA used provides information of the amount free fatty acids in the pulp.

Retention time [min]	Compound	Group
3.85	Palmitic acid	Saturated fatty acid
4.27	Heptadecanoic acid	Saturated fatty acid
4.44	Internal standard	Saturated fatty acid
4.68	Linolenic acid	Triunsaturated fatty acid
4.84	Oleic acid	Unsaturated fatty acid
4.84	Linoleic acid	Diunsaturated fatty acid
5.01	Stearic acid	Saturated fatty acid
5.23	Isopimaric acis	Resin acid
5.31	Pimaric acid	Resin acid
5.42	Palustric acid	Resin acid
5.73	Dehydroabietic acid	Resin acid
9.90	Sitostanol	Phytosterol