

ABSTRACT

ADAMOPOULOS, LAMBRINI. *Understanding the Formation of Sugar Fatty Acid Esters.* (Under the direction of Dimitris S. Argyropoulos (chair) and John A. Heitmann (co-chair).)

This study aims at elucidating a variety of salient features that dictate the kinetics and chain length effects governing the formation and antimicrobial activity of sugar esters of fatty acids. To do this, anomerically pure glucose, sucrose and cellobiose sugars were transesterified with the methyl esters of fatty acids of variable chain lengths C_4 , C_8 , C_{12} , C_{16} , and C_{20} . The methyl esters of butyric, caprylic, lauric, stearic and arachidic acids were reacted with the above carbohydrates to give the respective penta and octaesters. The kinetics of these transesterification reactions were followed by quantitative ^{31}P NMR after phosphylation of the labile OH groups with 1,3,2-dioxaphospholanyl chloride. This approach proved to be a facile and quantitative means to follow the specific substitutions occurring at the various OH positions within the sugars as a function of degree of conversion, and incoming chain length. As anticipated, a variety of steric and hydrophobic effects were shown to play a key role in determining the reactivity of these systems. The various sugar esters were then adsorbed onto cellulose disks and their microbial activity was examined. Finally, cellulose esters of butyric acid were synthesized using the acyl chloride process.

Understanding the Formation of Sugar Fatty Acid Esters

by

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Her work in the Forest Biomaterials Laboratory deals with “*Stereoselectivity, Kinetics and Chain Length Effects Governing the Formation and Antimicrobial Activity of Sugar Esters of Fatty Acids*”. Her research interests include using biomass as a feedstock for chemicals, materials and energy, as well producing value added products from lignocellulosics.

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1. Introduction

Growing interest in the use of biomass as a feedstock for the development of a carbohydrate based economy, as an alternative to fossil resources, has put the spotlight on carbohydrates.

Carbohydrates are the most abundant organic compounds on the planet. They constitute a suitable replacement for fossil fuels since they contain a considerable amount of carbon and hydrogen. They are a renewable natural resource which is widespread and inexpensive, and from which a wealth of bulk and fine chemicals can be produced.

Nevertheless, in order to better utilize carbohydrates as raw materials, it is essential to understand their complex chemistry. They are polyfunctional compounds, with multiple hydroxyl groups, which are sensitive to heat/ acidic or basic conditions.

This multifunctionality allows for many products to be obtained from even the simplest reaction. Different degrees of substitution result in different physicochemical properties, which in turn will be vital to different applications.

Understanding the regioselectivity of carbohydrates is very important when using these to form interesting derivatives. This study aims at elucidating some of this complex chemistry while attempting to synthesize fatty acid esters of glucose, sucrose, cellobiose and cellulose.

Such derivatives have the potential to give value-added products and highly versatile materials with interesting characteristics. These compounds, derived from natural oils and sugars, are used as surfactants in the food and cosmetic industries, as insecticides and antimicrobial agents, and even as non-caloric fat substitutes.

This wide range of applications is made possible by the variety of available sugars and fatty acids. In fact, the properties of these esters are intimately related to the structure. As a result, in order to understand the structure/function relationship, it is important to take a step back and first examine their basic chemistry.

2. Literature Review

2.1 Carbohydrates

Carbohydrates are the most widely distributed, naturally occurring compounds on Earth. They are the product of photosynthesis, storing light energy in the form of chemical energy. Their general formula is $C_n(H_2O)_n$. They are polyhydroxy aldehydes or ketones which can be reduced to give sugar alcohols, oxidized to give sugar acids, substituted at one or more of the hydroxyl groups to give other compounds or derivatized at the hydroxyl groups. Thus, they are polyfunctional compounds, with multiple hydroxyl groups, which are sensitive to heat, acidic or basic conditions.

This polyfunctionality allows for many products to be obtained from even the simplest reaction. Different degrees of substitution result in different physicochemical properties, which in turn will be vital to different applications. In order to control the degree of substitution, the regioselectivity of the sugar should be well understood. Depending on the reaction conditions and catalysts, the transformation can be oriented towards many different positions.

2.1.1 Glucose

Glucose is a carbohydrate found in many materials. It is present in combined forms in cellulose, starch, sucrose, lactose. It may also be found in the free state in materials such as honey or grapes. In the body, the cells use it as a source of energy and metabolite intermediate.

Glucose is a monosaccharide. It contains six carbon atoms and an aldehyde group. It can exist in an open-chain (acyclic) and ring (cyclic) form, the latter being the result of an intramolecular reaction between the aldehyde carbon atom and the C-5 hydroxyl group to form an intramolecular hemiacetal. In an aqueous solution, both forms are in equilibrium, and at pH 7 the cyclic one is the predominant. The ring contains 5 carbon atoms and one oxygen atom, and resembles the structure of pyran. In this ring, each carbon is linked to hydroxyl side group with the exception of the fifth atom, which links to a sixth carbon atom outside the ring, forming a CH₂OH group.

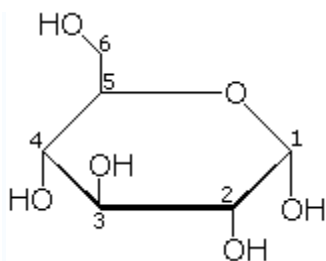


Figure 1: α - Glucose

Glucose has 4 chiral centers. In theory, glucose may have 15 optical stereoisomers. Only seven of them are found in living organisms. The most important among these are galactose and mannose.

When glucose is in its ring form, an additional asymmetric carbon, the anomeric carbon atom, is created at C1. This leads to the formation of two ring structures, the anomers α -Glucose and β -Glucose. In the α form, the hydroxyl group attached to C-1 is below the plane of the ring, in the β form it is above. The α and β forms interconvert over a timescale of hours in aqueous solution, to a final stable ratio of α : β 36:64, in a process called mutarotation (Figure 2).

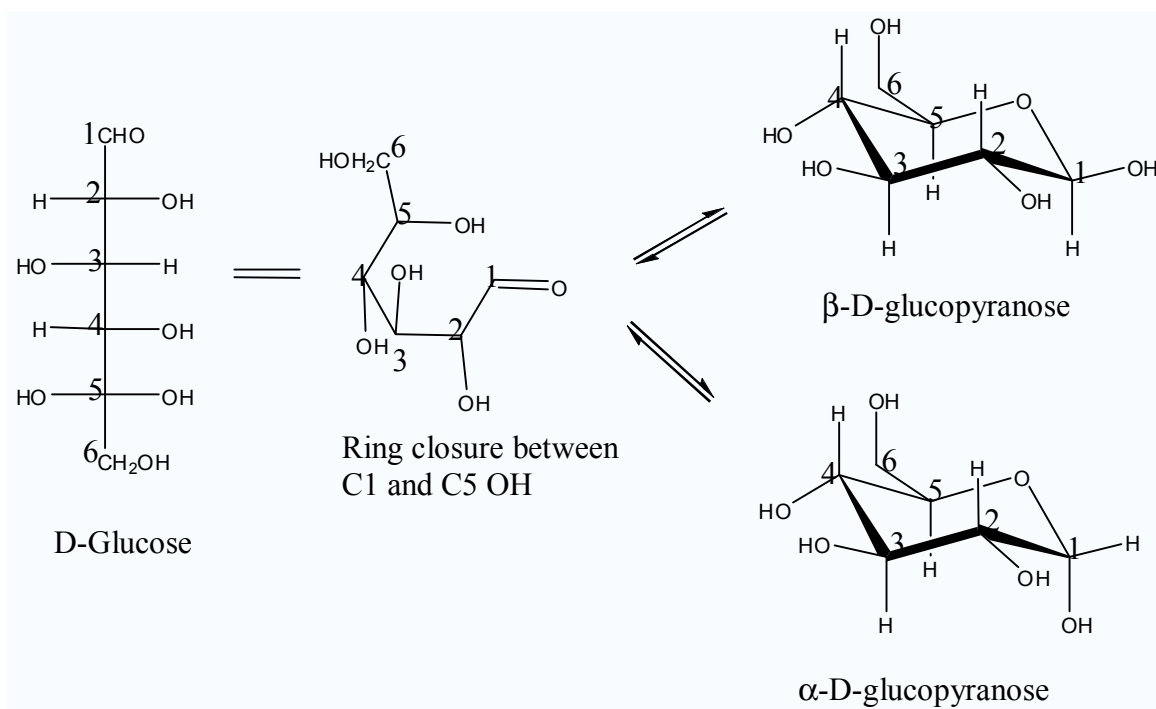


Figure 2: D-glucose ring closure and mutarotation

2.1.2 Sucrose

Sucrose ($C_{12}H_{22}O_{11}$) is a naturally occurring carbohydrate, found in sugar cane, sugar beet and many other plants. It also occurs in honey and in the sap of maple trees. Sucrose is a disaccharide of α - glucose and fructose. These two moieties are connected at their anomeric carbon atoms. It does not contain a hemi-acetal linkage and so, it is a non-reducing sugar. Its chemistry is then limited to its eight hydroxyl groups, of which three are primary (C1, C6' and C6) and five are secondary.

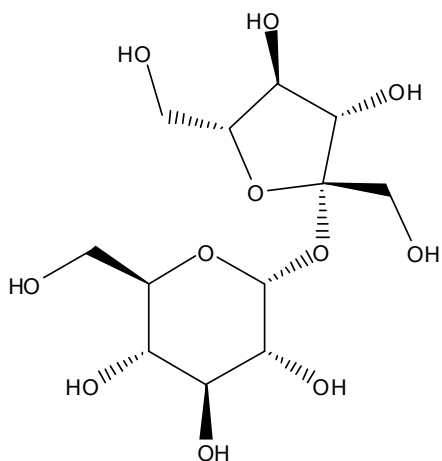


Figure 3: Sucrose

2.1.3 Cellobiose

Cellobiose ($C_{12}H_{22}O_{11}$) is the basic repeat unit of cellulose, the major structural component of plant cell walls. It is a disaccharide, formed by two glucose moieties in β -(1,4) linkage. The bonding between the glucopyranose rings in cellobiose is from the anomeric carbon in ring A to the C-4 hydroxyl group on ring B. This leaves the anomeric carbon in ring B free, so cellobiose may assume alpha and beta anomers at that site. Thus, cellobiose is a reducing sugar. Furthermore, it presents eight hydroxyl groups which may also react.

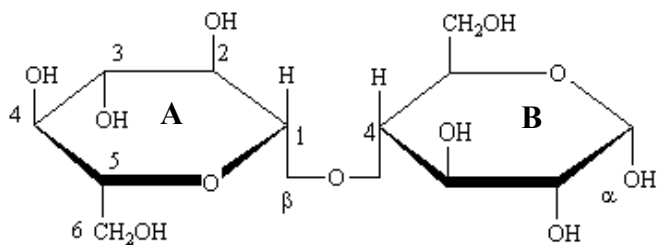


Figure 4: Cellobiose

2.2 Fatty Acids and their Methyl Esters

2.2.1 Fatty acids

Fatty acids are carboxylic acids, often with long aliphatic tail that may be either saturated or unsaturated. They are found in animal and vegetable fats and oils and have the general formula $C_nH_{2n+1}COOH$. Saturated fatty acids do not contain any double bonds or other functional groups along the chain. In other words, the omega (ω) end contains 3 hydrogens (CH_3-) and each carbon within the chain contains two hydrogens ($-CH_2-$). Saturated fatty acids form straight chains and, as a result, can be packed together very tightly, allowing living organisms to store chemical energy very densely. The fatty tissues of animals contain large amounts of long-chain saturated fatty acids.

The saturated fatty acids used in this work are:

- Butyric: $CH_3(CH_2)_2COOH$
- Caprylic: $CH_3(CH_2)_6COOH$
- Lauric (dodecanoic acid): $CH_3(CH_2)_{10}COOH$
- Stearic (octadecanoic acid): $CH_3(CH_2)_{16}COOH$
- Arachidic (eicosanoic acid): $CH_3(CH_2)_{18}COOH$

2.2.2 Fatty Acids as Antimicrobial Agents

The antimicrobial activity of plant oils and extracts has been recognized and studied for many years^{1,2,3,4}. Kabara has set forth a number of points that generalize the antimicrobial action of fatty acids^{5,6,7}.

The action of fatty acids results from the undissociated molecules rather than their corresponding anions. Consequently, the antimicrobial activity of fatty acids is affected by the pH, since the hydrogen ion concentration determines the degree of dissociation of the acid. More rapid antimicrobial activity has been observed at lower pH, in circumstances where pH alone is not lethal. Moreover, changes in pH affect the minimum inhibitory concentrations of fatty acids. The effect varies with the length of the acid and its degree of saturation.

As a result, there seems an intimate structure- function relationship that should be considered when examining the fatty acids as antimicrobial agents. The most effective saturated, unsaturated and polyunsaturated fatty acids were found to be those with chain lengths of C₁₂, C_{16:1} (16 carbons and one degree of unsaturation) and C_{18:2} (18 carbons and two double bonds), respectively. For fatty acids longer than twelve carbons, the number and position of double bonds is more important than for smaller fatty acids. In addition, cis isomers were found to be more effective than trans isomers. Moreover, the chain length, the degree and type of unsaturation as well as the geometric isomerism are important determinants of the biocidal activity of a fatty acid.

Nevertheless, certain general characterizations concerning the effect of fatty acids can be made⁸. For instance, fatty acids with greater than eight carbons are generally ineffective against gram- negative bacteria. Some short-chain (i.e., < 8 carbon atoms) fatty acids inhibit both gram-negative and gram-positive bacteria. The C₈ and C₁₀ fatty acids are generally more active against yeasts and molds. Gram positive bacteria are more affected by slight longer chain lengths, with lauric acid (C₁₂) being the most active saturated fatty acid against most bacteria.

The effect of the unsaturation of a fatty acid is influenced by the number, position, and geometrical isomerism of the double bonds. Monounsaturated fatty acids are generally more active than saturated fatty acids, but even this depends on the chain length of the fatty acid. Optimum activity is found in palmitoleic acid (C_{16:1}), whereas short chain unsaturated fatty acids are generally less active. A second double bond increases antimicrobial activity but its place in the chain is not significant.

Furthermore, esterification of fatty acids with a monohydric alcohol, such as methanol or ethanol, reduced their activity. In contrast, esterification of fatty acids to polyhydric alcohols such as glycerol or sucrose increased their effectiveness. Also, it is important to note that monoester forms are more potent than polyester forms.

2.3 Sugar Esters of Fatty Acids

In the United States, interest in the synthesis of sugar esters of fatty acids began in 1952, when the Sugar Research Foundation saw their surfactant potential.

Sugar fatty acid esters with degrees of substitution of 1 to 3 are nonionic, digestible, absorbable, and biodegradable detergents of low toxicity. Carbohydrate fatty acid polyesters (DS 4-14) are lipophilic, nondigestible, nonabsorbable molecules.⁹

Sugar esters have a wide variety of applications. Mixtures of regioisomers, as well as mono-, di- and trimesters are used as emulsifiers, whose resulting physicochemical properties depend on the average degree of substitution and fatty acid chain length. They are used as non-ionic surfactants, bleaching boosters and food additives. Sucrose esters of fatty acid with a low degree of substitution can be used as food and cosmetic emulsifiers.

Also, sugar polyesters have applications as fat substitutes. Olestra® (Figure 5), developed by Proctor and Gamble, is a polysubstituted fatty acid ester used as a non-caloric fat. It presents similar properties to those of triglycerides but is non-digestible. As the degree of substitution increases, hydrolysis by lipolytic enzymes decreases due to steric hindrance and the type of fatty acid acids substituted¹⁰.

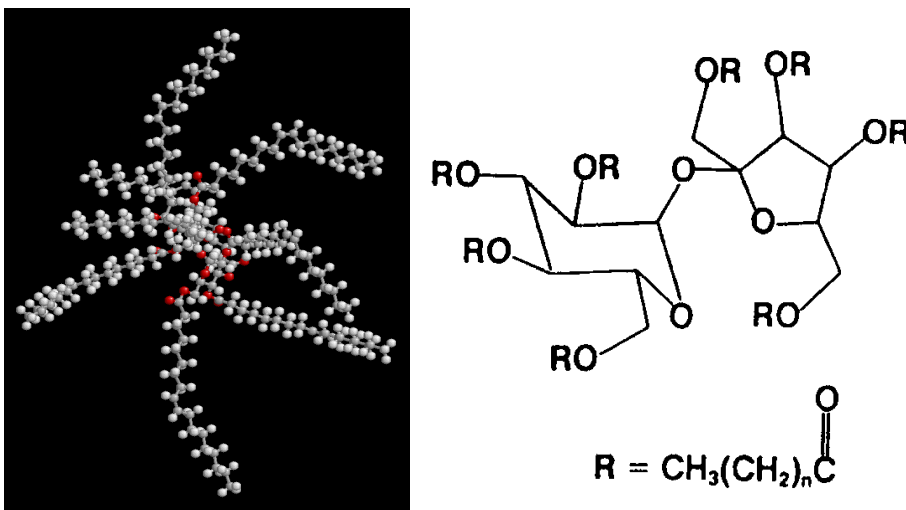


Figure 5: Olestra® - ball and chain model and chemical structure

2.3.1 Synthesis and Kinetics

– Chemical Synthesis

Carbohydrate polyesters may be synthesized by various methods and techniques. In fact, the patent literature is abundant in this rapidly growing field.

Carbohydrate polyesters can be synthesized by interesterification, but high temperatures and toxic solvents limit the use of such processes.

Polyesters of sucrose with degrees of substitution from 4-8 can be prepared using acylating agents such as acid chlorides or aryl esters¹⁶.

Feuge et al. developed a solvent free interesterification process involving molten sucrose and long chain fatty acid methyl esters¹¹. The temperatures employed are between 170°C and 187°C and catalysts such as lithium, potassium or sodium soaps are required.

Lithium palmitate mainly produced sucrose polyesters with DS greater than 3. Lower esters were best produced using combinations of lithium oleate with potassium or sodium oleate at a level of 25% based on sucrose weight. In this case vegetable oil is a solvent as well as a fatty acid source. The reaction is carried out at 110°C to 140°C with potassium carbonate as a catalyst. The product can be used without further purification.

For sugar polyesters, the interesterification involves the reaction of the alkyl ester of sucrose with fatty acid methyl esters in the presence of sodium methoxide (NaOCH_3) or sodium or potassium metal as catalyst.

Direct esterification of sugars with fatty acids is limited because it requires high temperatures. At temperatures above 185°C, sucrose caramelizes.

Akoh and Swanson synthesized sucrose polyesters by a simple ester-ester interchange reaction by a solvent-free process where sucrose octaacetate, a sodium metal catalyst and the fatty acid methyl esters of vegetable oils were mixed prior to heating¹². Twenty to thirty minutes after heat was applied, a one phase melt was formed. High yields of sucrose polyesters were obtained at temperatures as low as 105°C, and synthesis times were as short as 2 hours by applying a vacuum of 0-5mm Hg.

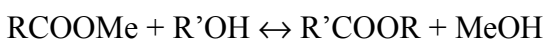
– Transesterification

Fatty acid esters of carbohydrates can be obtained by the transesterification of fatty acid alkyl esters with carbohydrates.

The term transesterification refers to an important class of organic reactions where an ester is transformed into another through interchange of the alkoxy moiety. When the original ester is reacted with an alcohol, the transesterification process is called alcoholysis.¹³

The transesterification is an equilibrium reaction, where the transformation occurs by mixing. The presence of a catalyst (strong acid or base) accelerates the reaction considerably.

The general equation for the transesterification reaction is:



Transesterification reactions are commonly employed at an industrial scale, for instance in the production of polyethylene terephthalate as well as for the production of a large number of acrylic acid derivatives.

Initial reports of sugar fatty acid ester synthesis by transesterification involved the use of toxic solvents such as dimethylformamide (DMF), dimethylsulfoxide (DMS) or dimethylpyrrolidone (DMP) at 90-95°C under 80-100 mmHg pressure for 9 to 12 hours. These solvents were used to solubilize both the sugars and the free fatty acids. This process, known as the Hass-Snell process used solvents that are not approved in food technology and yielded products which smelled and contained toxic materials¹⁵.

As a result, propylene glycol or water were employed as solvents. Soap or the product itself acted as an emulsifier to conduct the reaction in a microemulsion system

with potassium carbonate as the catalyst.¹⁴ The reported yield is 85% for the sucrose monoester and 15% for the diester, after purification. Since the Hass-Snell process was developed, in 1959, a number of studies have reported synthesis methods for sugar esters of fatty acids.

Typically, sugar polyesters are prepared through the base catalyzed transesterification of carbohydrates with fatty acid methyl esters, using sodium methoxide as a catalyst. The base catalyzed transesterification reaction proceeds faster than its acid catalyzed counterpart. (15)

However, some of these methods require very high temperatures (above 100°C) and toxic, low-volatility solvents such as DMF and DMSO. Nevertheless, solvent-free processes have also been developed. Reaction yields of about 90% can be obtained by a two-stage solvent free process using sodium hydride, Na-K alloy and soaps as catalyst (16). High temperatures are required and purifying the final product is difficult. Sucrose polyesters are available by a two stage transesterification process. Potassium soaps provide a homogeneous melt of sucrose and fatty acid methyl esters.

The reaction proceeds as follows (Figure 6). Initially, the base reacts with the alcohol, producing an alkoxide and the protonated catalyst. The alkoxide then attacks the carbonyl group of the fatty acid methyl ester. This nucleophilic attack generates a tetrahedral intermediate. The alkyl ester and the anion of the fatty acid are then formed. The anion deprotonates the catalyst and generates an active species which reacts with a molecule of the alcohol, starting another catalytic cycle¹³.

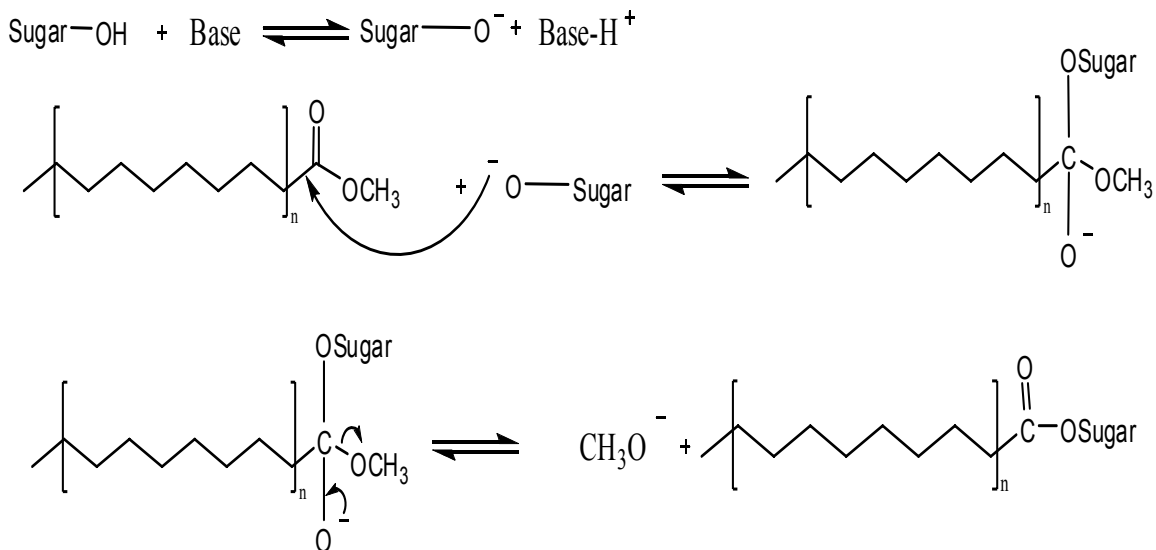


Figure 6: Transesterification- Mechanism of Reaction

Alkaline metal alkoxides (such as CH_3ONa) give very high yields (>98%) in short reaction times (30 min), even at low molar concentrations (0.5 mol %). However, these most active catalysts require the absence of water, making them difficult to use industrially. Alkaline metal hydroxides (KOH and NaOH) are less active than metal alkoxides but are a good alternative. They allow for equally high yields by increasing the catalyst concentration to 1 or 2 mol%.

Also, it is worth mentioning that the reaction of the hydroxide with the alcohol will introduce some water in the system, which will hydrolyze some of the produced ester, with consequent soap formation. This reduces the final conversion. The use of potassium carbonate, in a concentration of 2 or 3 mol% reduces the soap formation and allows for increased yields. In fact, the addition of potassium carbonate allows for the formation of bicarbonate instead of water, and the esters are not hydrolyzed.

When fatty acid methyl esters are transesterified with sucrose, methanol is formed and can be removed by distillation. This drives the equilibrium in favor of the sucrose ester and improves the yield of the desired product.¹⁷ Also, vacuum may be applied to evacuate the methanol formed during the reaction.

The transesterification reaction does not allow regioselectivity and the degree of hydroxyl group substitution on the sugar is uncontrolled¹⁸. Nevertheless, the reaction may be oriented towards a specific substitution pattern by carefully choosing the catalyst, as well as the reaction conditions. Most studies have shown that the yield of sucrose mono and diesters increases with the molar ratio of sucrose: fatty acid. This is due to the increased chance of substitution of the three primary hydroxyl groups of sucrose. However, as the molar ratio of sucrose: fatty acid decreases, that is the fatty acid methyl ester is in excess, the more highly substituted derivatives (DS 4-8) of sucrose are preferentially synthesized.¹⁹

To make Olestra, natural oils-such as cottonseed or soybean - are heated in a base-catalyzed reaction with methanol to detach the fatty acids as methyl esters. Afterwards, the glycerine settles out and is drawn off, and the fatty acid methyl esters are distilled. Then, sucrose and another base catalyst are added to the fatty acid methyl esters, with emulsifiers. Under high temperature, sucrose polyesters form and methanol is removed. Further processing removes leftover fatty acid esters and emulsifiers. Then, the new fat is bleached and deodorized.

– Enzymatic Synthesis

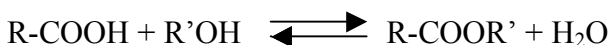
The peculiar properties of enzymes, especially their high selectivity and their activity under mild conditions, have made them quite popular. These biocatalysts are very

well suited to support natural product synthesis, and more specifically carbohydrate modifications.²⁰

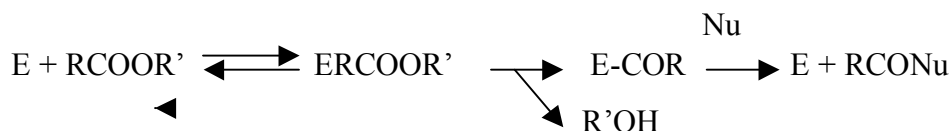
As a result, enzymes are being used to react hydroxyl groups selectively and catalyze the synthesis of sugar esters. Operating conditions are milder, side reactions like sugar caramelization are limited, and the only contaminants present at the end are the sugar and residual fatty acids.

Klibanov demonstrated that hydrolytic enzymes could in fact be used to perform esterification and transesterification reactions.^{21,22}

In dry organic solvents, lipases and proteases reverse their normal hydrolytic activity, and are able to catalyze esterifications and transesterifications according to the following reactions.



The mechanism of action of serine proteases is as follows:



In the usual hydrolytic pathway, water is the nucleophile that attacks the acyl-enzyme intermediate. In organic solvents, the attack is performed by alcohols, amines, and thiols, leading to the formation of esters, amides, and thioesters, respectively²³

In order to optimize conditions for biocatalyzed reactions, the amount of water present must be closely monitored. In fact, a minimum amount is required to ensure that the enzyme remains in its active form²⁴. This amount varies depending on the lipase. Also,

the nature of the organic solvent is important. Solvents should be non polar or of low polarity so that the enzyme hydration is not affected, but they should be sufficiently polar to dissolve the sugars. Moreover, reaction yields, cost advantages as well as toxicity also come into play. Reaction conditions (temperature, pH, reaction time, molar ratios between substrates) as well as the amount of enzyme used and whether or not it is immobilized are all factors that need to be tweaked in order to optimize the synthesis.

The use of supercritical fluids, such as CO₂, has also been studied as an alternative to the use of organic solvents^{25,26}. Pressure and temperature changes allow for variations in the properties of supercritical CO₂. However, solubility issues arise for very polar compounds. Possible solutions include the use of more polar supercritical fluids, which would require higher critical temperatures, or the use of more polar co-solvents such as ethanol, acetone or water. Also, complexation agents (e.g. organoboronic acids) may be used or the polar compound may be pre-adsorbed onto an inert material with high internal surface area²⁷

2.3.2 Selectivity and Kinetics

Esterifications and transesterifications are usually performed with the help of a basic catalyst. The derivatives are mostly substituted at the primary positions.

Magoffin et al. found that only the reactive sites of sucrose, that is the three primary hydroxyl groups at C6 of glucose, and C1' and C6' of fructose, can be substituted to yield mono, di- and tri- esters²⁸. Nevertheless, due to steric hindrance, the C1' position of fructose is substituted less readily.

The transesterification reaction of the Snell process was shown to follow first order kinetics, and the rate of reaction is independent of the sucrose concentration and the molecular weight of the fatty acid²⁹. Also, the mono-substitution in sucrose takes place preferentially at C-6 in the D-glucose unit, with the ratio of the substitution of the glucose to fructose moiety being 4:1.

In support, Molinier et al also found that the glucose moiety of sucrose seems to be more reactive³⁰. The transesterification of sucrose with N- decanoylthiazolidinethione in DMF was performed using different bases. In all cases, the reaction was rather selective on the glucose moiety. However, the use of potassium carbonate led to more complex mixtures. Studies of mono-O-substitution during base catalyzed reactions showed that migrations from one position to another may occur. In fact, although most base catalyzed transesterifications lead finally to primary esters, migrations are catalyzed by base and the actual kinetic reactivity of the hydroxyl groups may be different.

According to Molinier et al, in an organic medium, the formation of the ester at OH-2 occurs first, after which extensive acyl transfer to OH-3 and OH-6 occurs³⁰. This OH-2 selective reaction would be due to electronic factors. Moreover, the use of potassium carbonate shows a significantly larger amount of esterification at position 1'. Finally, primary OH groups can also be selectively reacted based on steric hindrance.³¹

Moreover, Molinier et al performed the transesterification of sucrose with different acyl donors in DMF, in the presence of catalytic butyl lithium. The major ester formed from the transesterification using methyl esters as acyl donors (as well as cyanoethylesters and methylthioethylesters) was found to be formed at OH-6. This can either be the result of selective esterification at OH-2 followed by migrations to OH-3

and OH-6 or a direct selective esterification at OH-6, without concomitant esterification at the other primary positions 6' and 1'³⁰.

Dzulkefly et al. studied the selectivity and kinetics of the solventless interesterification of glucose pentaacetate with fatty acid methyl esters. Their results indicated that the C1 and C6 positions were preferred for substitution of fatty acid groups onto the glucose pentaacetate ring³². Moreover, they studied the fatty acid chain length with respect to selectivity. They found that for the short-chain C₆₋₁₀ mixed fatty acids, the C₁₀ was the most favored. Within the C₁₂ to C_{18:2} fatty acid series, C_{18:1} was the most favored.³²

2.4. Sugar Esters of Fatty Acids: Applications

2.4.1 Fatty acid esters: Emulsifying properties

Sucrose esters are non ionic surfactants with a wide range of hydrophilic-lipophilic balance (HLB) values. HLB is calculated using “hydrophilic group numbers” assigned to the various hydrophilic and lipophilic moieties appearing in the surfactants. In theory, HLB relates the molecular weight of the hydrophilic portion of the surfactant molecule to the total molecular weight. The HLB scale ranges from 0 to 20. In the range of 3.5 to 6.0, surfactants are more suitable for use in water/oil emulsions. Surfactants with HLB values in the 8 to 18 range are most commonly used as in oil/water emulsions.³³

The surface active properties of sucrose esters are derived from the original hydrophilic group of sucrose and the original lipophilic group of fatty acids. By varying

the degree of substitution or the fatty acid chain lengths, wide ranges of functionality can be obtained. As a result, HLB values can range anywhere from 1 to 16.

However, only sucrose esters with toxicological clearance have been approved for specific uses in the United States since 1983. At present, the U.S. FDA has approved only sucrose esters blended with mono-di- and tri- glycerides of palmitic and stearic acids. These surfactants are completely biodegradable and can be completely metabolized.

Sucrose esters in which six or more hydroxyl groups have been esterified are non absorbable fat mimicking materials. They are used as non caloric fat substitutes and their non-absorbability has the potential benefit of lowering cholesterol.

What is more, sucrose esters surfactants of three or fewer fatty acids of reduce surface tension when added to an oil-water mixture. They are adsorbed at the interface between water and oil and orient themselves so that the hydrophilic portions are towards the water and the lipophilic portions are towards the oil.

Sucrose fatty acid esters from lauric ($C_{12:0}$) to docosanoic acid ($C_{22:0}$) have surfactant properties and can reduce the surface tension of water. Fatty acids of lower chain length do not have significant surfactant properties.

Monoesters are soluble in many organic solvents but only slightly in water. Moreover, surfactant properties depend on the degree of esterification as well as the fatty acid chain length and degree of saturation. Sucrose esters with a shorter, more unsaturated fatty acid and less esterified groups show a more hydrophilic function.³⁴ On the other hand, sucrose polyesters are not good surfactants of oil and water emulsions. However, they are excellent stabilizers of water-oil emulsions because of their lipophilic

nature.³⁵ Low and high HLB value sucrose esters are more functional in water-oil and oil-water emulsions, respectively.³⁶

The functionality of sucrose esters in foods is complex, due to multiple interactions with starch, proteins, and oils and fats. In fact, Osman et al³⁷ observed a reduced iodine affinity of amylose, indicative of complex formation, in the presence of sucrose esters. The ability of sucrose esters to form complexes was suggested, and these were related to the percentage of the fatty acid portion. Later, Matsunaga and Kainuma³⁸ reported that sucrose esters are capable of preventing starch retrogradation by forming a helical complex with amylose.

Also, in the presence of sucrose esters with HLB between 9 and 16, the migration of amylose from the starch granule is retarded by the formation of an inclusion complex.³⁹ Furthermore, sucrose esters can function as dough conditioners by interactions with flour protein. This happens through two mechanisms⁴⁰: (1) hydrophilic and/or hydrophobic binding with gluten protein, and/ or (2) interaction in bulk form with the water phase of the dough.

2.4.2 Fatty acid esters: Antimicrobial properties

The antimicrobial activity of sucrose esters comes from the interaction of the esters with cell membranes of bacteria, causing autolysis. The lytic action is assumed to be due to stimulation of autolytic enzymes rather than to actual solubilization of cell membranes of bacteria.⁴¹

Conley and Kabara conducted several experiments using sucrose esters⁴². They determined the minimal inhibitory concentration of a series of fatty acid esters of

polyhydric alcohols against gram-negative and gram-positive organisms. Gram-negative organisms were not affected. Gram-positive organisms were. Also, sucrose esters were more inhibitory than the free fatty acid. Lauric acid was the exception.

However, although the effectiveness of fatty acids increased when esterified, the spectrum of antimicrobial action of the esters is narrower when compared with the free acids. In addition, very similar organisms do not have the same susceptibility to comparable sucrose esters⁴³.

In fact, the antimicrobial activity of the sugar esters is determined by the structure of the esterified fatty acids. Monoesters are more potent than polyesters and diesters of sucrose appear to be more active than monoester forms, with sucrose dicaprylate having the highest activity^{44,45} Also, sucrose esters have a substantial effect against toxicogenic and spoilage molds. However, they show no inhibitory activity against yeasts.

2.4.3 Fatty Acid Polyesters

When four or more fatty acids are esterified onto sucrose, the polyester behaves as a fat and has physical and organoleptic properties similar to those of fats. However, because a large number of fatty acids are attached to the central sugar molecule, the digestive enzymes can't get in place to break them off, and so, they are absorbed and digested to a lesser extent. As a result, the caloric properties are very different from those of regular fats.

Sucrose polyesters are not hydrolyzed by pancreatic lipase and not absorbed in the small intestine. Their characteristics are similar to those of conventional oils but they

do not contribute any significant calories (41). Olestra, developed by Procter and Gamble, is a synthetic, non-absorbable sugar polyester, approved by the U.S. FDA for use in snacks as a fat substitute.

2.5 Purification

One of the most challenging issues in the synthesis of carbohydrate fatty acid esters and polyesters is the development of a uniform assay procedure to isolate, purify and analyze the product. The methods may vary depending on the starting materials, mode of synthesis, catalyst used and product formed.

Most patents on the subject consider washing the reaction mixture with a variety of organic solvents^{46,47,48,49,50}. The purification of the crude ester product often involves neutralization, bleaching to reduce coloring materials and washing and distillation to remove free fatty acids, methyl esters and solvents. Deodorization and chromatographic processes are also used.

Wagner et al use a two step process to separate the sugar ester from the crude sugar ester reaction product⁴⁹. In the first step, a precipitate of sugar ester is formed from a mixture of alcohol, water and crude sugar ester reaction product. In a second subsequent step, the recovered sugar ester precipitate is washed with an organic solvent. Washing with methanol and hexane removes the unreacted fatty acid methyl esters and sucrose fatty acid esters with a degree of substitution of about 1 to 3^{12, 51}.

Akoh and Swanson neutralized the raffinose polyester reaction mixture with acetic acid, dissolved in hexane, stirred and bleached with activated charcoal⁵¹. The charcoal particles were then removed by filtration, and the filtrate was washed with methanol and allowed enough time for phase separation. The raffinose polyester was in

the denser methanol insoluble layer. The methanol insoluble products were redissolved in hexane and washed in methanol. Extraction of the raffinose polyesters gave a less dense hexane layer containing the polyesters and a denser methanol layer containing the residual fatty acid methyl esters. The raffinose polyester was dried over anhydrous sodium sulfate and filtered. Methanol and hexane were removed by rotary evaporation to yield neat oil.

Sometimes, further purification is desirable. Furthermore, if gram quantities of carbohydrate polyesters are to be purified, chromatography columns may be used.

Cormier et al. describe the separation of mono- and diesters of sucrose stearate and tallowate by high-pressure liquid chromatography using a mixture of praseodymium chloride (PrCl) and methanol as eluant⁵².

Kaufman and Garti separated mono-, di-, tri-, and polyesters of sucrose prepared by transesterification with methyl stearate or palmitate and tallow fat by high-performance liquid chromatography⁵³. This was done with both UV and refractive index detectors using methanol- isopropanol and aqueous methanol as eluents.

Jaspers et al. described two procedures for quantitative analysis of sucrose mono- and diesters, by high-performance liquid chromatography on reversed-phase columns⁵⁴. Methanol and water were used for the separation of the monoesters, while methanol-ethyl acetate-water was used for the separation of di-esters. These methods gave information about the amount of monoesters and di-esters in the product, and the number of the most important structural isomers. However, a complete separation of all the possible di-ester products was not possible, due to the presence of more complex structural isomers.

Finally, Owens et al successfully separated sucrose polyesters on an open-tubular column using supercritical CO₂ as the mobile phase⁵⁵. Room-temperature splitting injection was used with an uncoated, fused silica inlet tube to focus solute on the head of the column, similar to the retention gap method in gas chromatography. Artifact-free detection was possible by the use of a thin-walled, tapered-capillary restrictor as the interface between the column and a flame ionization detector. The column temperature had a pronounced effect on the selectivity and resolution of the chromatogram. Also, the interpretation of the lab chromatograms was done by calculating and plotting a model chromatogram.

2.6 Fatty Acid Cellulose Esters

The chemical modification of natural cellulose fibers has been the object of much attention^{56, 57, 58}. Many efforts have been directed towards using natural cellulose fibers as reinforcing agents in polymer composites. Natural cellulose fibers are low cost, biodegradable alternatives to traditional reinforcing agents. However, the very important hydrophilicity of cellulose presents certain challenges, as it is the main cause of poor compatibility between the cellulose fibers and the polymer matrix, leading to composites with unsatisfactory properties^{58,59}. In order to improve the adhesion of the fibers to the matrix, chemical modification of the fiber surface properties is often undertaken.

Following this line of thought, esterification reactions can be carried out to modify the fiber surface and decrease the natural hydrophilic character of cellulose. In fact, cellulose esters represent a class of commercially important thermoplastic polymers with excellent film forming characteristics. Meanwhile, long-chain aliphatic esters of cellulose have the potential to become important biodegradable plastics⁶⁰. The ester bond

is enzymatically labile and both fatty acids and cellulose are naturally abundant and relatively inexpensive materials.

Cellulose esters of short chain fatty acids (length of four carbons or less) are commercially synthesized using acid anhydrides and a sulfuric acid catalyst in a heterogeneous phase reaction. Cellulose esters of higher acid chain lengths have been prepared using acid chlorides and pyridine^{56,57}, acid chlorides under vacuum⁶⁰ and aliphatic acids with trifluoroacetic acid.

Kinetics studies have claimed a maximum degree of substitution (DS) for the pyridine- acid chloride method⁶¹. However, this maximum DS of 3.0 is not practically attainable by the vacuum acid chloride process⁶⁰. Moreover, reaction rates and degrees of substitution are hindered by a low accessibility of cellulose to the reagents⁶⁰.

Nevertheless, the careful selection of a cellulose solvent system allows for an improved accessibility of cellulose in solution, allowing all of the cellulose to be exposed to reactants simultaneously. This in turn results in uniformly distributed substituents along the cellulose backbone⁶².

Wang and Tao reported the synthesis of fatty acid cellulose esters (FACE)⁵⁷ using hydrolyzed soybean oil and thus soybean fatty acids (mainly oleic, linoleic and linolenic acid). They prepared the fatty acid chlorides with phosphorous pentachloride in benzene and reacted these with activated (by mercerization) α -cellulose. The esterification reaction was performed in DMF and pyridine was also added to chelate the condensation reaction by-product HCl.

Kwatra et al. studied the synthesis of long chain fatty acid cellulose esters by the vacuum acyl chloride method. In this process, the HCl is removed by vacuum distillation,

thus eliminating the need for additional solvents and producing less complex solvent waste streams⁶⁰.

The acyl chloride is prepared by treating the carboxylic acid with thionyl chloride (SOCl₂) in the presence of a base.

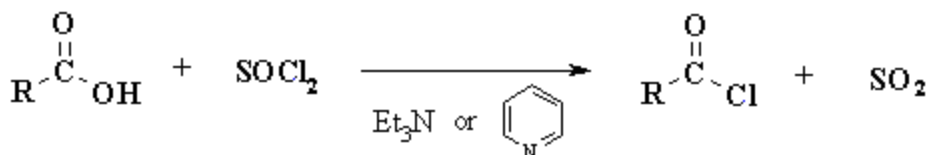


Figure 7: Acyl chloride preparation

Acyl chlorides are the most reactive of the carboxylic acid derivatives and therefore can be readily converted into other carboxylic acid derivatives. They are sufficiently reactive that they react quite readily with cold water and hydrolyze to the carboxylic acid.

The esterification reaction occurs by nucleophilic addition/elimination. The acyl chloride reacts with the alcohol to produce the ester and HCl.

The reaction mechanism is as follows:

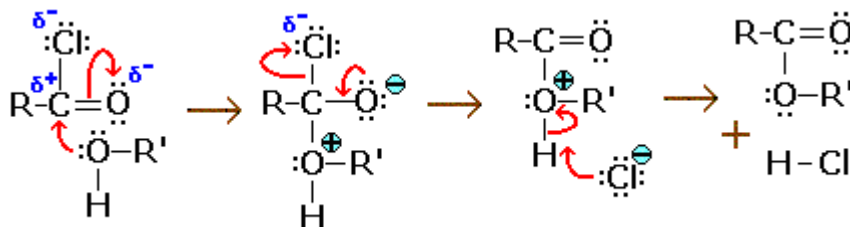


Figure 8: Esterification- Mechanism of Reaction

The acyl chloride carbonyl is highly polarized and the positive carbon is attacked by the nucleophilic alcohol. The alcohol adds to form a highly unstable ionic intermediate via a C-O bond and the π electron pair of the C=O double bond moves onto the oxygen atom to give it a full negative charge. The C-Cl bond electron pair then moves onto the chlorine atom which leaves as a chloride ion. Parallely, one of the lone pairs of electrons from the negative oxygen atom shifts to reform the C=O carbonyl bond. Finally, the previously formed chloride ion abstracts a proton to form the oxonium ion and the ester product.

2.7 Characterization

Infrared (IR), proton NMR and carbon NMR have routinely been used to confirm or elucidate the structure of the synthesized carbohydrate polyesters. In the IR spectrum, characteristic functional group absorption bands to look for are: 3420-3500 cm^{-1} (OH), 1740-1750 cm^{-1} (ester C = O) and 1460-1470 cm^{-1} (C-H stretch in CH_3 and/or CH_2).

The characteristic signals in the ^{13}C -NMR spectrum may vary depending on the starting material. Nevertheless, the methyl carbons of the acid will be in the 10-20 ppm range, the CH_2 groups will be between 20 and 35 ppm, the methoxy group will be around 50 ppm and the sugar carbons between 50 and 100 ppm.

3. Research Objectives

This study aims at elucidating a variety of salient features that dictate the kinetics and chain length effects governing the formation and antimicrobial activity of sugar polyesters of fatty acids. This will allow for a better understanding of their bioactivity, especially their structure/function relationships.

The objectives of this work are as follows:

1. Synthesize glucose, sucrose and cellobiose esters of butyric acid (C4), caprylic acid (C8), lauric acid (C12), stearic acid (C18) and arachidic acid (C20) by transesterification. The goal is to use saturated fatty acids and vary the chain length in order to observe related effects.
2. Study the transesterification reaction by phosphorous nuclear magnetic resonance (^{31}P NMR)
3. Follow the specific substitutions occurring at the various OH positions within the sugars by ^{31}P NMR
4. Quantify the degree of conversion and relate it to the sugar and the incoming fatty acid chain length
5. Consider the antimicrobial activity of these compounds
6. Synthesize cellulose esters of fatty acids

4. Experimental

To do this, anomerically pure glucose, sucrose and cellobiose sugars are transesterified with the methyl esters of fatty acids of variable chain lengths (C_4 , C_8 , C_{12} , C_{18} , C_{20}). The methyl esters of butyric, caprylic, lauric, stearic and arachidic acids were reacted with the above carbohydrates to give the respective penta and octaesters. The transesterification reaction is performed in such a way so as to yield a variety of substituted sugars varying in degrees of substitution. The kinetics of these transesterification reactions were followed by quantitative phosphorous (^{31}P) NMR after phoshitylation of the labile OH groups with 1,3,2- dioxaphospholanyl chloride. This route has seldom been used, and shows great promise as an analytical technique for characterizing the actual chemistry and regiospecificity of these polyfunctional and otherwise very complex compounds.

This approach proved to be a facile and quantitative means to follow the specific substitutions occurring at the various OH positions within the sugars as a function of degree of conversion, and incoming chain length. As anticipated, a variety of steric and hydrophobic effects were shown to play a key role in determining the reactivity of these systems.

Various sugar esters were then adsorbed onto cellulose disks and their microbial activity was examined.

Finally, cellulose esters were synthesized via the acyl chloride process. These were studied by FT-IR.

4.1 Sugar esters of fatty acids

4.1.1 Synthesis of Sugar Esters of Fatty Acids

As described by Volpenhein⁶³, 85% KOH pellets (0.055 moles) are dissolved in some methanol and added to (0.347 moles) of fatty acid methyl ester. The mixture is refluxed for 2 hours.

At this time, (0.073 moles) of sugar and 1g potassium carbonate are added and the condenser is removed. The methanol is then evaporated from the mixture under a gentle stream of nitrogen. A vacuum is then applied and the reaction temperature is set.

Reaction conditions are maintained for one to four hours.

In this manner, 15 different sugar esters of fatty acids are synthesized using anomerically pure glucose, sucrose and cellobiose and the methyl esters of butyric acid (C4), caprylic acid (C8), lauric acid (C12), stearic acid (C16) and arachidic acid (C20).

4.1.2 Characterization

The transesterification reactions were followed by quantitative phosphorous (³¹P) NMR.

The labile OH groups are phoshtylated with 1,3,2- dioxaphospholanyl chloride. A phosphorous peak then appears in the spectrum, characteristic of each hydroxyl group.

15 to 20 mg the crude reaction mixture are dissolved in 500µL anhydrous pyridine (PyD₅)- deuterated chloroform(CDCl₃) mixture (1/1, V/V). 100 µL of internal standard 2-nitrophenol (13.8mg/mL in PyD₅/ CDCl₃ (1/1, V/V)) are added. 50 µL of relaxation reagent chromium (III) acetylacetonate (11.4mg/mL in PyD₅/ CDCl₃ (1/1, V/V)) are added next. Finally, 80 µL of 1,3,2- dioxaphospholanyl chloride (Reagent I) are added.

The sample is mixed until dissolution. Complete dissolution is difficult and occurs only after addition of Reagent I.

Two dimensional long range heteronuclear NMR was also used. The samples were prepared in the same manner.

4.2. Antimicrobial Studies

The antimicrobial effect of the sugar esters was examined by adsorbing 10^{-3} moles of purified sugar esters onto cellulose disks.

These were then tested by the disk diffusion method. A series of sterile Petri plates (100 mm x 15 mm) containing a tryptic soy agar with 5% sheep's blood nutrient was used for growing bacterial lawns. E.coli bacterial culture was used to swab the plates after which 3 x 2 cm cellulose-ester disks were symmetrically arrayed on the plate. They were then incubated overnight at 37°C to encourage the growth of a bacterial lawn. The zones of inhibition were determined by measuring the bacteria-free region from the edge of the disk to the edge of the lawn.

4.3 Cellulose Ester Synthesis

4.3.1 Cellulose Esters from FAME

450 g of peanut oil and hempseed oil (used as is from Natural Oils International Inc.) was dissolved into 500 mL of ACS grade methanol in a three-necked flask. 0.5 g of NaOH was added to this solution. The mixture was then heated to 70°C for 30 min. (with stirring), and allowed to cool. The upper layer of the two layers that appeared had an off-yellow color and contained the ester. The latter was extracted and dissolved in an alkaline solution (0.5 g KOH) of 500 mL methanol. It was then refluxed for 2 hours producing the

fatty acid methyl ester soap as indicated again by TLC. This was dried overnight in a vacuum oven, and a dark yellow oil was produced.

Next, 100 g of FAME and of 10 g of cellulose disks were dissolved in 500 mL methanol. A catalytic amount of potassium carbonate was added (100 mg, 1 mmol), after which the solution was heated and the methanol was driven off. At 100°C, a vacuum was applied to the reaction and it was heated for 2 hrs. at 130°C.

The cellulose disks were recovered and a Soxhlet extraction with hexane was performed. The disks were then dried overnight at 40°C under reduced pressure.

4.3.2 Cellulose Esters from Acyl Chloride

- Making the acyl chloride

4.96 grams of stearic acid (20 mmol) were added to a 100 mL flask and heated to 70°C in order to obtain a melt. 3mL of thionyl chloride (40 mmol) were then added dropwise and allowed to react for 30 minutes as previously described by Hwang and Fowler⁶⁴.

After this time the excess, unreacted thionyl chloride was removed by rotary evaporation under high vacuum (vacuum pump).

The stearyl chloride was recovered and reacted with cellulose as follows.



Figure 9: Experimental setup

– Making the cellulose ester

Twenty five grams of microcrystalline cellulose fibers (Avicel) (predried for 1 night at 40°C under reduced pressure) were added to a 100 mL flask containing 30 mL dry THF and 1 mL of pyridine (dried with molecular sieves). 12 mmol of acyl chloride (stearyl C18) were added dropwise to the reaction vessel. The reaction was conducted at 70°C for 5 hours. The fibers were recovered by filtration and a Soxhlet extraction with hexane was performed. The fibers were then dried overnight at 40°C under reduced pressure.

– Characterization

Fourier Transform Infrared Spectroscopy (FT-IR) was used to determine the existence of an ester linkage. Spectra were obtained on a NEXUS spectrometer. A total of 256 scans for each sample were taken with a resolution of 4cm⁻¹. The cellulose disk spectra were

collected in reflectance mode. Spectra were recorded at wavenumbers 4000 - 600 cm^{-1} by a Continuum IR scope coupled to a ThermoNicolet Nexus 670 FT-IR and using OMNIC software (ThermoNicolet, Madison WI). Spectra were compared to a control disk of underivatized cellulose and a cellulose disk dipped in FAME. For the microcrystalline cellulose samples, KBr pellets were prepared. The cellulose powder and KBr were powdered and mixed in a mortar. They were then pressed under vacuum to make pellets. The FT-IR spectra were then recorded in transmittance mode.

5. Results and Discussion

5.1. ^{31}P NMR

Argyropoulos et al. have found that the structures of carbohydrate molecules can be very well investigated by using phosphorous NMR⁶⁵. Carbohydrates form stable compounds with reagents based on trivalent phosphorous, and so, derivatization with 1, 3, 2-dioxaphospholanyl chloride (Reagent 1) yields molecules that can be detected and monitored by ^{31}P NMR. The reaction of Reagent 1 with the hydroxyl groups in carbohydrates is as follows (Figure 10):

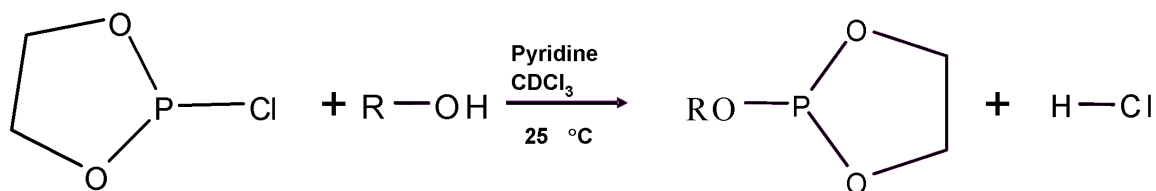


Figure 10: Derivatization reaction of carbohydrates with 1,3,2-dioxaphospholanyl chloride

As a result, the transesterification reaction, where a hydroxyl group is replaced by an ester linkage, can be monitored by observing a decrease in the hydroxyl content of a carbohydrate and of specific hydroxyl groups on the sugar.

In order to monitor the transesterification of α - D- glucose, sucrose and cellobiose with various fatty acid methyl esters (methyl butyrate (C4), methyl caprylate (C8), methyl laurate (C12), methyl stearate (C16) and methyl arachidate (C18)), we observe and quantify the hydroxyl peaks of the sugar in the phosphorous NMR spectrum at times $t=$ zero, $t=30$ min, $t=2$ hours and $t=4$ hours.

5.1.1 Internal Standard Selection

The quantification of the NMR results is done through the use of an internal standard, used in known quantities. It provides a peak in the spectrum, well separated from the other peaks, whose integral can be set to a value of 1. The integrals of the other peaks are then taken compared to the internal standard.

As a result, the selection of an internal standard is a matter which requires caution and care. The internal standard must be a pure crystalline solid possessing a reactive functional group whose ^{31}P NMR signal, after derivatization with Reagent 1, will give a sharp signal in the outer limits of the sugar region. A variety of compounds were tested (Table 1) and finally, 2-Nitrophenol was determined to be suitable.

Table 1: Chemical shift for compounds tested as internal standards

Internal Standard	Chemical Shift (ppm)
2-Fluorophenol	130.08, 129.88
Trifluoroacetic Acid	133.29
Nitrophenol	128.8
Chloroacetic Acid	127.52
Dichloroacetic Acid	133.28
Pentafluorophenol	133.61
Benzanilide	132.74
2-Phenylbenzimidazole	132.99, 127.3
N-Phenylbenzylamine	132.9, 127.48
Endo-N-hydroxy-5-norbornene-2,3-dicarboximide	136.88
2-Nitrophenol	129.73
3-Nitrophenol	129.25
4-Nitrophenol	128.8

5.1.2 Glucose Esters

As for the phosphorous NMR spectra of the various sugars, the derivative of α -D-glucose, which contains five hydroxyl groups, gives a spectrum that contains five well separated signals of similar intensity in the range 132.4-137.8 ppm⁶⁶ (Figure 11).

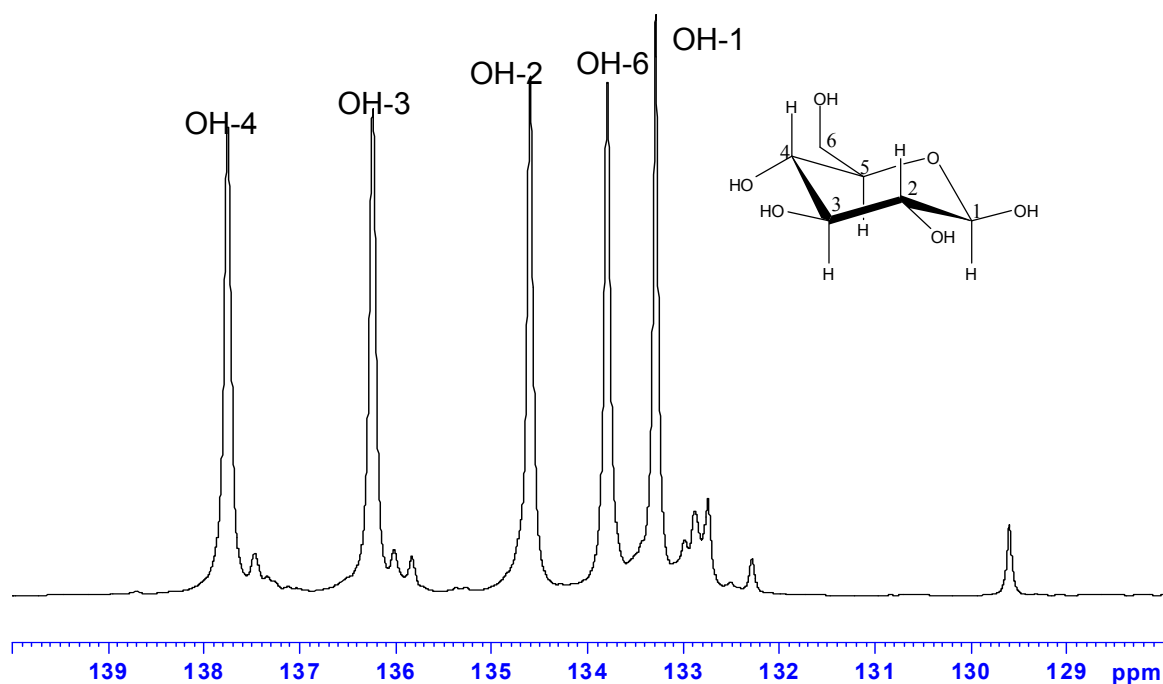


Figure 11: ^{31}P NMR spectrum of α -D-glucose phosphitylated with Reagent 1

The various hydroxyl group resonances have been assigned by Argyropoulos et al.⁶⁶ by substituting specific hydroxyls with methyl groups and observing the disappearance of the hydroxyl peak in the spectrum. It is important to note that the NMR integrations provide quantities in mmol OH per gram of material. However, since the

fatty acids differ in size and molecular weight, the values obtained must be converted in mmol OH per gram of glucose, in order to be comparable.

The chemical shift values for each hydroxyl as found in this work, as well as the literature values are presented in the following table (Table 2).

Table 2: ^{31}P NMR -Chemical shifts for derivatized hydroxyls of α -glucose

Hydroxyl Group	Chemical Shift (ppm) From Literature⁶⁶	Chemical Shift (ppm)
OH-4	137.8	137.75
OH-3	136.3	136.24
OH-2	134.4	134.6
OH-6	134	133.8
OH-1	133.4	133.29

Moreover, small signals also appear in the α - D- glucose spectrum. These weak signals correspond to the β -anomer of glucose, where all hydroxyl groups are in the equatorial conformation.

As the reaction proceeds, we notice an increase in the peaks that originate from the β -anomer of glucose relative to those of the α -anomer. This is evident in the following spectra (Figure 12), taken for the transesterification of glucose with methyl laurate, 1 hour after the start of the reaction and 4 hours after.

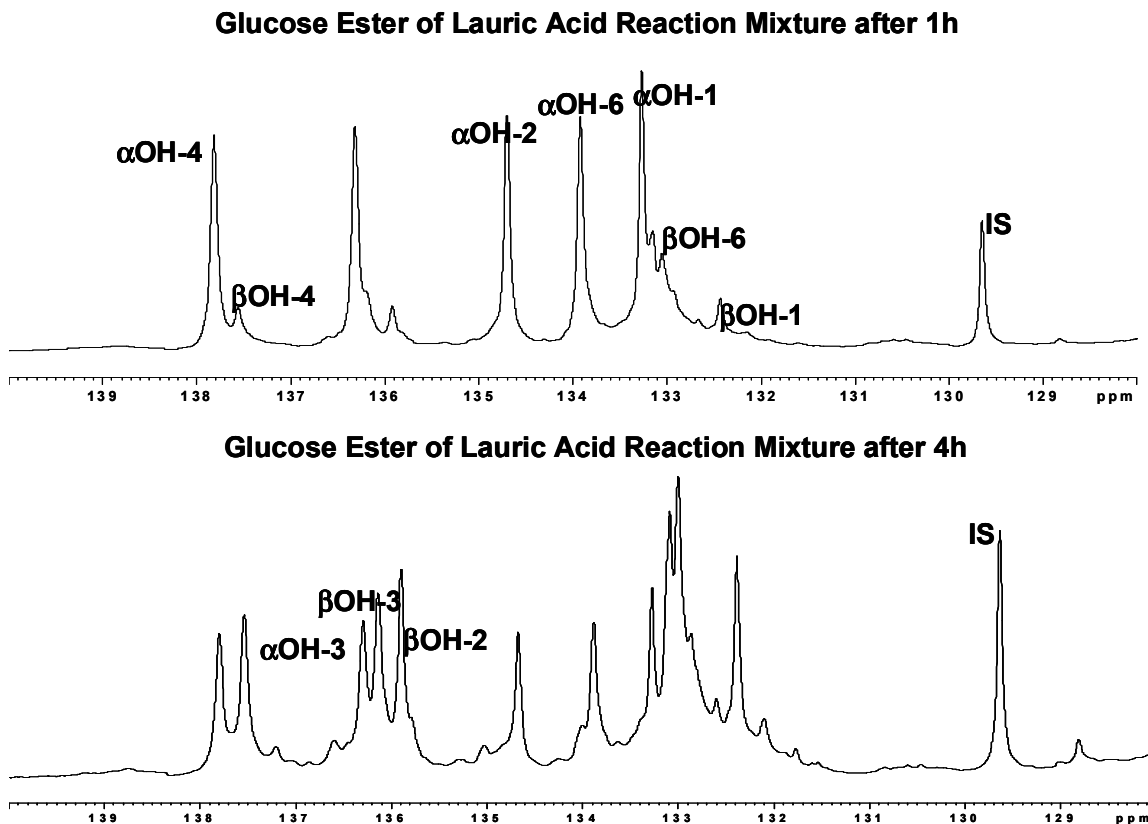


Figure 12: ^{31}P NMR spectra for transesterification of glucose with methyl laurate after $t=1$ hour and $t=4$ hours

In effect, when quantitating the hydroxyl at OH-1 for α -D-glucose, as well as the same hydroxyl for the β -anomer for the reaction of glucose with methyl caprylate, we observe a decrease in the α -form and an increase in the β - form (Figure 13).

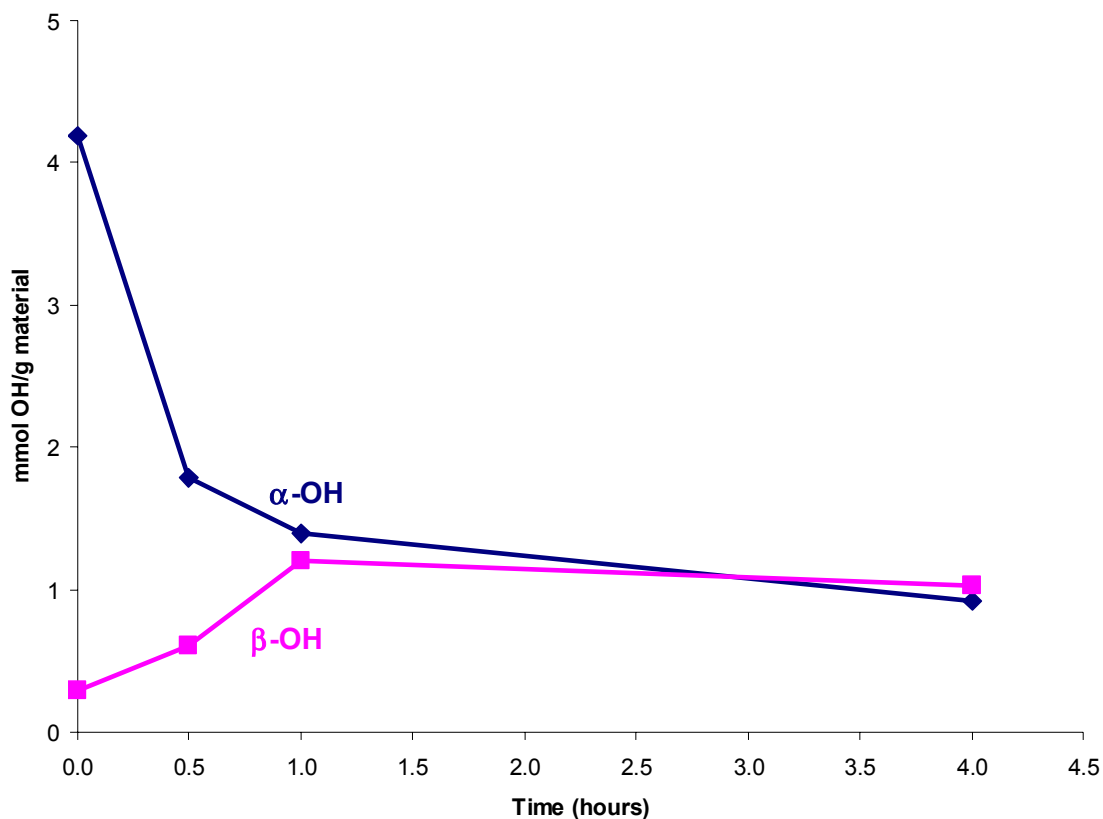


Figure 13: α and β OH-1 during transesterification of glucose with methyl caprylate

Moreover, we can also look at each hydroxyl group individually and study its reactivity. This is shown in the following graphs, depicting the unreacted hydroxyl groups in the reaction mixture for the glucose esters of butyric (C4), caprylic (C8), lauric (C12), stearic (C18) and arachidic (C20) acids.

Hydroxyl Groups in Glucose Ester of Butyric Acid (C4) Reaction Mixture

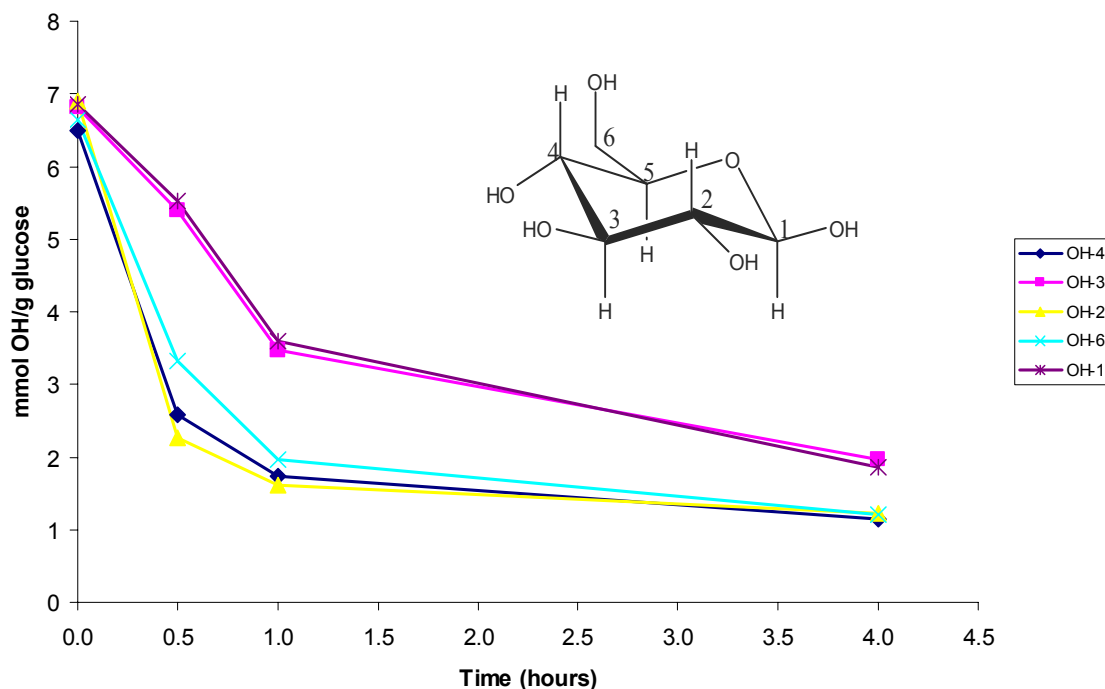


Figure 14: Hydroxyl groups in the reaction mixture of glucose esters of butyric acid

When looking at the reactivity of the hydroxyl groups in the reaction mixture of glucose esters of butyric acid (C4) (Figure 14), it is evident that the hydroxyl groups at C1 and C3 appear less reactive. In fact, there is a 0.9 mol difference at the end of the reaction between OH-1 and OH-3 and the other three hydroxyl groups.

When examining the reaction mixture of glucose esters of caprylic acid (C8) (Figure 15), the hydroxyl at C1 again appears to be less reactive, it is approximately 0.6 mol more abundant at the end of the reaction than the hydroxyl at C4 and 0.3 mol more abundant than the other hydroxyls.

Hydroxyl Groups in Glucose Ester of Caprylic Acid (C8) Reaction Mixture

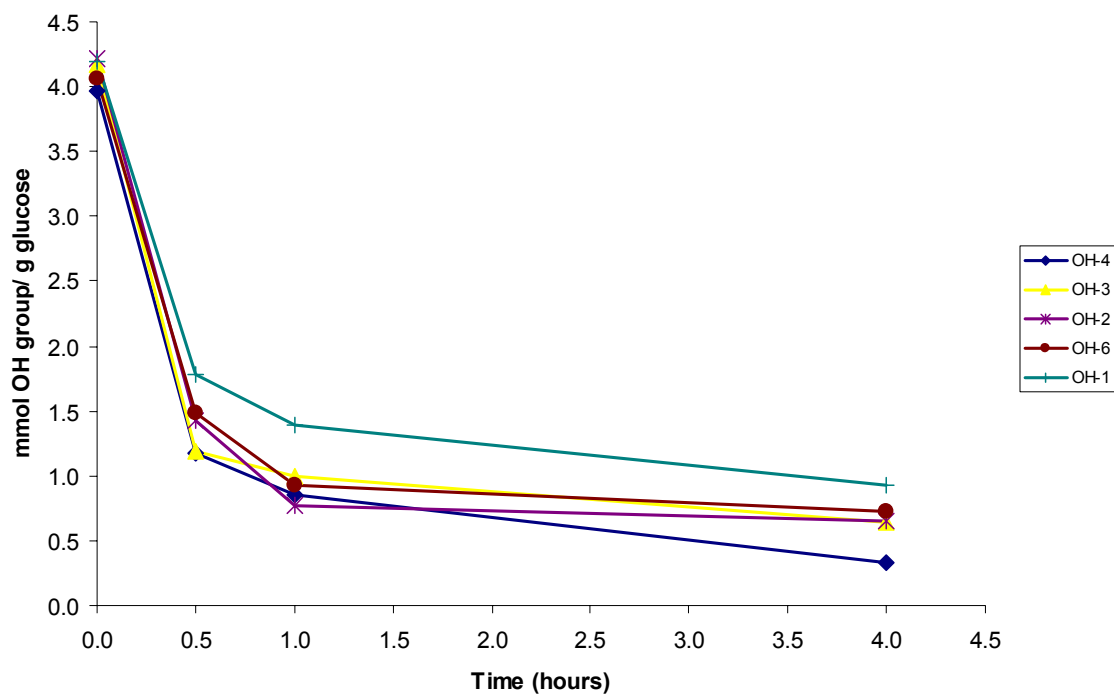


Figure 15: Hydroxyl groups in glucose ester of caprylic acid (C8) reaction mixture

The reaction mixture of glucose esters of lauric acid (C12) also shows a similar trend. That is, the hydroxyl group at C1 again appears to be less reactive and is about 0.15 mol more abundant than the other hydroxyls at the end of the reaction (Figure 16).

Hydroxyl Groups in Glucose Ester of Lauric Acid (C12) Reaction Mixture

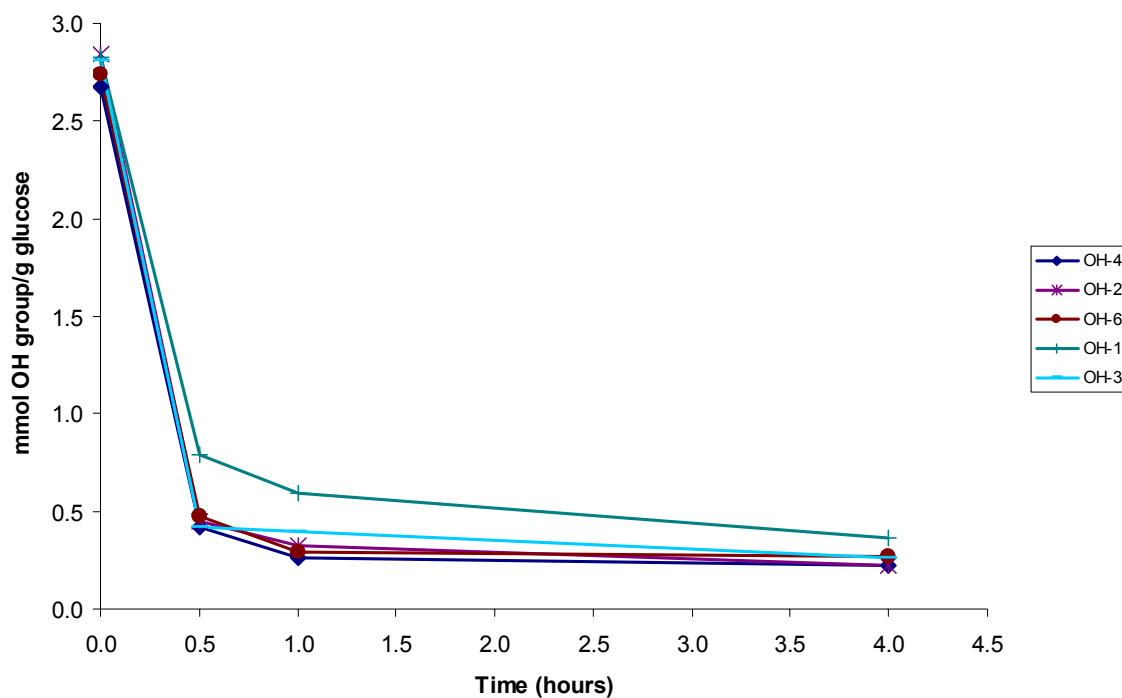


Figure 16: Hydroxyl groups in glucose ester of lauric acid (C12) reaction mixture

At longer fatty acid chain lengths, the hydroxyls appear to react more uniformly. That is, for the esters of stearic acid, OH-1 lags behind the other hydroxyls by an insignificant 0.05 mol (Figure 17) and for the esters of arachidic acid, all hydroxyls react uniformly (Figure 18).

Hydroxyl Groups in Glucose Ester of Stearic Acid (C18) Reaction Mixture

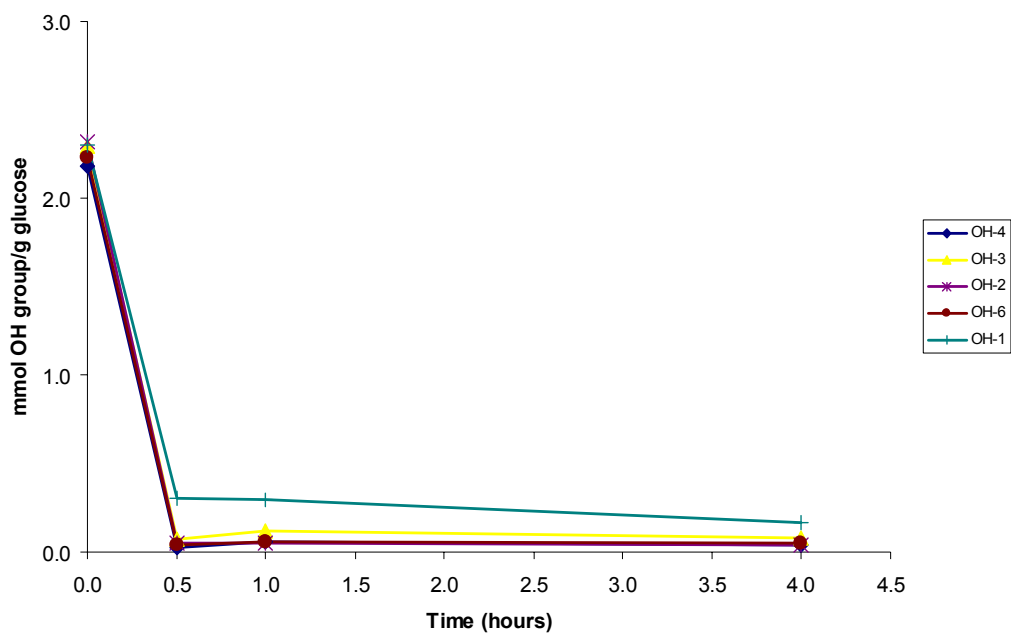


Figure 17: Hydroxyl groups in glucose ester of stearic acid (C18) reaction mixture

We can speculate that the lag that OH-1 shows may be related to the mutarotation of glucose. As seen previously, the β -form of glucose increases during the reaction. This means that ring opening and closing is taking place, and when the glucose ring is in the open form, there is no hydroxyl group present at C1 to react with the fatty acid methyl esters.

Hydroxyl Groups in Glucose Ester of Arachidic Acid (C20) Reaction Mixture

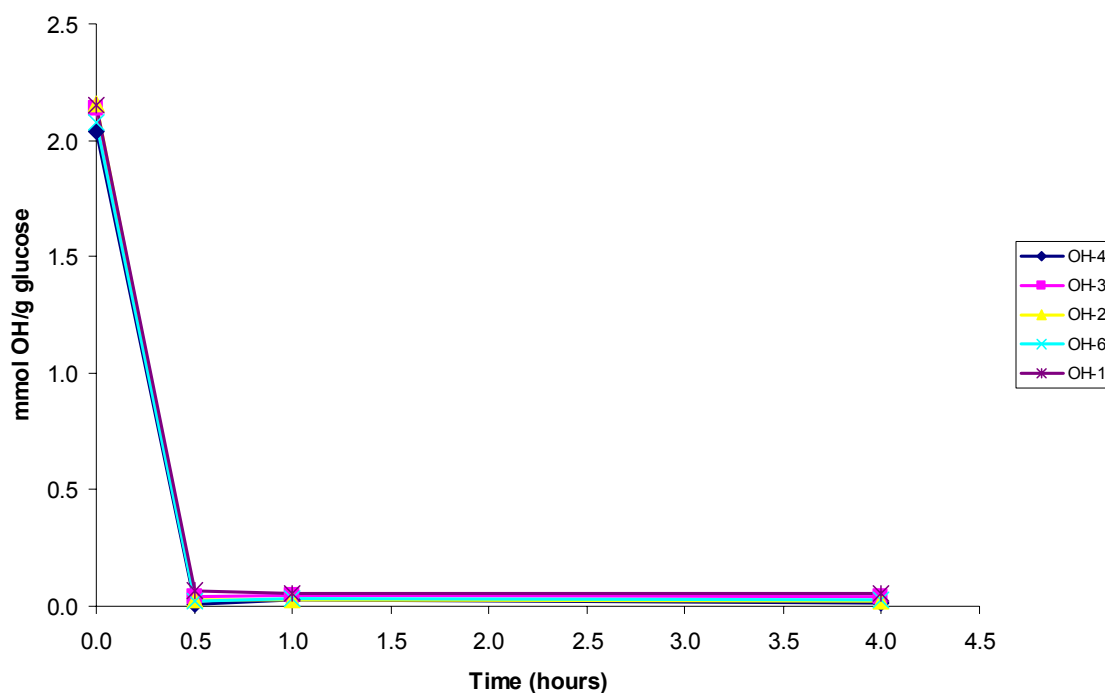


Figure 18: Hydroxyl groups in glucose ester of arachidic acid (C20) reaction mixture

When looking at total hydroxyl conversion for the various fatty acids employed (Figure 19), we see that the hydroxyl conversion increases with time, especially for the short chain fatty acids. However, it also appears to increase with fatty acid chain length. Indeed, more hydroxyl groups appear to be esterified as the fatty acid chain length increases.

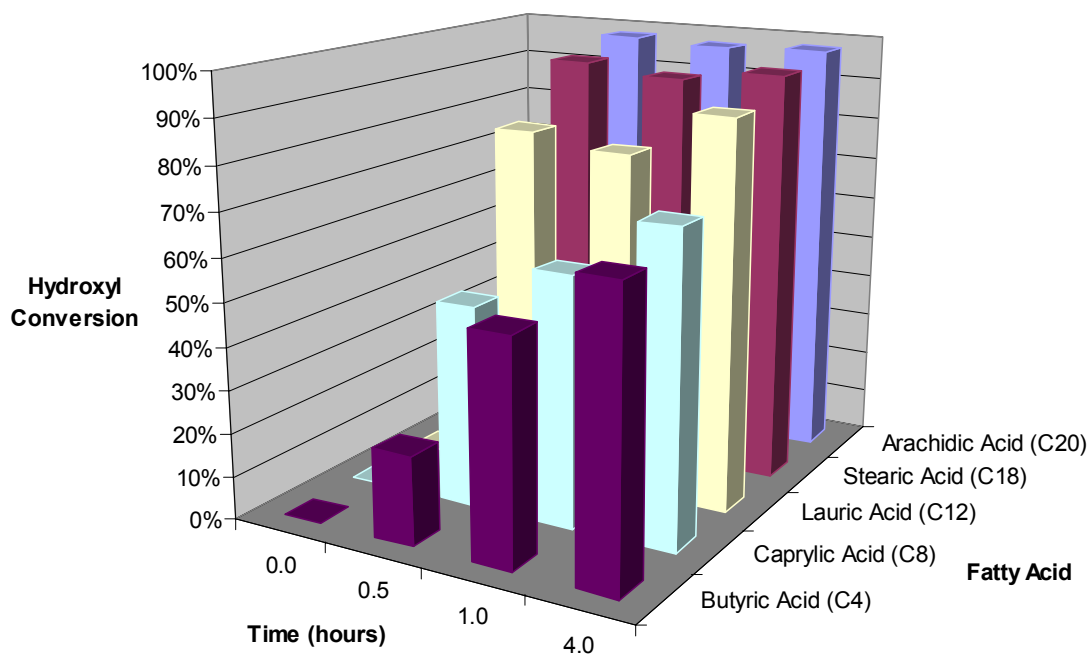


Figure 19: Glucose ester reaction mixture- Hydroxyl group conversion

The effect observed with varying fatty acid chain lengths leads us to believe that hydrophobic interactions play a key role in this reaction. Moreover, the hydroxyl group reactivity showed that as the fatty acid chain length increases, that is, when hydrophobic interactions are maximal, there is no distinction between rates.

Similar observations have been made for the lipase catalyzed esterification of saccharides. Such systems have shown higher enzyme activity at high hydrophobicities^{67,68}. However, a hydrophobic solvent is not necessarily a good choice for achieving optimal rates and degrees of conversion because the solubility of the

hydrophilic substrate must also be taken into consideration. Optimal reaction conditions are a compromise between enzyme activity and substrate solubility.

Shintre et al.⁶⁹ studied the effect of various reaction parameters, amongst which the acyl chain length on the progress of the esterification reaction using Lipolase 100 L, the lipase from the genetically engineered species of *Aspergillus oryzae*, to form benzyl esters of fatty acids. When fatty acids such as caprylic, capric, lauric and myristic acids were esterified with benzyl alcohol, the initial rate of reaction was found to be linearly proportional to the chain length of the fatty acid. Moreover, as the chain length of the fatty acid increases from 8 to 14, the conversion of the acid at any given time also increases.

Similar observations were made by Claon and Akoh⁷⁰, who found that the affinity of *Mucor miehei* lipase is higher for longer chain fatty acids in terpene ester synthesis and Iwai et al.⁷¹, who found that conversion of geraniol and farnesol esters of C2–C6 acids increased as the fatty acid chain length increased.

Shintre et al. explain this phenomenon as follows. They claim that the ability of various substrates to bind at the active site of the lipase and release a sufficient amount of binding energy, required for effecting a change in conformation of the lipase to a form which is a more efficient catalyst, is different. Smaller substrates, which are not able to release enough energy, are not able to change the conformation of the native lipase to the desired catalytically active form and as a result the reaction proceeds slowly. However, substrates with a larger chain length are able to release sufficient binding energy in order to bring about the desired change in the conformation and hence the reaction proceeds faster and the conversion values are higher.

Janssen et al.⁷²(1996) and Parida and Dordick⁷³ have also reported the influence of the acyl donor chain length on the reaction rate and on enzyme specificity. Ibrahim et al.⁷⁴ studied the synthesis of glycerides from different fatty acids by a lipase from *Humicola lanuginosa*, and found that the enzyme presents specificity towards fatty acids with carbon number ranging from C12 to C18.

Moreover, Janssen et al.⁷⁵ presented a detailed study on the esterification of glycerol and fatty acids with different chain lengths. Their results indicated that with short-chain fatty acids, higher mole fractions of monoester were obtained, while with long-chain fatty acids, the higher mole fractions were for the mono- and diesters. They found that the ester mole fraction depends on the fatty acid chain length in reaction systems without solvents. Nevertheless, in experiments in the presence of a high concentration of solvents, the equilibrium ester mole fraction is rather constant and independent of the fatty acid chain length.

In our system, we are attempting to synthesize polyesters without solvents. The increase in hydroxyl conversion as the fatty acid chain length is increased is in accord with the previous observations.

Selmi et al.⁷⁶ described the synthesis of tri-acylglycerol with different fatty acid chain length (C10–C18) in an open reaction system. The studies included C18 with different unsaturation number (0–2). They presented results of reaction rates and product yields and concluded that better yields are obtained with higher fatty acid chain lengths, and that a double bond in the acid does not affect the triacylglycerol yield. However, the presence of two double bounds (linoleic acid, C18:2) showed a lower yield.

Flores et al⁷⁷ studied the equilibrium position in lipase mediated esterification of butanol with either saturated or unsaturated fatty acids of different chain lengths, or mixtures of them. They found that the ester mole fraction at equilibrium increases with the fatty acid chain length and for fatty acids with the same carbon number, the highest values are found for unsaturated fatty acids. For reaction systems containing two saturated fatty acids, a slightly higher mole fraction is obtained for the fatty acid with the higher chain length, while for mixtures consisting of saturated and unsaturated fatty acids, the mole fractions of the unsaturated esters are lower than those of the saturated ones, regardless the chain length of the fatty acid.

These observations lead us to believe that the reactivity of a fatty acid is clearly related to its structure. The different reactivities of saturated and unsaturated acids may be due to molecular geometries. In fact, saturated fatty acids are known to allow facile packing and close intermolecular interactions. The tetrahedral bond angles on carbon results in a molecular geometry for saturated fatty acids that is relatively linear although with zigzags. On the other hand, the introduction of double bonds in the hydrocarbon chain produces one or more bends in the molecule. These molecules cannot be packed as closely together as straight molecules and their intermolecular interactions are much weaker.

Meanwhile, Baldwin et al.⁷⁸ have reported the hydrophobic acceleration of Diels-Adler reactions. When substances with non-polar regions are dissolved in water, they tend to associate so as to diminish the hydrocarbon-water interfacial area. This “hydrophobic effect” is a principal contributor to substrate binding in enzymes and to the self-association of amphiphiles in micelles or membranes. The Diels-Alder reaction

brings together two non-polar groups. The reaction of cyclopentadiene with butenone shows more than a 700-fold acceleration in water compared with the rate in 2,2, 4-trimethylpentane. The rate in methanol is intermediate, but closer to that in the hydrocarbon solvent. Moreover, they found that the Diels-Alder reaction of anthracene-9-carbinol with N-ethylmaleimide is slower in polar solvents than it is in non-polar hydrocarbon solutions with the exception of water in which the rate is very fast. Only the hydrophobic effect seems capable of explaining this exceptional behavior of water.

Although the reactions we have performed are not in aqueous media, the hydrophilicity of the sugar and hydrophobicity of the fatty acid chain are factors that must be taken into consideration. We can then speculate that the increased conversion with increasing fatty acid chain length may be related to both steric and hydrophobic considerations.

Moreover, amphiphilic molecules may also present effects associated with micelle formation. Over a certain small concentration range, termed the critical micelle concentration, such molecules form aggregates called micelles. These micelles are responsible for altering the rates of organic reactions in aqueous solutions of surfactants⁷⁹. Basically, surfactants capable of forming aggregates in aqueous solution are molecules of the type **RX**, in which **R** is a hydrophobic moiety, usually a straight-chain alkyl group of 8 to 18 carbon atoms, and **X** is a hydrophilic group. These surfactants can lead to rate increases of 5 to 1000- fold⁷⁹.

In our experimental system, we observe an obvious increase in reaction rate for fatty acids with an alkyl chain length above 8 carbons. Accordingly, we may be detecting an increased reactivity with increasing chain length due to micelle formation and

changing solution properties. Increasingly hydrophobic surfactants have decreasing values of the critical micelle concentration, thus forming micelles more easily. Furthermore, increasingly hydrophobic surfactants form increasingly large micelles (in terms of both molecular weight and number of molecules per micelle)⁷⁹.

The kinetics of organic reactions occurring in micellar systems are dominated by two factors: *electrostatic* interactions and *hydrophobic* interactions between the micellar phase and reactants, transition states, and products. For instance, the degree of rate augmentation experienced on micellation for the hydrolysis of sodium alkyl sulfates increases very substantially as the length of the alkyl chain is increased from 10 to 18 carbon atoms.⁸⁰

Also, the attack of N-myristoyl-L-histidine on p-nitrophenyl esters is markedly accelerated in the presence of micelles formed from hexadecyltrimethylammonium bromide while the attack of N-acetyl-L-histidine is not and, furthermore, the reaction of the former nucleophile with p-nitrophenyl hexanoate is much faster than that with the corresponding acetate.⁸¹ All in all, kinetic effects in micellar systems are accentuated when hydrophobic interactions between substrate and surfactant are accentuated.

This effect of like attracts like, and of hydrophobic interactions, was also observed by Sealy et al.⁶² who studied cellulose esters having alkyl substituents in the range of C12 to C20 and found that side chain crystallization took place. Side-chain crystallization has been reported in other polymers with stiff backbones and long n-alkyl side chains, such as poly(n-alkyl thiophenes)⁸² and poly(n-alkyl α ,L-glutamates)⁸³. These systems showed the first appearance of side-chain crystallization when the substituent size reached a length of C10 or C12

A dimensionally accurate molecular model of a section of a cellulose eicosanoate chain demonstrates the ability of the side chains and the molecule as a whole to adopt a conformation favorable for side-chain crystallization.

Dimensionally accurate molecular model of the structure of cellulose eicosanoate (C20) with the ester substituents aligned regularly as they might be in a crystal lattice. The illustration was constructed by repeating the trialkanoyl cellobiose unit which was drawn using the computational chemistry software package "Hyperchem" (Autodesk Inc., Sausalito, CA). The cellobiose unit was depicted in an all-chair β -1,4 conformation optimized by MM+ calculation. The alkyl chains drawn in all-anti conformation were also optimized using MM+ calculation before being attached to the optimized cellobiose unit

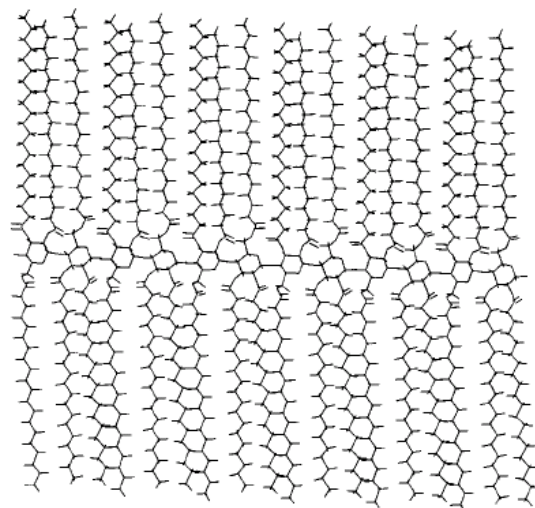


Figure 20: Molecular model of the structure of cellulose eicosanoate (C20)⁶²

It has also been found^{82,83} that crystallization is limited to the part of the side chain extending beyond the first eight or nine methylene units, and that crystals incorporate overlapping or interdigitated side chains from neighboring main chains.

Perhaps the effect observed with increasing chain length is due to longer fatty acids being able to “feel each other” better during the reaction. As a result, once the first fatty acid attaches itself to the sugar, the sugar becomes more “welcoming” for the other fatty acids. Consequently, the longer the hydrophobic tail, the less hydrophilic the sugar becomes, the more attractive the environment around the sugar becomes to other fatty acid chains, which are then closer in proximity and more likely to react.

In fact, fatty acid chain interaction is a topic that is very much of interest in self assembly chemistry, but also in biology as the interactions of fatty acids with proteins, serums and enzymes can affect varied human functions. Consequently, it seems plausible

that the chain length effect observed in the transesterification reaction is principally due to attractive forces.

Consequently, hydrophobic interactions are clearly at play when dealing with long chain alkyl groups, and must be considered in terms of how they may help or hinder a particular process.

Nevertheless, it should be noted that contradictory results have also been reported. Pederson et al.⁸⁴ studied a mixed reaction medium, favoring the solubility of the carbohydrate in order to evaluate the effect of fatty acid chain length on a lipase catalysed esterification of native disaccharides using an immobilized preparation of *C. antartica* lipase B. With sucrose as the acyl acceptor the 6-*O*-acyl and 6-*O*-acyl monoesters were formed with fatty acids of chain length C-4 and C-10 while the 6,6-*O*-acyl diester was formed only with butanoic acid (C-4:0) as acyl donor. The highest initial reaction rate and yield were obtained with the shortest chain length of the acyl donor. Initial reaction rates and ester yields were affected by the solubility of the disaccharide, with higher reaction rates and yields with maltose than with sucrose, while no formation of esters were observed with either cellobiose or lactose as acyl acceptors.

5.1.3 Sucrose Esters

Subsequently, the same transesterification reaction that was used to make glucose esters was carried out using sucrose as the sugar alcohol and was again monitored by ³¹P NMR. The spectrum of sucrose shows 7 peaks (Figure 21), the most intense of these peaks actually corresponds to two hydroxyl groups whose resonances overlap.

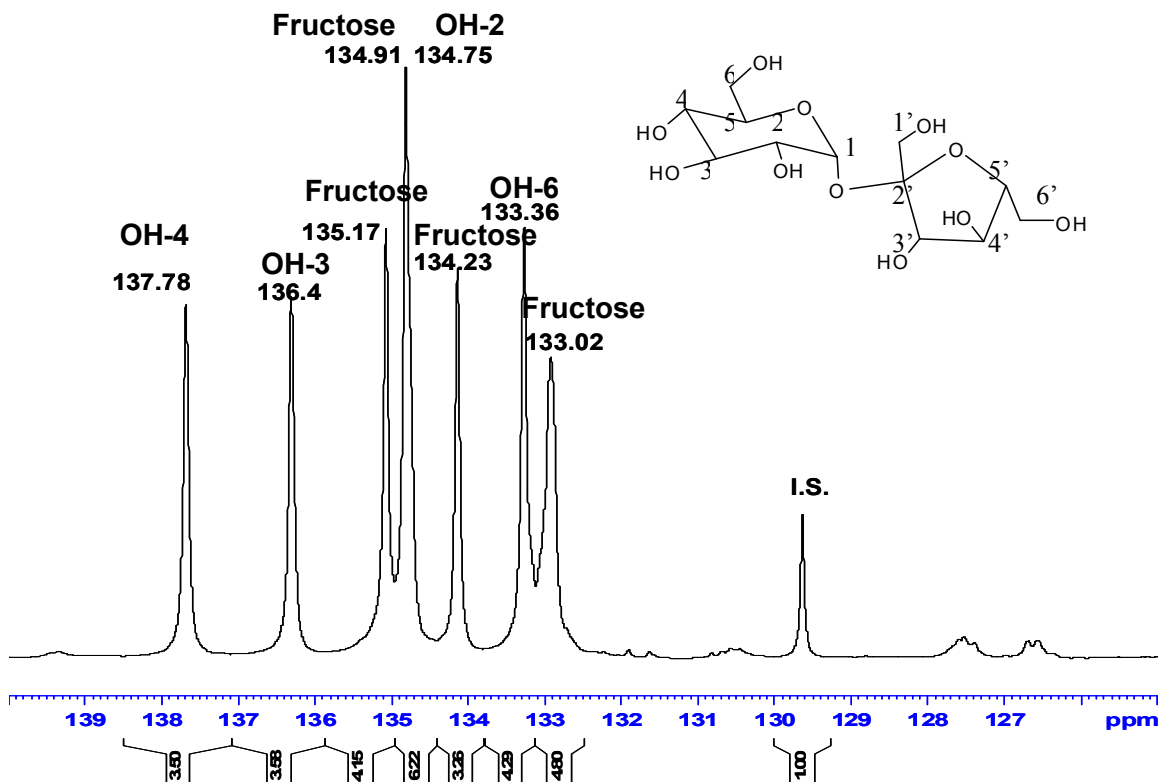


Figure 21: ^{31}P NMR spectrum of sucrose phosphitylated with Reagent 1

Table 3: ^{31}P NMR-Chemical shifts for derivatized sucrose

Hydroxyl Group	Chemical Shift (ppm)
OH-4 from glucose	137.78
OH-3 from glucose	136.4
From fructose	135.17
From fructose	135.02*
OH-2 from glucose	134.87*
From fructose	134.23
OH-6 from glucose	133.36
From fructose	133.02

*The two hydroxyls overlap and their chemical shift appears at 134.91ppm.

Fructose is composed of a glucose moiety and a fructose moiety. As a result, the peaks can be assigned as coming from fructose or glucose. Furthermore, the glucose peaks can be assigned to specific hydroxyls based on the work of Argyropoulos et al.⁶⁶

We also attempted to identify the hydroxyl from the fructose moiety of sucrose by applying 2 dimensional long-range (3 bond) HETCOR ^1H - ^{31}P NMR (Figure 22).

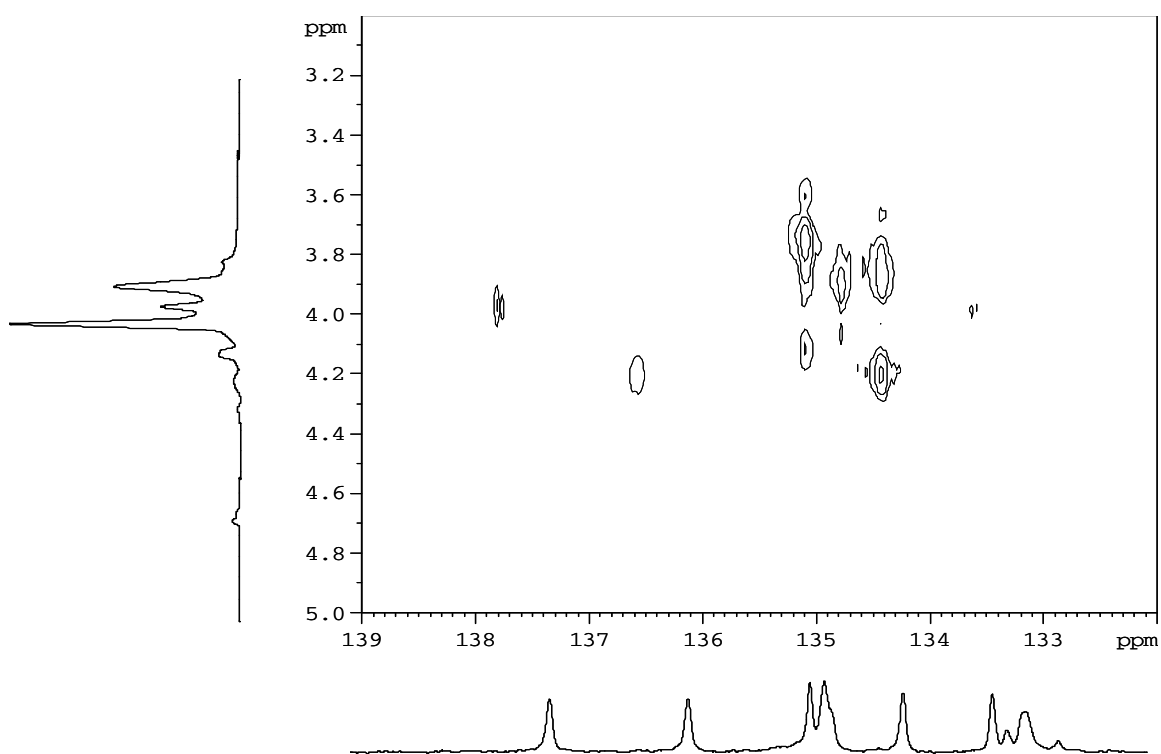


Figure 22: 2D ^1H - ^{31}P NMR spectrum for sucrose

Unfortunately, the 2D NMR spectrum did not help with the identification of the hydroxyls from fructose. In fact, when looking at the proton spectrum, we see that the hydrogen resonances overlap and that no distinction can be made. It seems that by phosphitylating the sugar, the chemical environment of all hydrogen atoms becomes very

similar due to their neighboring phosphorous and oxygen atoms. Consequently, we were unable to assign resonances to the fructose hydroxyls and will simply refer to them as hydroxyls from fructose in the following experiments.

As for glucose, we studied the hydroxyl group reactivity of sucrose throughout the transesterification reaction.

For the reaction mixture of sucrose esters of butyric acid (C4), we notice that all hydroxyl groups seem to react uniformly (Figure 23).

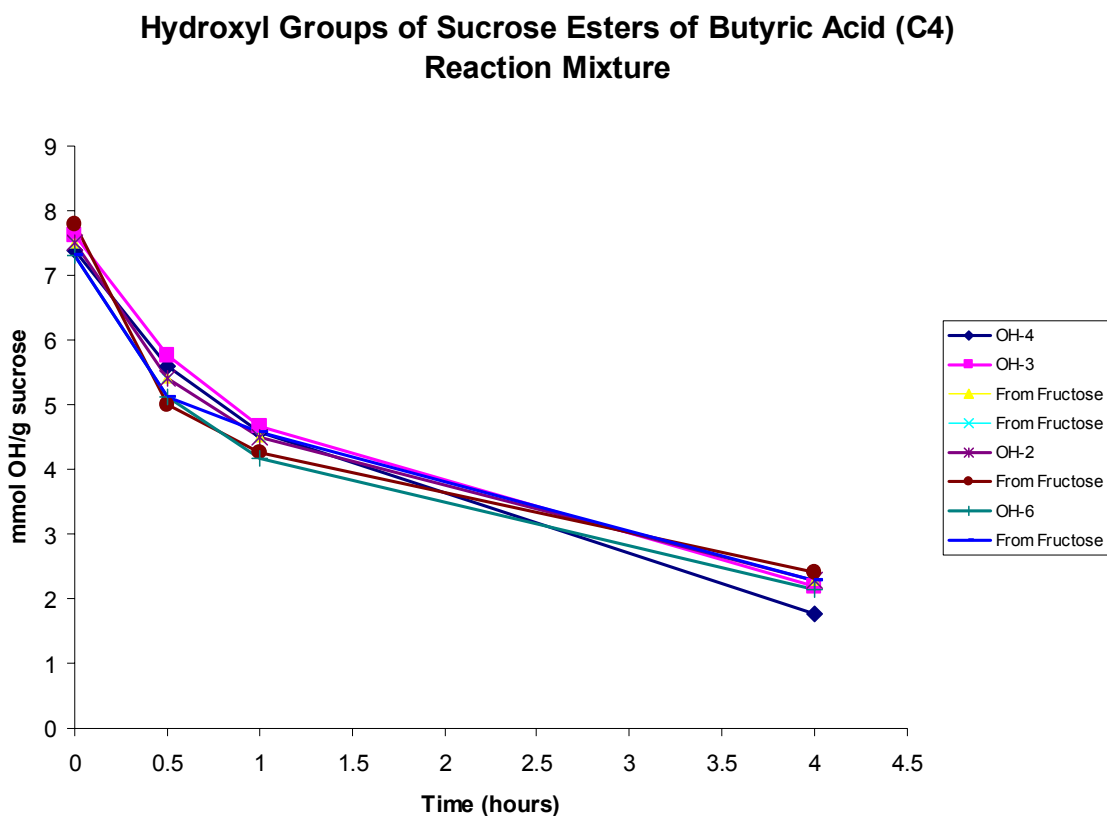


Figure 23: Hydroxyl groups of sucrose esters of butyric acid (C4)

In the reaction mixture of sucrose esters of caprylic acid (C8), we notice two hydroxyl groups, both originating from the fructose moiety of sucrose that are slower to react than the others. At the end of the reaction, they appear to be 0.3 mmol OH/g sucrose and 0.7 mmol OH/g sucrose, respectively, more abundant than the other hydroxyl groups.

Hydroxyl Groups of Sucrose Esters of Caprylic Acid (C8) Reaction Mixture

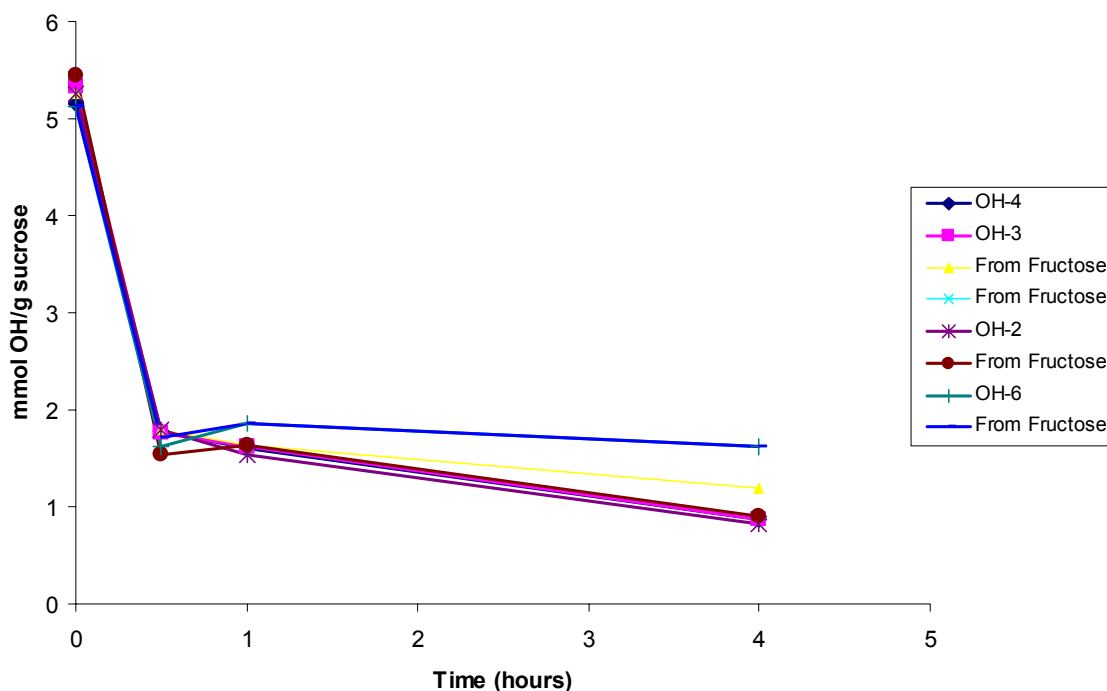


Figure 24: Hydroxyl groups in sucrose ester of caprylic acid (C8) reaction mixture

This observation can also be made when looking at the reaction mixture of sucrose esters of lauric (C12) and stearic acids (C18) (Figure 25 and Figure 26 respectively)

Hydroxyl Groups in Sucrose Ester of Lauric Acid (C12) Reaction Mixture

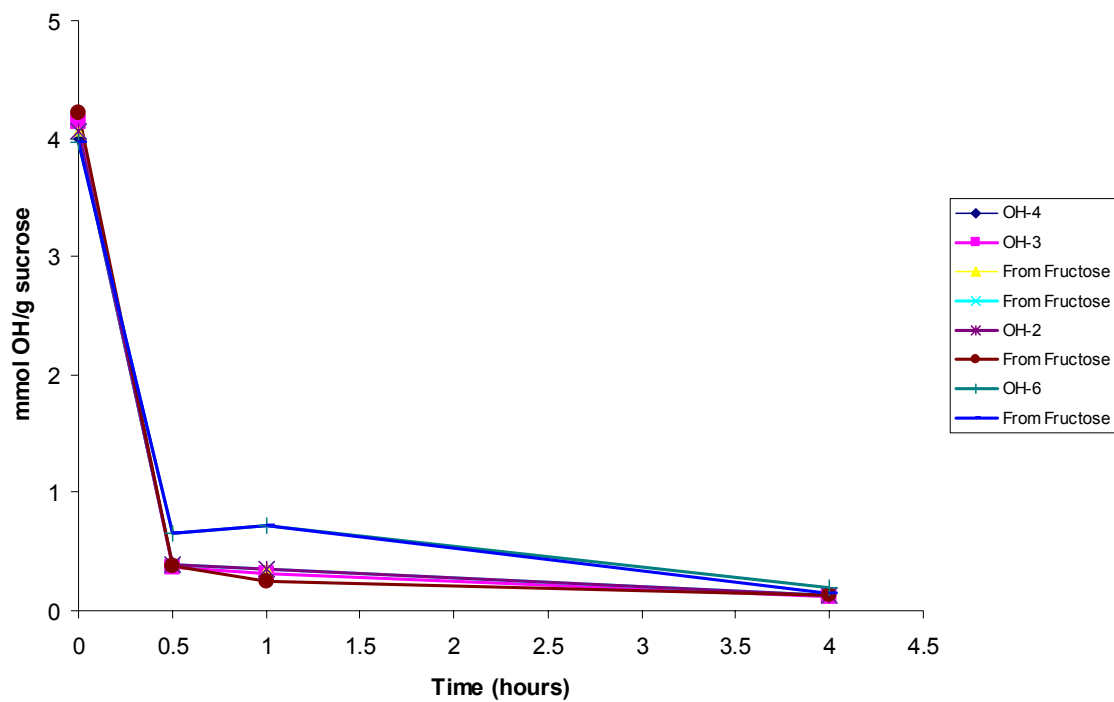


Figure 25: Hydroxyl groups in sucrose ester of lauric acid (C12) reaction mixture

Hydroxyl Groups of Sucrose Esters of Stearic Acid (C18) Reaction Mixture

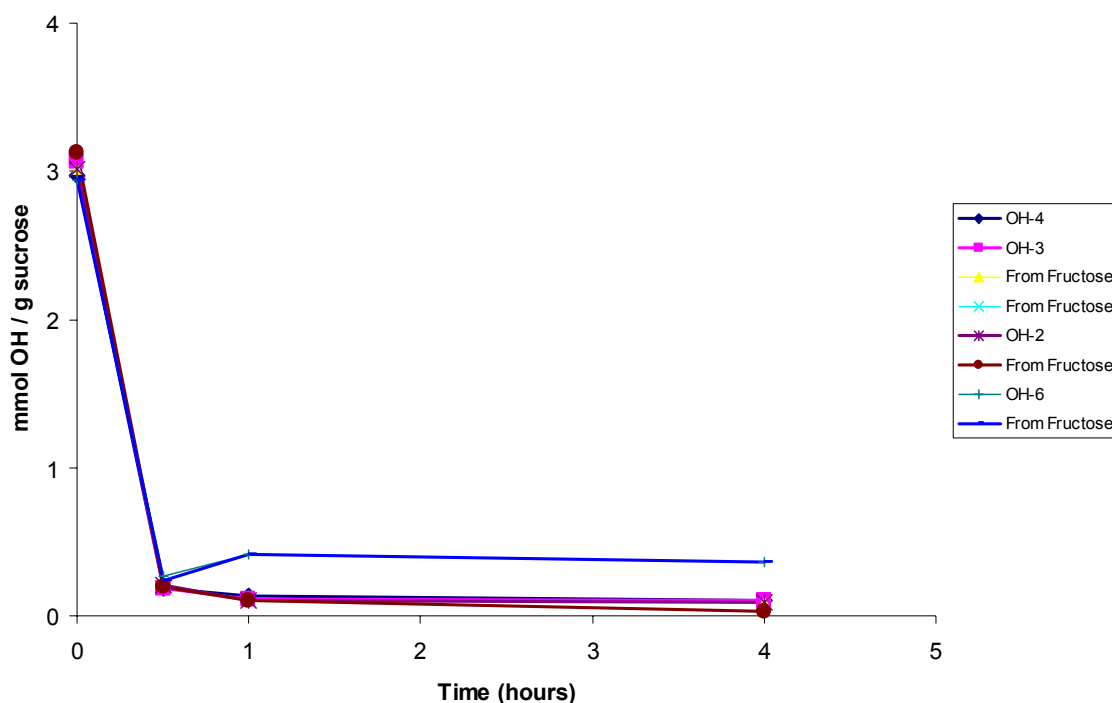


Figure 26: Hydroxyl groups in sucrose ester of stearic acid (C18) reaction mixture

Consequently, we can conclude that the fructose moiety of sucrose appears to be less reactive. This observation has previously been made by Molinier et al.³⁰, who found that in the transesterification of sucrose with N-decanoylthiazolidinethione in DMF using different bases, the reaction was rather selective on the glucose moiety.

The reaction mixture of sucrose esters of arachidic acid (C20) (Figure 27) shows hydroxyl groups which react quite uniformly, as observed for glucose.

Hydroxyl Groups in Sucrose Ester of Arachidic Acid (C20) Reaction Mixture

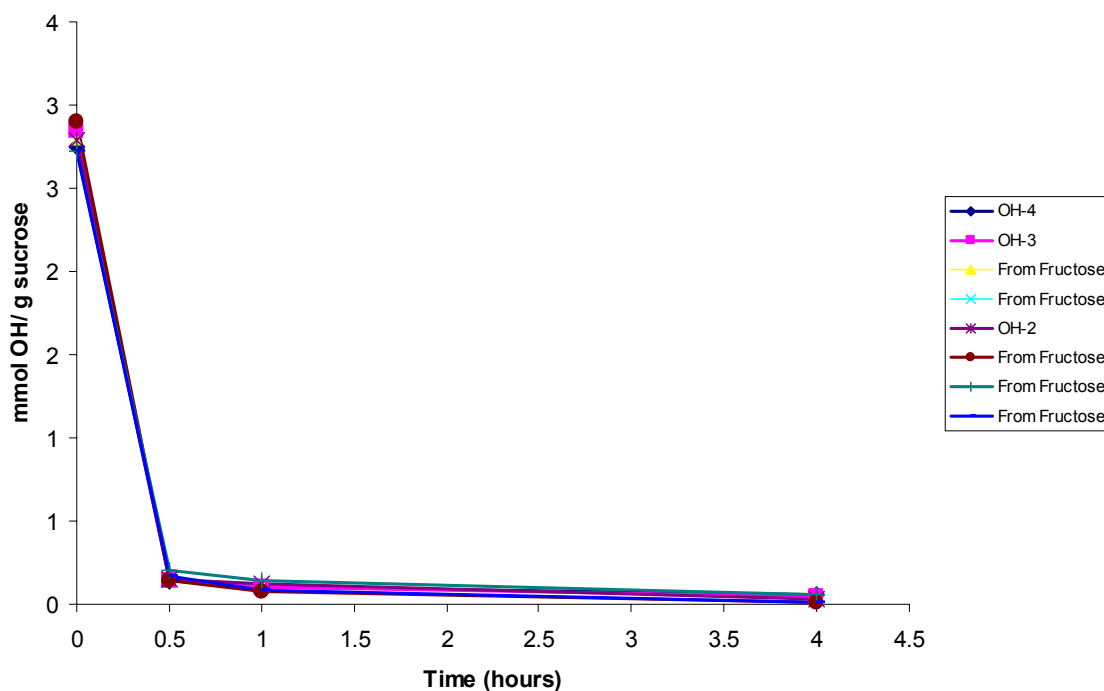


Figure 27: Hydroxyl groups in sucrose ester of arachidic acid (C20) reaction mixture

Finally, the total hydroxyl conversion for the sucrose esters (Figure 28) also shows the same trend apparent in the glucose ester reaction mixtures. Indeed, we once again notice that more hydroxyl groups are esterified as the fatty acid chain length increases.

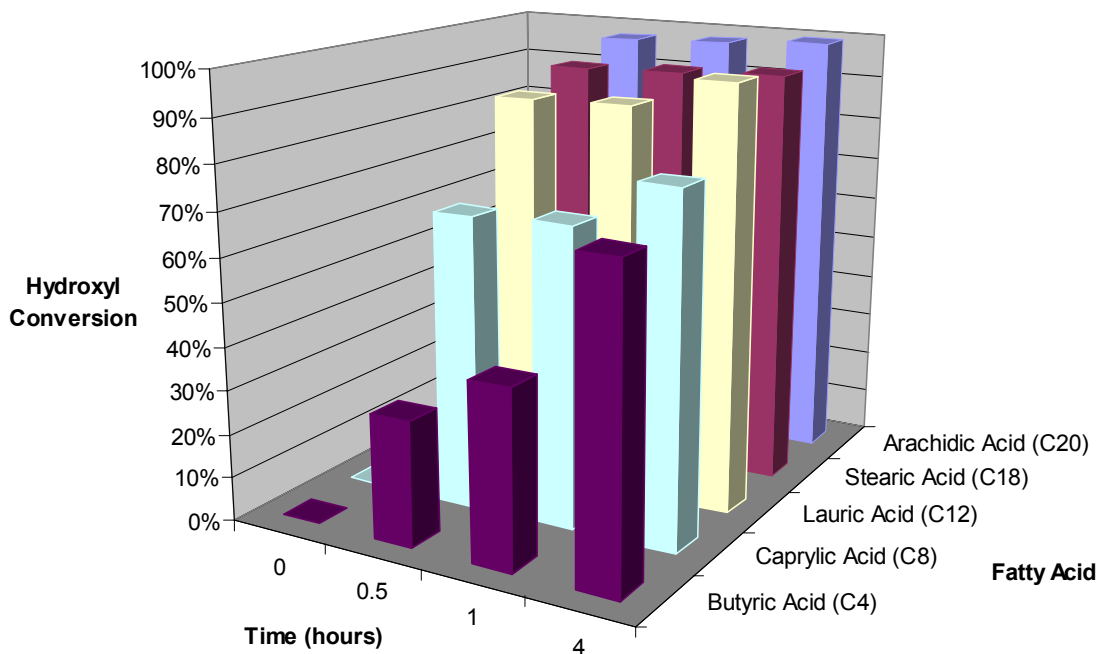


Figure 28: Sucrose ester reaction mixtures- Hydroxyl group conversion

5.1.4. Cellobiose Esters

The transesterification reaction was also studied with cellobiose as the sugar substrate. Cellobiose has 8 hydroxyl groups which can be clearly distinguished in the phosphorous NMR spectrum (Figure 29). They have been previously assigned⁶⁶ and although the experimental shifts vary quite a bit from those in the literature, they can be well followed during the reaction (Table 4). It is important to note that as for glucose, cellobiose also has a free anomeric carbon, so cellobiose may assume alpha and beta anomers at that site. The small peaks visible in the phosphorous spectrum are due to the α anomer.

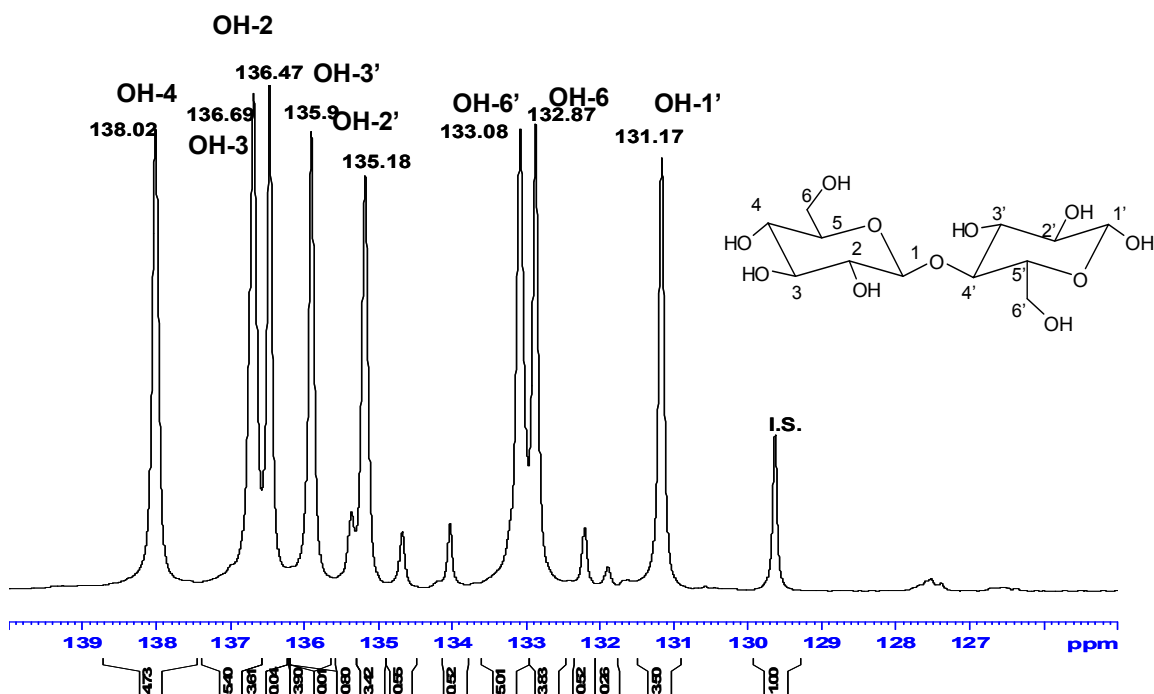


Figure 29: ^{31}P NMR spectrum of cellobiose

Table 4: ^{31}P NMR- Chemical shifts for derivatized cellobiose

Hydroxyl Group	Chemical Shift (ppm) From Literature ⁶⁶	Chemical Shift (ppm)
OH-4	138.3	138.02
OH-3	137.1	136.69
OH-2	136.6	136.47
OH-3'	136.2	135.9
OH-2'	135.6	135.18
OH-6'	133.8	133.08
OH-6	133.4	132.87
OH-1'	131.9	131.17

When looking at the reaction mixture for cellobiose esters of butyric acid (C4) (Figure 30), we notice that the hydroxyls all seem to react similarly, although differences in relative abundance are apparent at the end. In fact, it appears that OH-1' and OH-3'

react slightly more than the others and that OH-2, OH-3 and OH-4 are slightly less reactive.

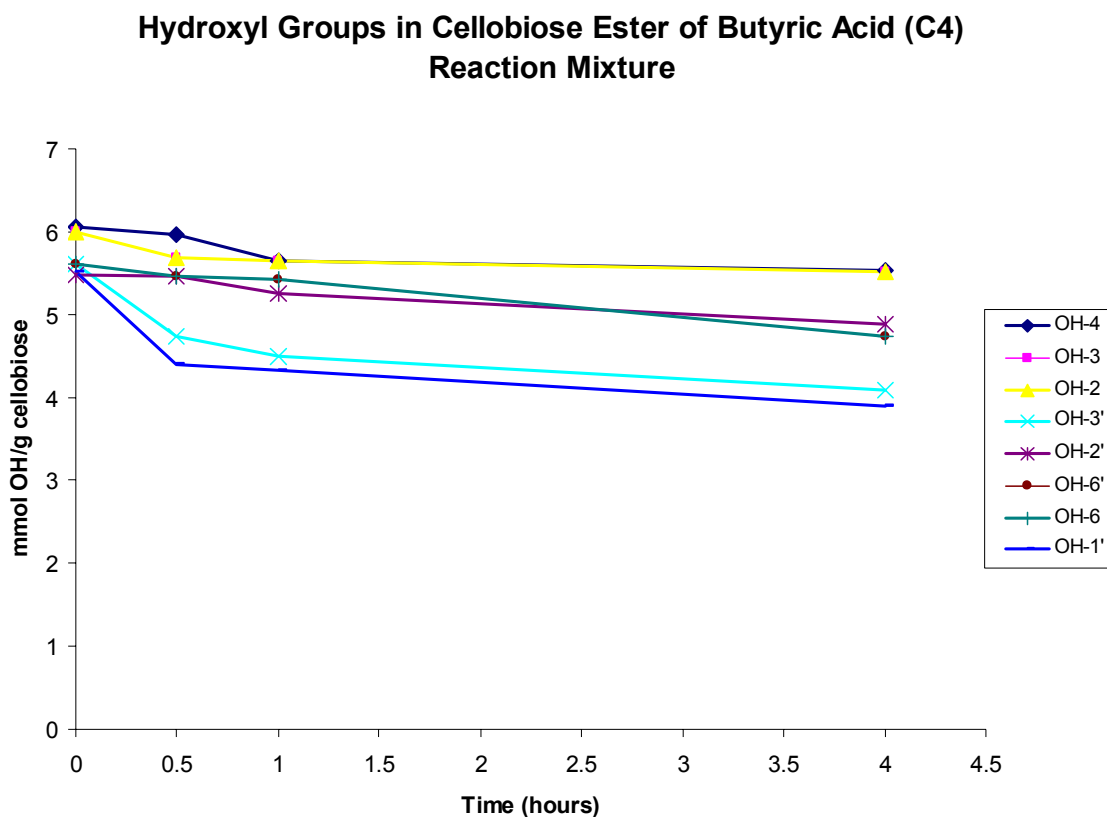


Figure 30: Hydroxyl groups in cellobiose ester of butyric acid (C4) reaction mixture

The reaction mixture of cellobiose esters of caprylic acid (C8) (Figure 31) shows similar trends. The hydroxyls appear to react similarly, and again OH-1' and OH-3' appear to be the most reactive. However, we should take into account the difference in relative abundance of hydroxyl groups at time $t=0$, and consider the possibility that the final values are affected by experimental error.

Hydroxyl Groups in Cellobiose Ester of Caprylic Acid (C8) Reaction Mixture

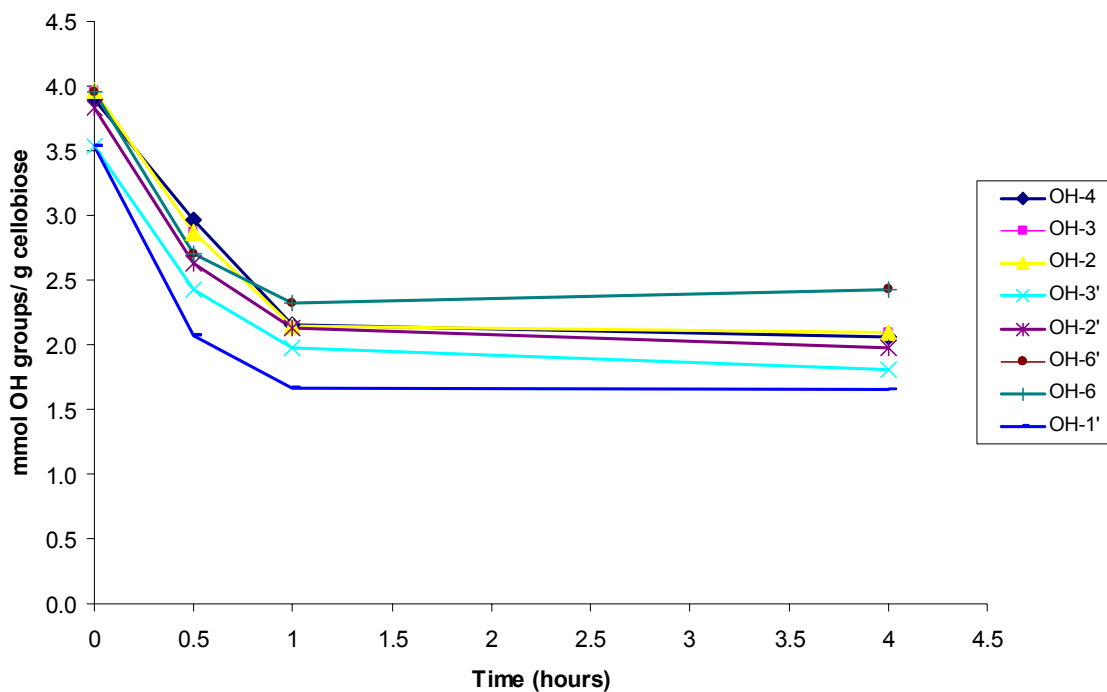


Figure 31: Hydroxyl groups in cellobiose ester of caprylic acid reaction mixture

When the reaction is performed with lauric acid (Figure 32), the hydroxyl groups appear to react comparably, except for OH-6 which lags behind initially. Nevertheless, at the end of the reaction, the unreacted hydroxyls all seem to be present in similar quantities.

Hydroxyl Groups in Cellobiose Ester of Lauric Acid (C12) Reaction Mixture

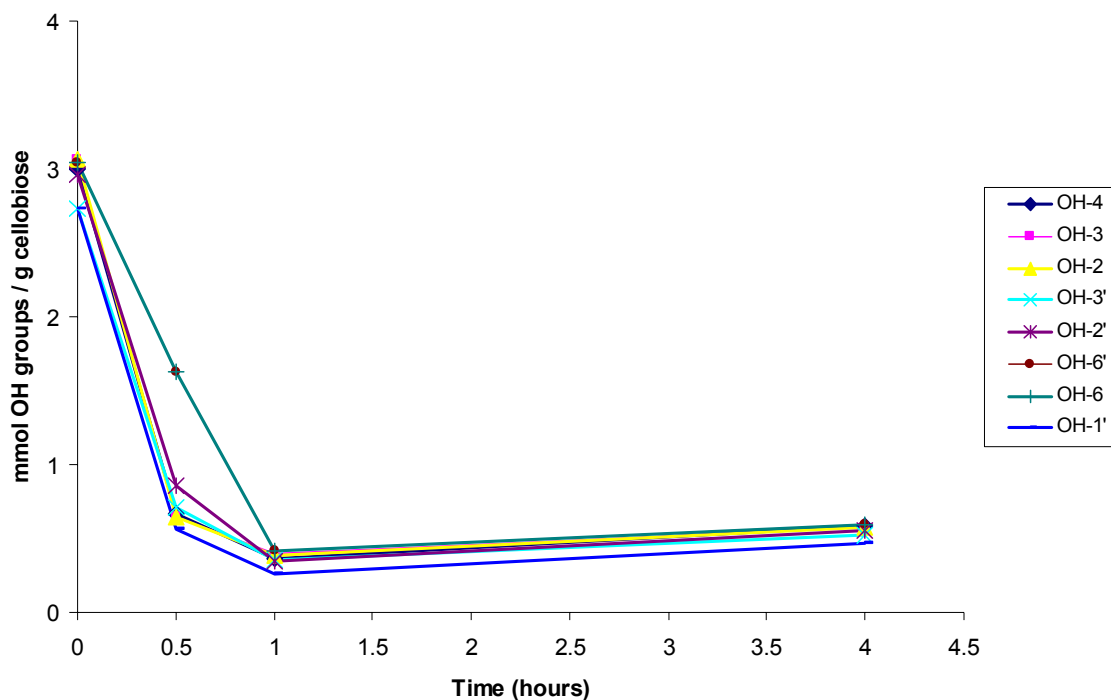


Figure 32: Hydroxyl groups in cellobiose ester of lauric acid (C12) reaction mixture

Similarly, for the reaction mixture of stearic acid (C18) and for that of arachidic acid (C20), the hydroxyls react uniformly, with negligible differences in molar amounts visible at the end of the reaction.

Hydroxyl Groups in Cellobiose Ester of Stearic Acid (C18) Reaction Mixture

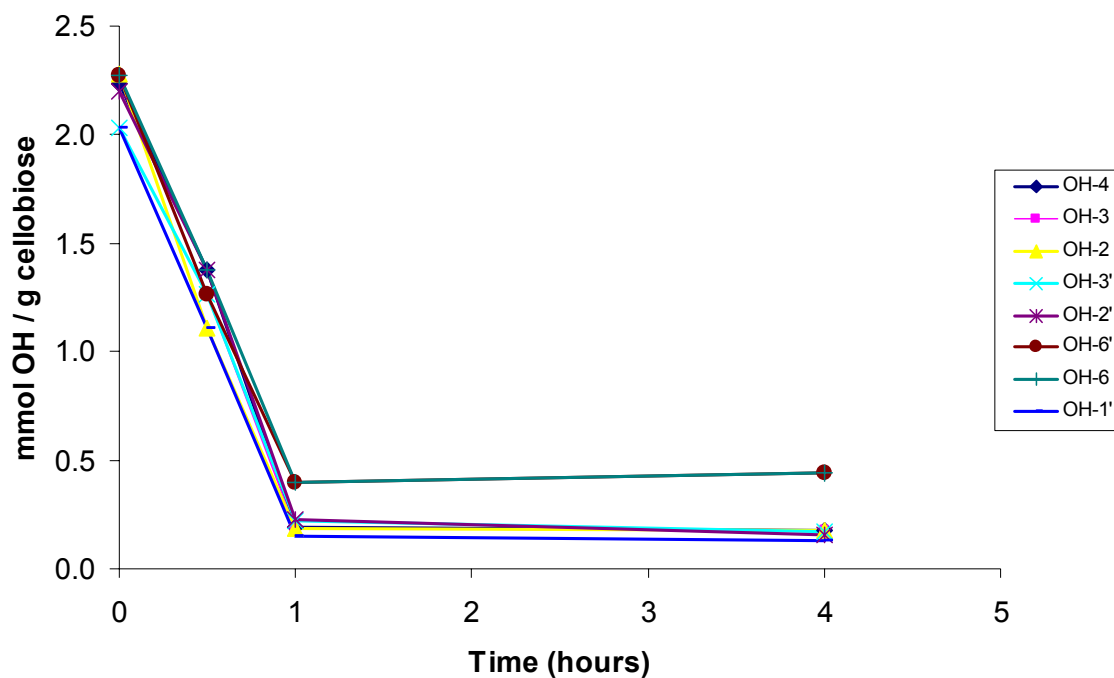


Figure 33: Hydroxyl groups in cellobiose ester of stearic acid (C18) reaction mixture

In the end, it appears that cellobiose hydroxyls react in the same way, without great variations in relative reactivities. This may be linked to the regularity of cellobiose, as a molecule. It is composed of two glucose moieties, making it a homogeneous compound. This may explain why all hydroxyls seem to be equally favorable to react.

Hydroxyl Groups in Cellobiose Ester of Arachidic Acid (C20) Reaction Mixture

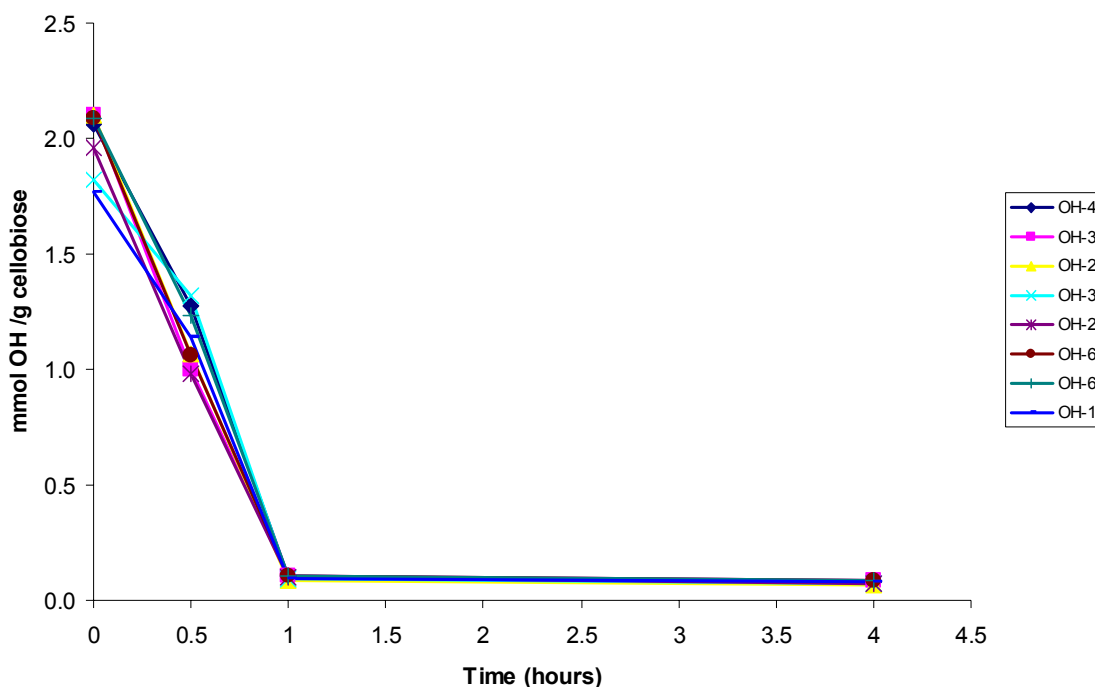


Figure 34: Hydroxyl groups in cellobiose ester of arachidic acid (C20) reaction mixture

The hydroxyl conversion for cellobiose shows the same trend as for glucose and sucrose. It appears that the longer chain fatty acids allow for a higher conversion of hydroxyl groups to ester linkages (Figure 35).

Finally, it appears that phosphorous NMR is a powerful tool for following hydroxyl groups during the course of a reaction, such as the transesterification reaction. Moreover, it appears that a variety of steric and hydrophobic forces play a key role in determining reactivity, and ultimately, it appears that longer chain fatty acids exert attractive forces and help the transesterification reaction proceed.

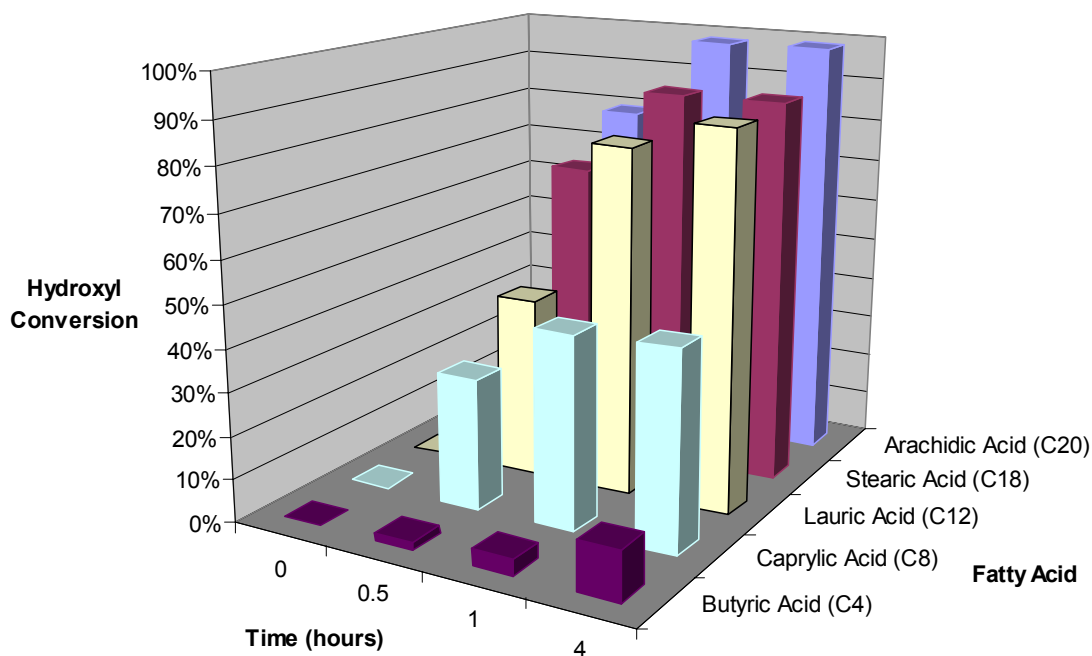


Figure 35: Cellobiose ester reaction mixture- Hydroxyl group conversion

5.2. Antimicrobial Activity

Glucose, sucrose and cellobiose esters of stearic acid (C18), as well as glucose and sucrose esters of lauric acid (C12) were obtained from the reaction mixtures using a two solvent methanol /hexane liquid/liquid extraction. The compounds were then dissolved in chloroform and a precise quantity was dropped onto cellulose disks. The chloroform was allowed to evaporate and the disks were then tested for antimicrobial activity against *E.coli*. using the disk diffusion method.

Glucose and sucrose esters of stearic acid (C18) showed no inhibition. Cellobiose esters of stearic acid showed no clear inhibition, still there may be some partial inhibition.

On the other hand, the esters of lauric acid (C₁₂) did show some partial inhibition. The glucose esters of lauric acid showed a 1 mm zone of asymmetric turbidity around disk. The sucrose esters of lauric acid had an even more pronounced effect exhibiting a 2 mm zone of asymmetric turbidity around the cellulose disk.

These observations can be rationalized as follows. First of all, we must consider the fact that we are dealing with sugar polyesters of fatty acids, which are not known for their antimicrobial properties, but rather their organoleptic properties. Consequently, we cannot expect an important inhibitory effect.

In addition, the tests were performed against *E.coli*, a gram negative bacterium. Sugar esters of fatty acids have not been proven to be effective against such bacteria⁴². Finally, we do see a dependence of the antimicrobial effect on the fatty acid used. Polyesters of stearic acid show little or no microbial activity whereas polyesters of lauric acid show partial inhibition of microbial growth. This observation is supported by findings that claim that lauric acid (C₁₂) is the most active saturated fatty acid against most bacteria⁸.

5.3. Cellulose Esters

We attempted to esterify cellulose by two different methods. In the first attempt, a transesterification reaction was employed and the fatty acids used were those of peanut oil and soybean oil.

5.3.1. Cellulose esters by transesterification

In previous experiments, natural oils were reacted to obtain the fatty acid methyl esters, which were used in a transesterification reaction with cellulose disks (Whatman paper #4) as the sugar substrate. These disks showed an interesting inhibitory effect against a series of bacterial cultures (*Bacillus megaterium*, *Bacillus subtilis*, *Staphylococcus aureus*, *Salmonella cholerae*, *Serratia marcescens*, *Enterobacter*, *Escherichia coli*), both gram positive and gram negative. However, the disk diffusion method, used to determine antimicrobial activity, is commonly used for compounds that diffuse from the solid support they are placed on, through the Petri dish where a bacterial lawn is growing, effectively stopping it.

The FT-IR spectrum for these disks clearly shows an ester carbonyl group present, as well as hydroxyls from cellulose. Nevertheless, the diffusion observed in the antimicrobial studies concerned us and we repeated this reaction in order to try and determine whether actual grafting of fatty acids onto cellulose occurs, or if the FAME is simply adsorbed on the cellulose disk.

The FT-IR spectrum run in reflectance mode for underivatized cellulose disks (Figure 36) shows a broad peak around 3300 cm^{-1} , characteristic of cellulose hydroxyls. The peaks that appear around 1000 cm^{-1} are due to C-OH stretching and the bands between 1100 cm^{-1} and 1200 cm^{-1} are due to C-O-C stretching. The FT-IR spectra of the cellulose disks, after reaction with FAME of peanut oil, washed with hexane and washed with methanol (Figure 37 and Figure 38), show cellulose hydroxyls, aliphatic -CH and -CH₂ groups from fatty acids, as well as an ester carbonyl. However, they also present a

peak around 2830 cm^{-1} characteristic of $-\text{OCH}_3$, which suggests that the fatty acid methyl esters have not been removed by washing.

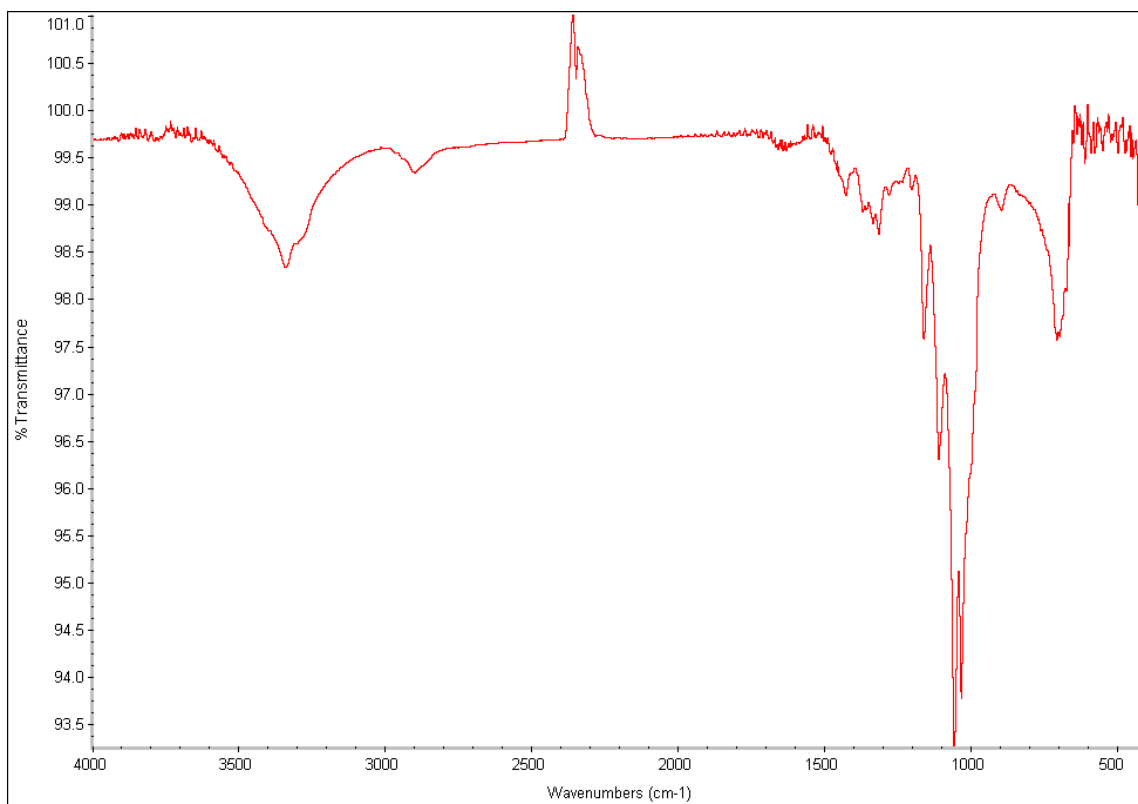


Figure 36: FT-IR for underivatized cellulose disks

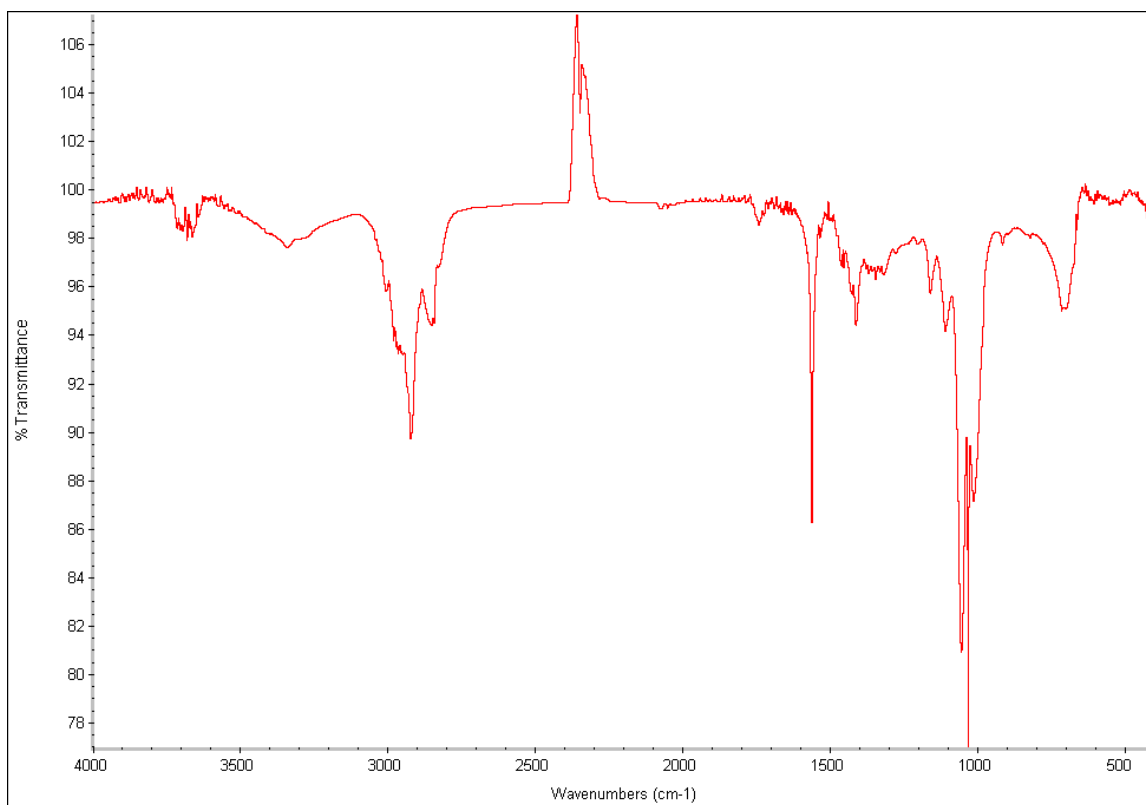


Figure 37: FT-IR spectrum of cellulose disks reacted with FAME of peanut oil , washed with hexane

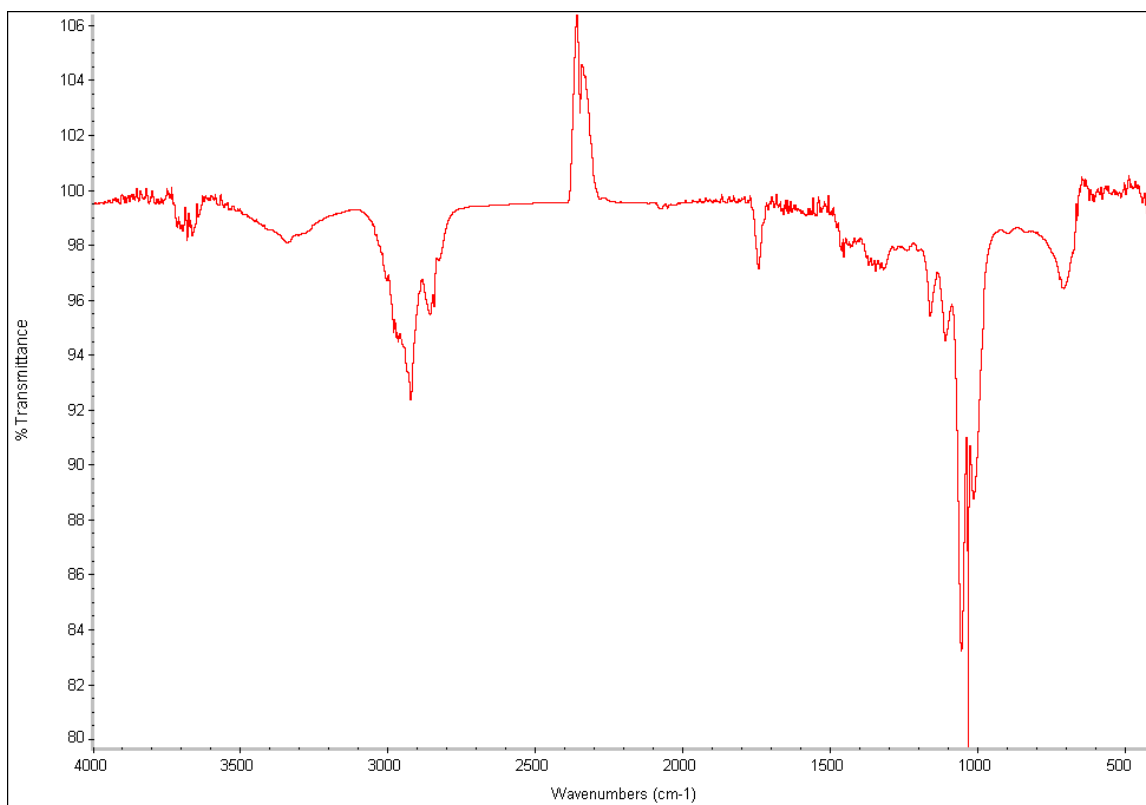


Figure 38: FT-IR spectrum of cellulose disks, reacted with FAME of peanut oil, washed with methanol

As a result, we decide to perform a hexane Soxholet extraction for the cellulose disks, reacted with peanut oil. The FT-IR spectra for these samples resemble those of unreacted cellulose, where the ester carbonyl is absent (Figure 39).

Consequently, it appears that the transesterification reaction performed with cellulose disks as sugar substrates was unsuccessful, and that the carbonyl esters in the FT-IR were due to unreacted FAME, which was on the disk. Furthermore, this explains the diffusion seen in the antimicrobial study. The zones of inhibition were effectively due to fatty acid methyl esters diffusing from the cellulose disk and not due to a cellulose ester of fatty acids.

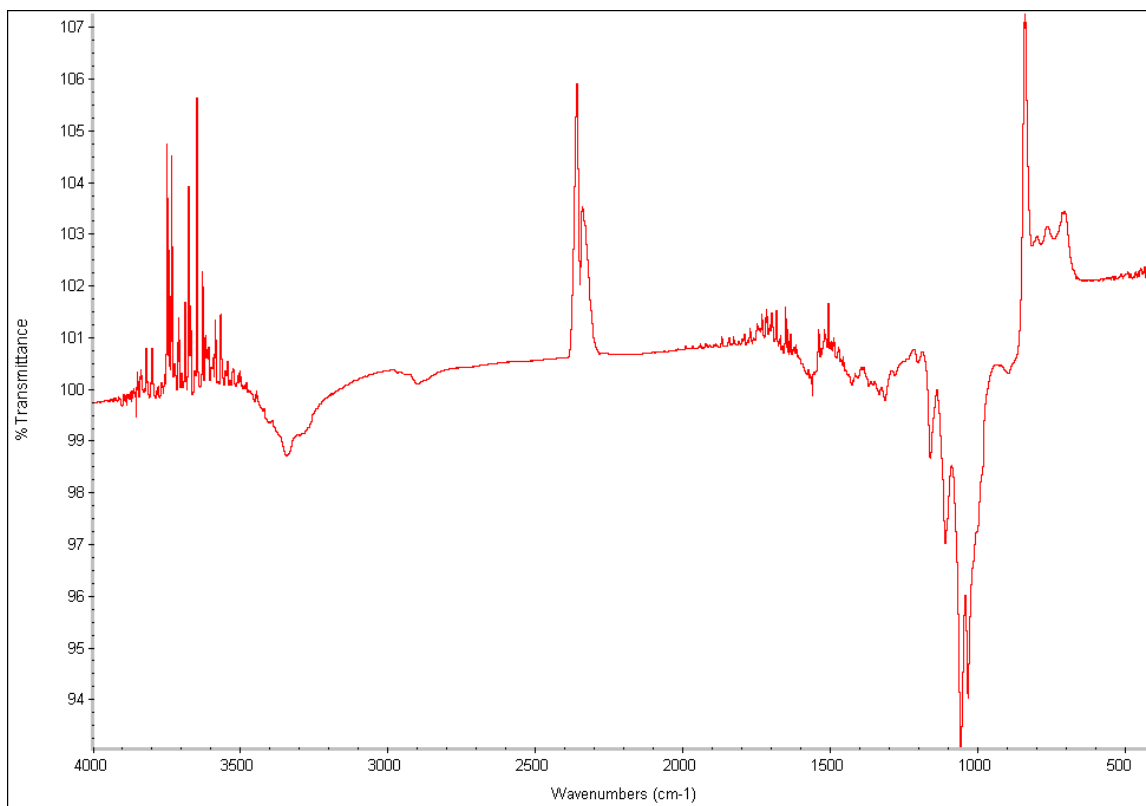


Figure 39: FT-IR spectrum of cellulose disks, reacted with FAME of peanut oil, after a hexane Soxholet extraction

5.3.2 Cellulose esters from the acyl chloride

We then attempted to graft stearic acid (C18) onto cellulose via its acyl chloride. The reaction was carried out as described previously and FT-IR spectra were taken in transmittance mode using KBr pellets. The FT-IR spectrum of underivatized microcrystalline cellulose is shown in Figure 40. Microcrystalline cellulose was used instead of filter disks in order to provide a larger available surface area for the reaction.

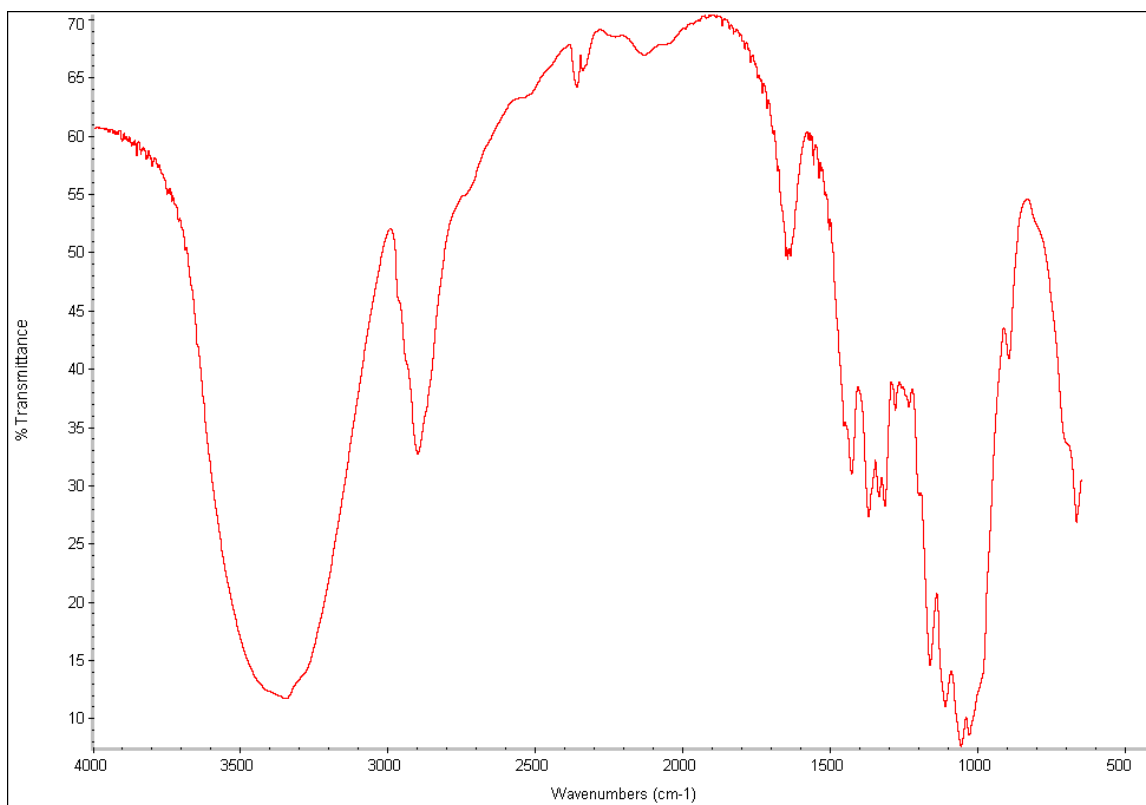


Figure 40: FT-IR spectrum of underivatized microcrystalline cellulose

The OH stretch of the cellulose hydroxyls can clearly be seen between 3600 cm^{-1} and 3200 cm^{-1} . Between 3000 cm^{-1} and 2800 cm^{-1} , we observe the -CH stretch and C-OH and C-O-C stretching is apparent between 1300 cm^{-1} and 1000 cm^{-1} . A small peak can also be seen around 1680 cm^{-1} due to the aldehyde in the reducing ends of cellulose.

The spectrum of stearic acid (Figure 41) shows a carbonyl stretching band which appears around 1700 cm^{-1} . The C-OH stretching band is very broad and appears like a shoulder at approximately 3400 cm^{-1} to 3100 cm^{-1} . The aliphatic -CH groups appear around 3000 cm^{-1} . Vibrations of medium intensity around 1430 cm^{-1} , 1240 cm^{-1} and 930 cm^{-1} are also associated with the C-OH group in carboxylic acids.

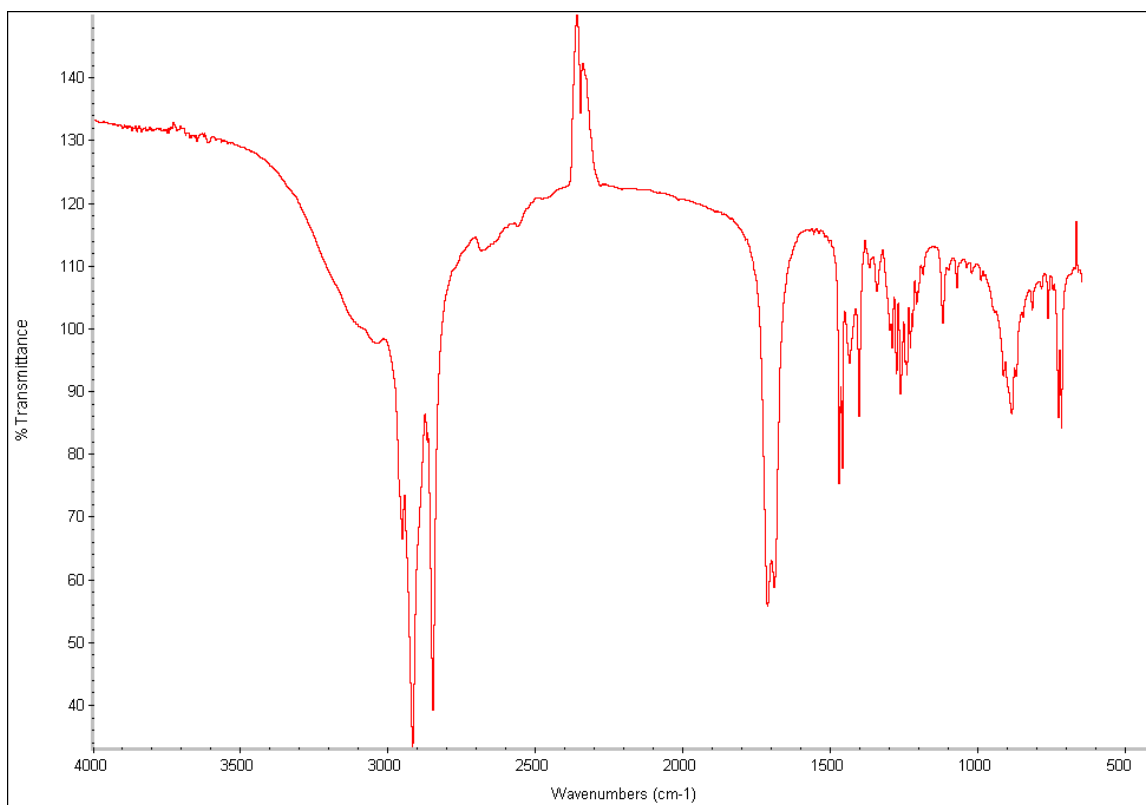


Figure 41: FT-IR spectrum of stearic acid

After having reacted 50mg of microcrystalline cellulose with the acyl chloride obtained from 5 g of stearic acid, we obtain a spectrum (Figure 42) with the following characteristics from stearic acid: aliphatic -CH and -CH_2 groups, a weak C-OH shoulder at a bit over 3100 cm^{-1} and vibrations of medium intensity around 1430 cm^{-1} , 1240 cm^{-1} and 930 cm^{-1} associated with the C-OH group in carboxylic acids. We also observe an antisymmetric C-O-C stretching mode near 1100 cm^{-1} that may be from cellulose but that is also characteristic of aliphatic esters. Finally, 3 bands appear in the carbonyl region. Of these, the band at 1720 cm^{-1} due to unreacted stearic acid. The band at 1800 cm^{-1} is due to the acyl chloride, and the band at around 1745 cm^{-1} is due to the ester. This confirms that the fatty acids were grafted onto cellulose.

The absence of the cellulose hydroxyls around 3500 cm^{-1} can be explained in two ways. They may not be visible in the infrared spectrum because of the relative amounts of cellulose and acid (1: 100 wt/wt) used. There are only 3 hydroxyls per glucose moiety in cellulose and some have been esterified, so the weak hydroxyl peak would not appear in the spectrum.

Another possibility is that the majority of the hydroxyls have reacted with the stearic acid to give cellulose esters. As a result, the ester carbonyl band is quite sharp, but no cellulose hydroxyls are left unreacted to be seen in the spectrum.

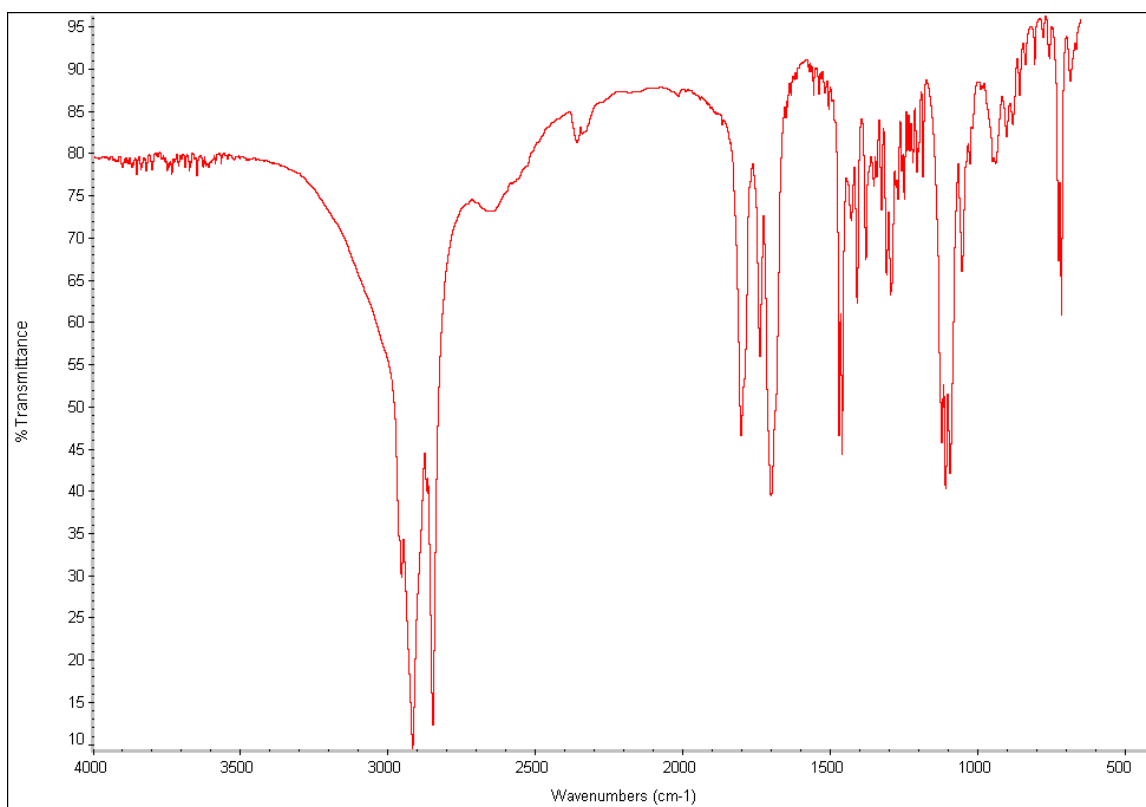


Figure 42: FT-IR spectrum for microcrystalline cellulose (50mg) reacted with stearyl chloride

The reaction was repeated using a larger weight ratio of cellulose to stearic acid (1/5 wt /wt). In this case, the FT-IR spectrum (Figure 43) shows the cellulose hydroxyls

(3300 cm^{-1}), the aliphatic -CH groups (2900 cm^{-1}), C-OH and C-O-C stretching is apparent between 1300 cm^{-1} and 1000 cm^{-1} . Three small peaks can also be seen in the carbonyl region around due to the aldehyde in the reducing ends of cellulose.

The stearic acid peaks probably overlap with those from cellulose and cannot be seen clearly.

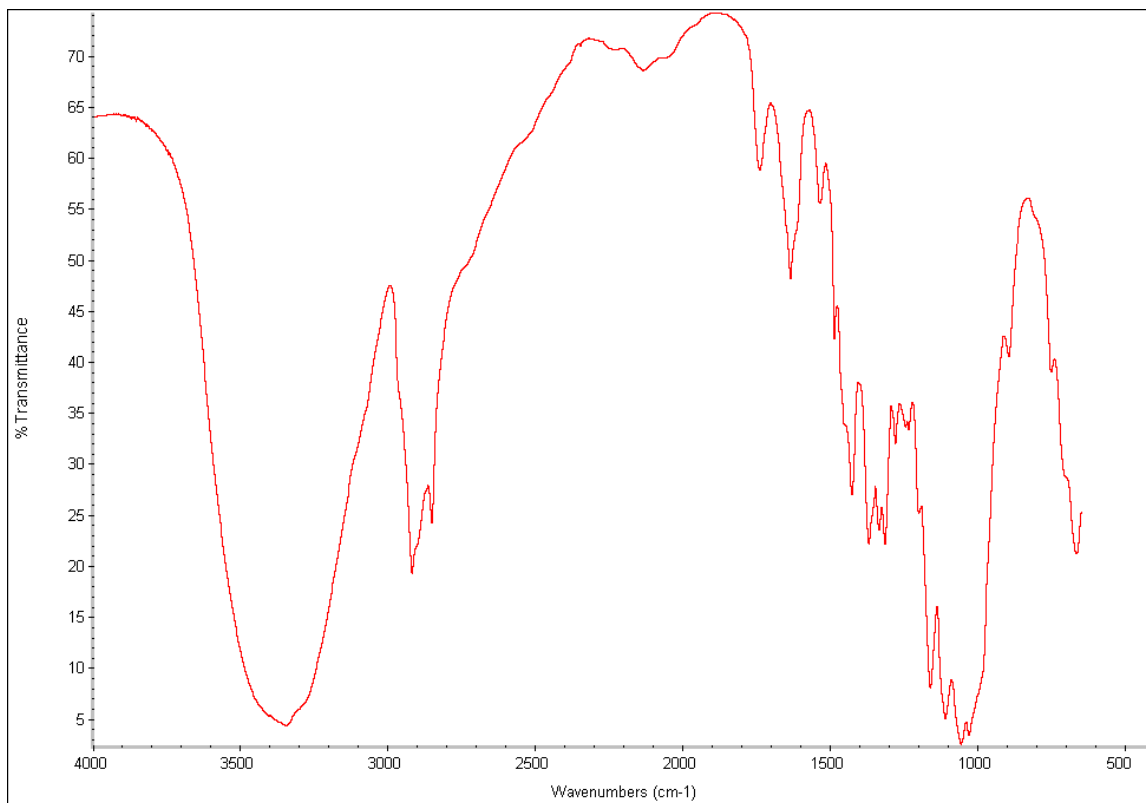


Figure 43: FT-IR spectrum of microcrystalline cellulose (1g) reacted with stearyl chloride

6. Conclusions

This study has allowed us to make the following conclusions.

Overall, for the study of the transesterification reaction:

- ^{31}P NMR proved to be a powerful tool for following the transesterification reaction
 - The reaction happens quite rapidly, with a large amount of hydroxyls being esterified during the first hour.
 - Hydrophobic interactions play a key role in the reactivity of the system.
- Hydroxyl group conversion increases as the fatty acid chain length increases.

For the glucose esters:

- The β form increases during the reaction
- OH-1 appears to be less reactive
- As the fatty acid chain length increases, the hydroxyls react more rapidly (hydrophobic interactions),
- More hydroxyl groups are esterified as the fatty acid chain length increases(hydrophobic interactions).

For the sucrose esters:

- The fructose moiety is less reactive.
- More hydroxyl groups are esterified as the fatty acid chain length increases.

For the cellobiose esters:

- The hydroxyl groups react uniformly.
- More hydroxyl groups are esterified as the fatty acid chain length increases

The antimicrobial study of sugar polyesters did not show any significant effects. However, esters of lauric acid appeared to be more effective antimicrobial agents. Finally, cellulose esters of fatty acids were successfully synthesized.

7. Suggestions for further research

Sugar esters of fatty acids are an interesting class of materials. They present appealing characteristics and their applications are many and varied.

One intriguing class of sugar esters are cellulose esters fatty acids. Natural cellulose fibers present high specific properties as well as advantages such as low cost, low density and biodegradability. Cellulose esters represent a class of commercially important, thermoplastic polymers with excellent fiber and film-forming characteristics. Long-chain aliphatic acid esters of cellulose (“fatty acid esters” or “waxy esters”) have been identified as potential biodegradable plastics due to the enzymatically labile ester bond and the natural abundance of both cellulose and fatty acids.

The modification of cellulose fiber surfaces by esterification with fatty acids is work that merits to be researched further. The effects of the fatty acid chain length, its degree of unsaturation, as well the degree of substitution on the cellulose are all factors that will affect the properties of the cellulose ester and, as a result, its applications.

Moreover, synthesis methods for such compounds should be researched. Methods which do not require using toxic solvents, that allow for easier product recovery and less cumbersome synthesis have tremendous potential for commercial applications.

Finally, research into synthesis methods using supercritical fluids may be the route to follow. It is a route that has proved invaluable in transesterification reactions producing biodiesel, and may present a potential synthesis pathway for sugar esters as well.

8. References

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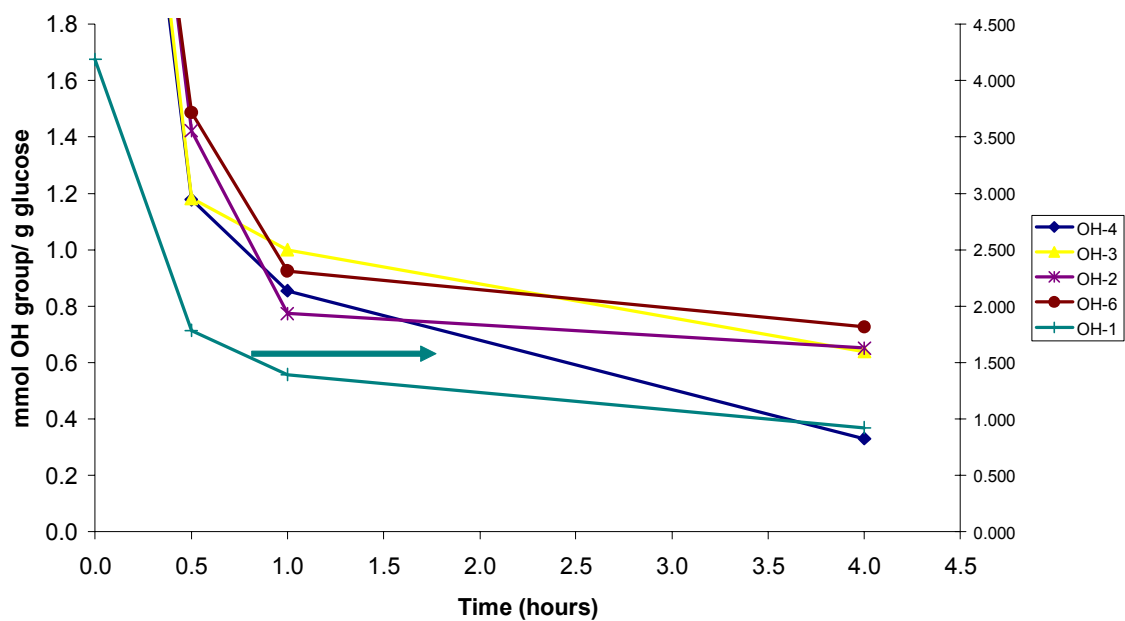
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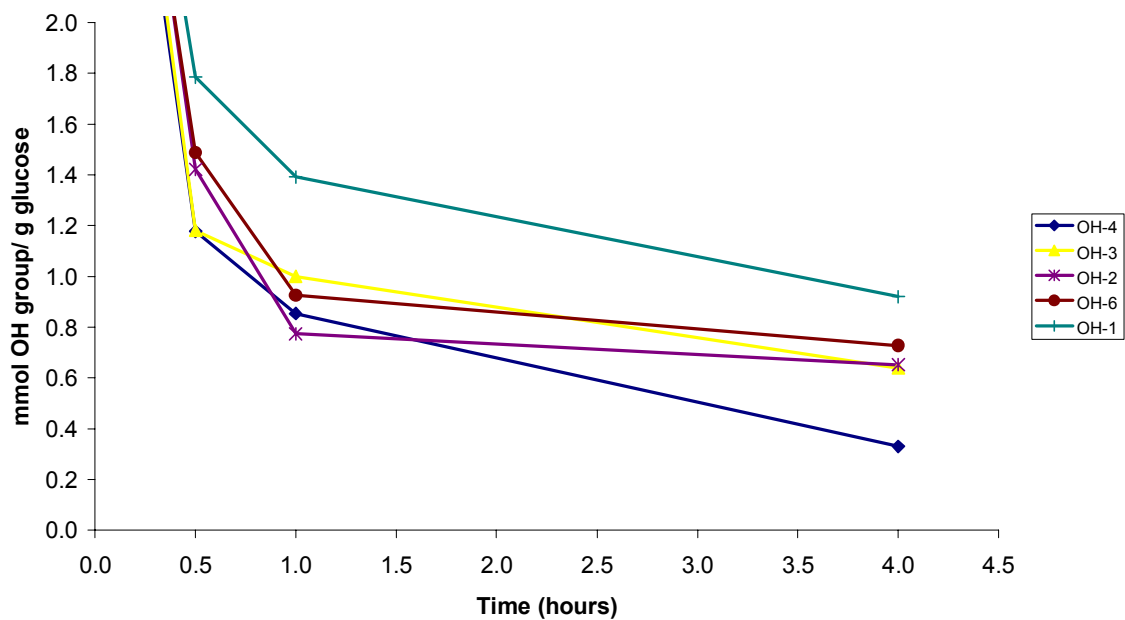
9. Appendices

Appendix 9.1: Glucose ester reaction mixtures

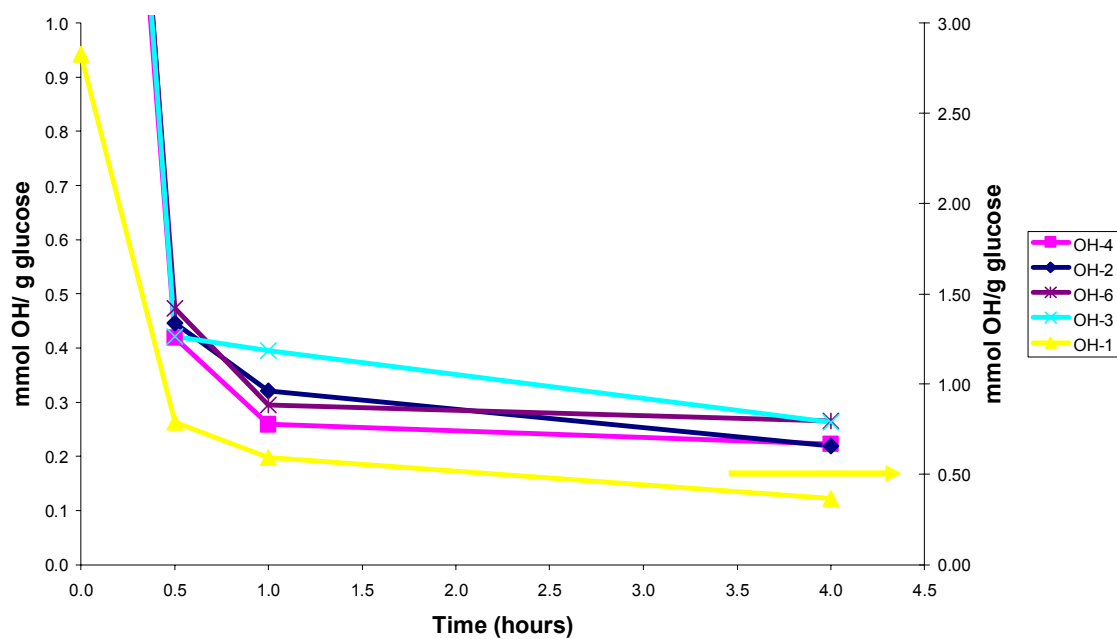
Hydroxyl Groups in Glucose Ester of Caprylic Acid (C8) Reaction Mixture



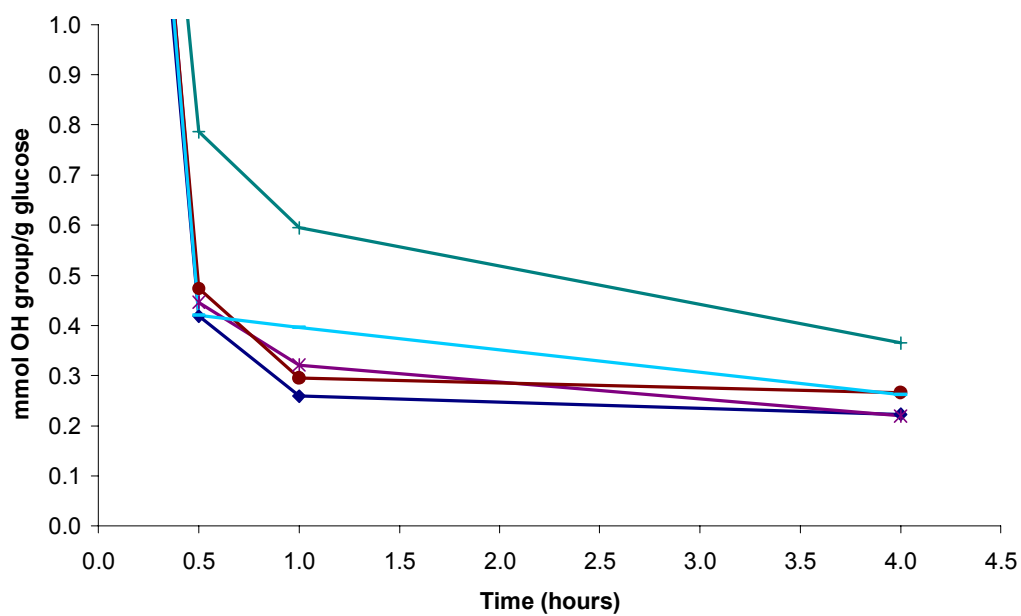
Hydroxyl Groups in Glucose Ester of Caprylic Acid (C8) Reaction Mixture



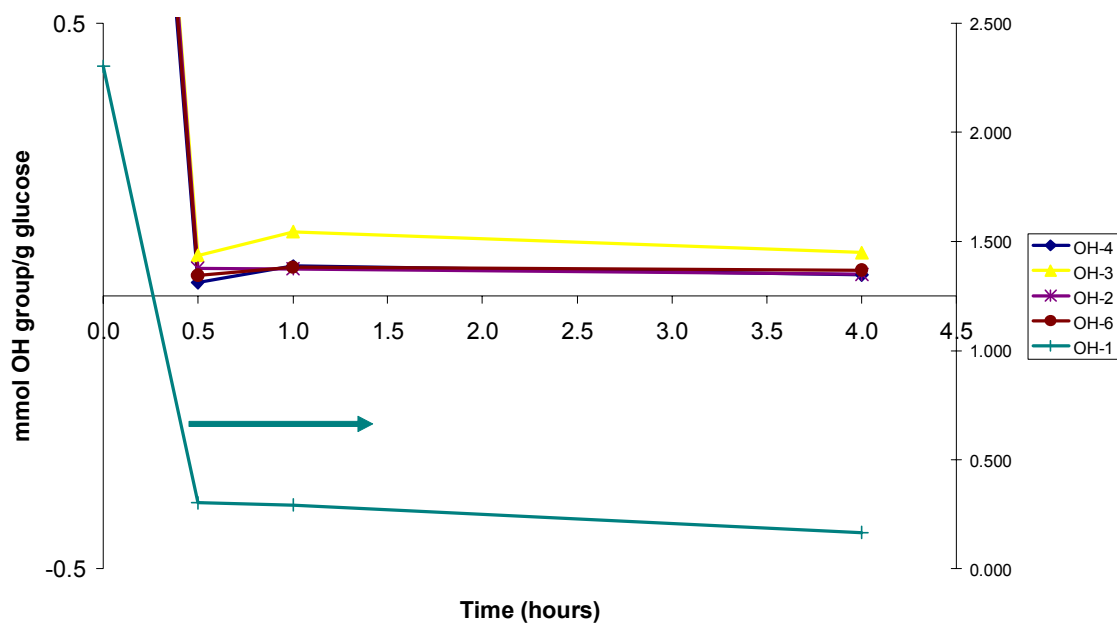
Hydroxyl Groups in Glucose Ester of Lauric Acid (C12) Reaction Mixture



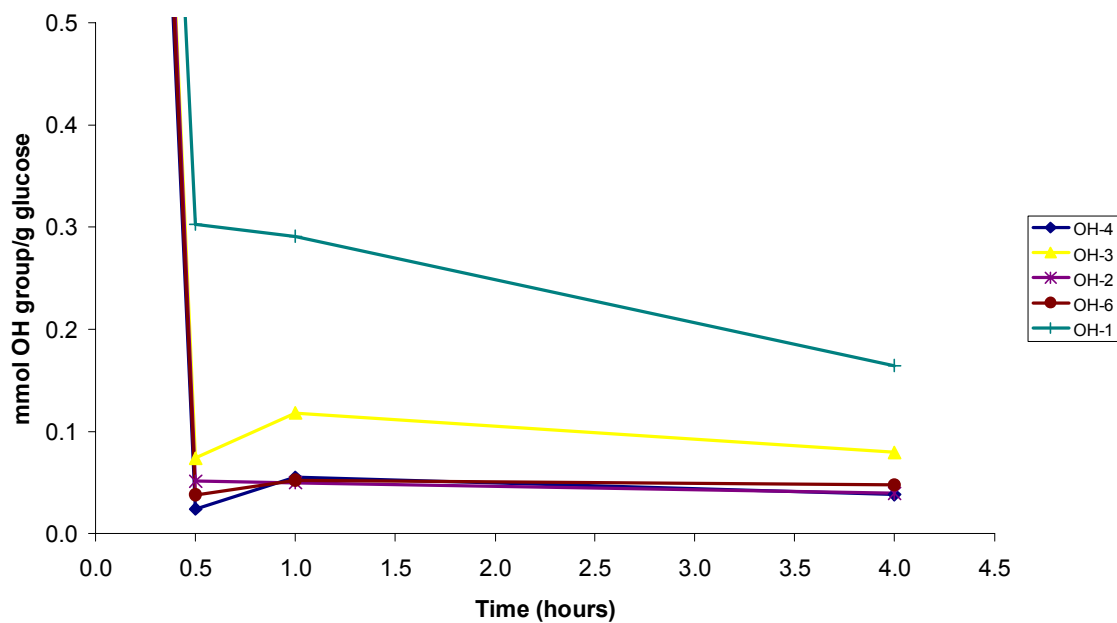
Hydroxyl Groups in Glucose Ester of Lauric Acid (C12) Reaction Mixture



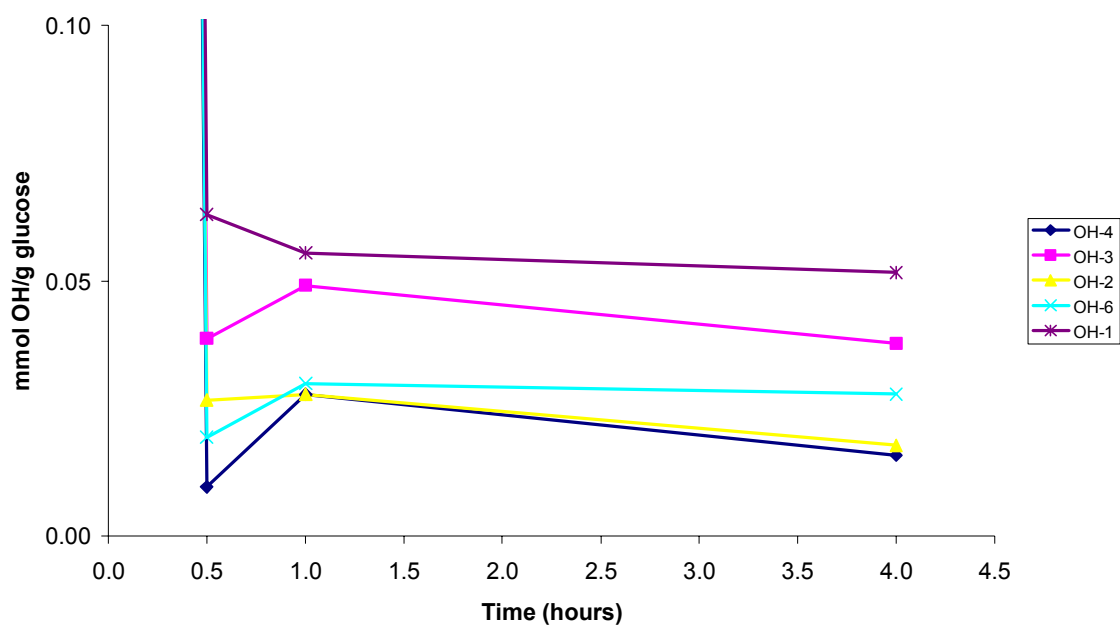
Hydroxyl Groups in Glucose Ester of Stearic Acid (C18) Reaction Mixture



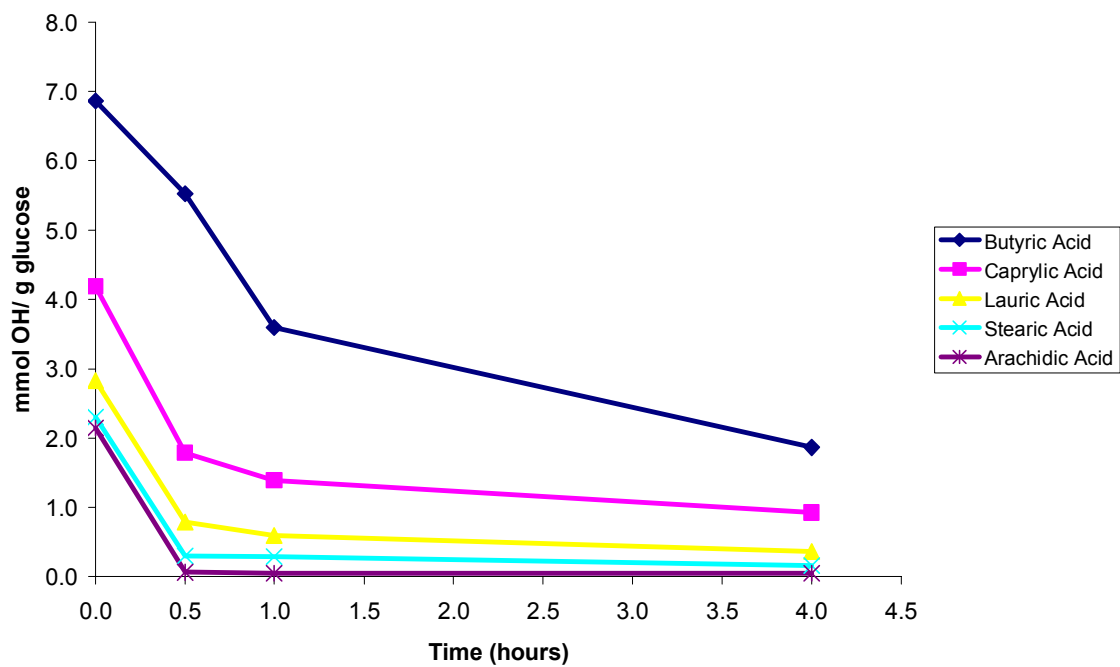
Hydroxyl Groups in Glucose Ester of Stearic Acid (C18) Reaction Mixture



Hydroxyl Groups in Glucose Ester of Arachidic Acid (C20) Reaction Mixture

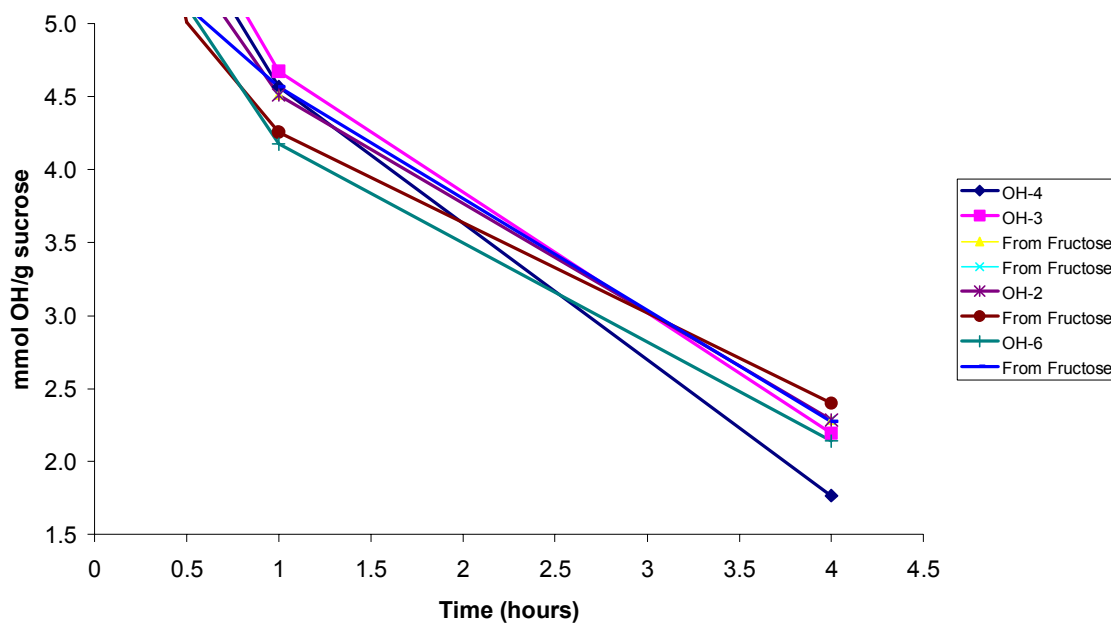


OH at C-1 in Glucose Ester Reaction Mixtures of Fatty Acids

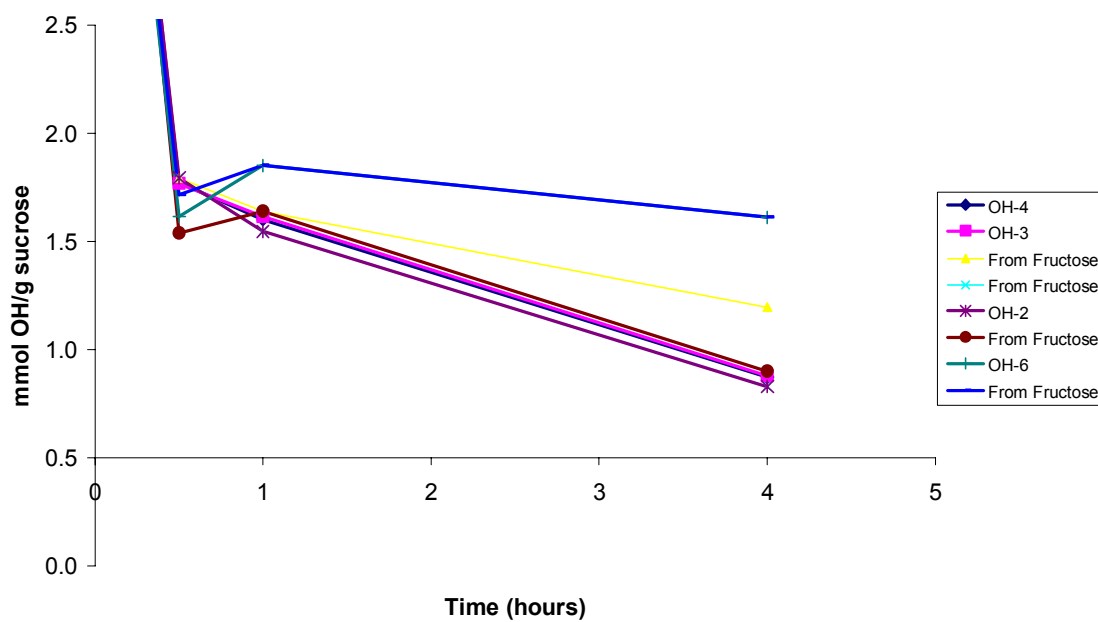


Appendix 9.2: Sucrose ester reaction mixtures

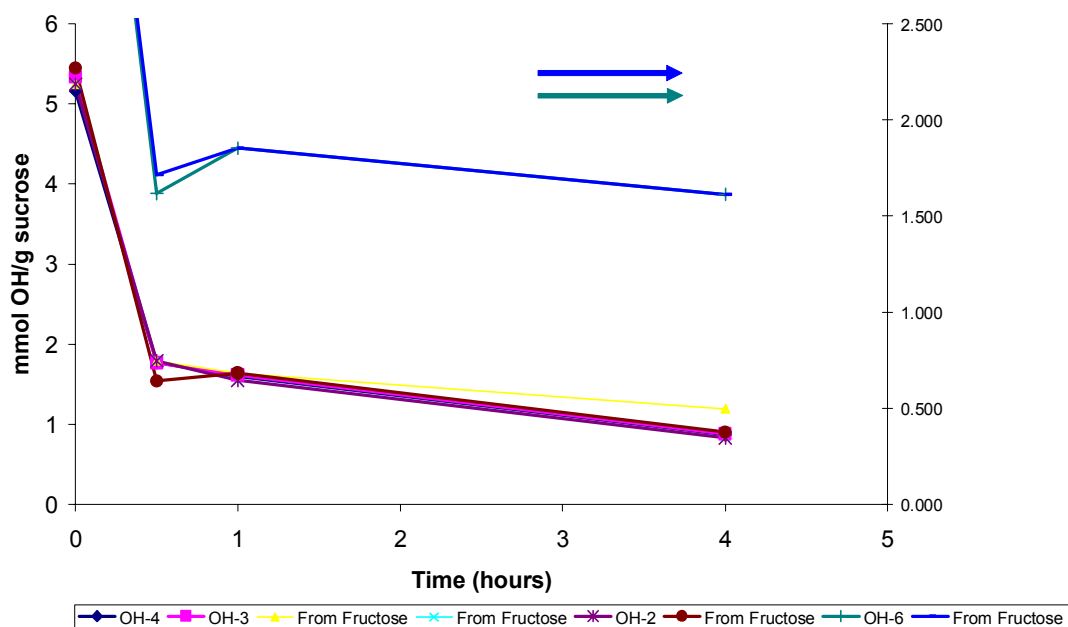
Hydroxyl Groups of Sucrose Esters of Butyric Acid (C4) Reaction Mixture



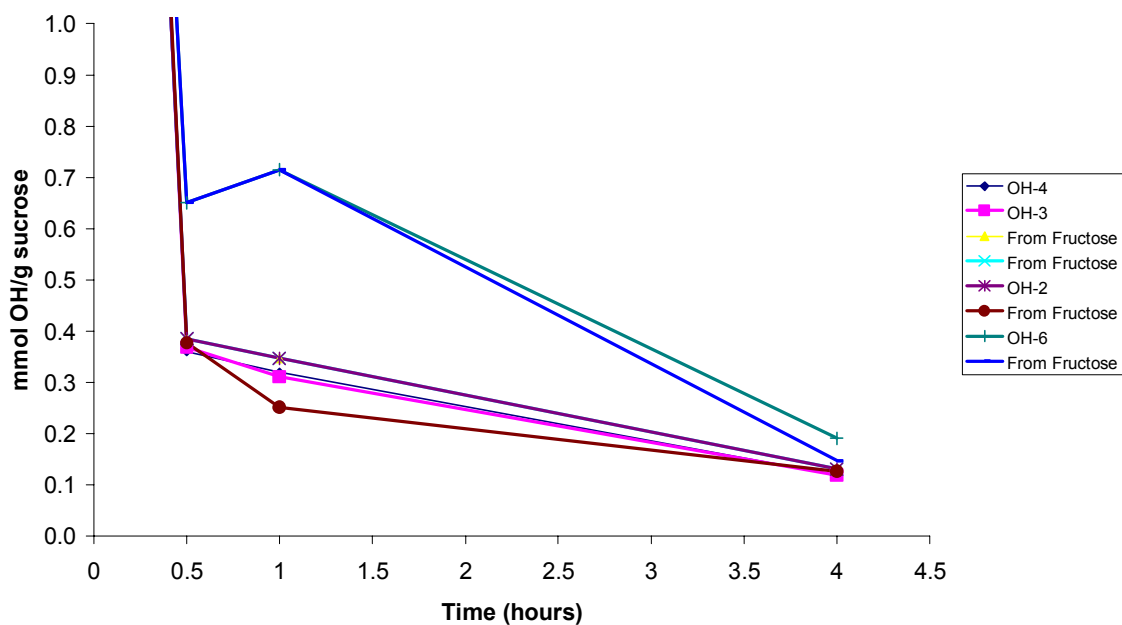
Hydroxyl Groups of Sucrose Esters of Caprylic Acid (C8) Reaction Mixture



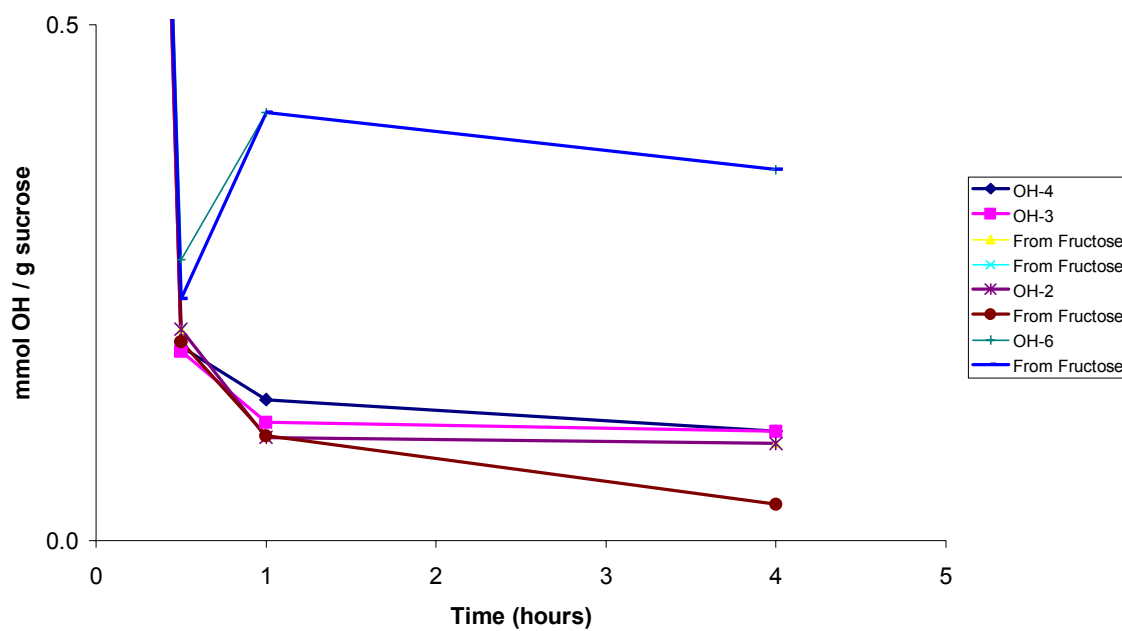
Hydroxyl Groups of Sucrose Esters of Caprylic Acid (C8) Reaction Mixture



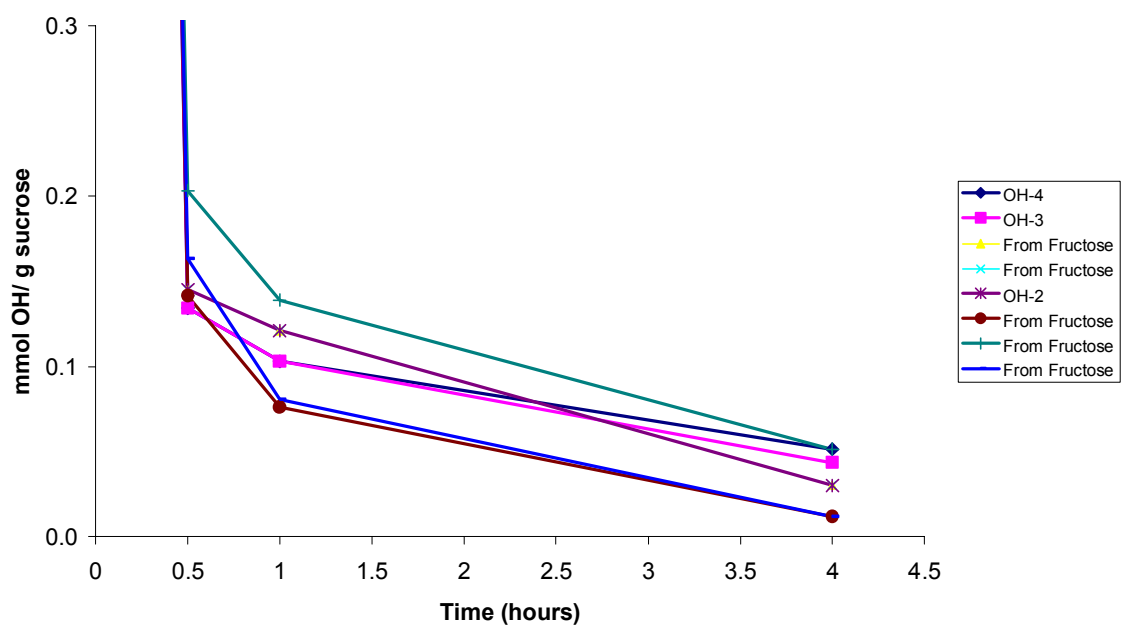
Hydroxyl Groups in Sucrose Ester of Lauric Acid (C12) Reaction Mixture



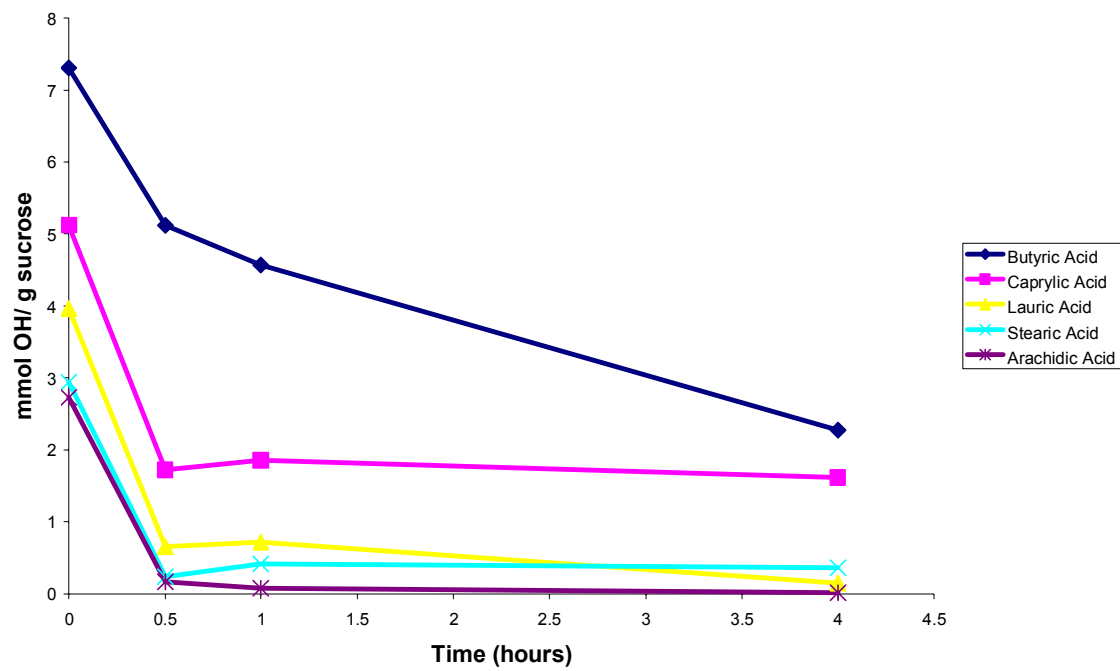
Hydroxyl Groups of Sucrose Esters of Stearic Acid (C18) Reaction Mixture



Hydroxyl Groups in Sucrose Ester of Arachidic Acid (C20) Reaction Mixture

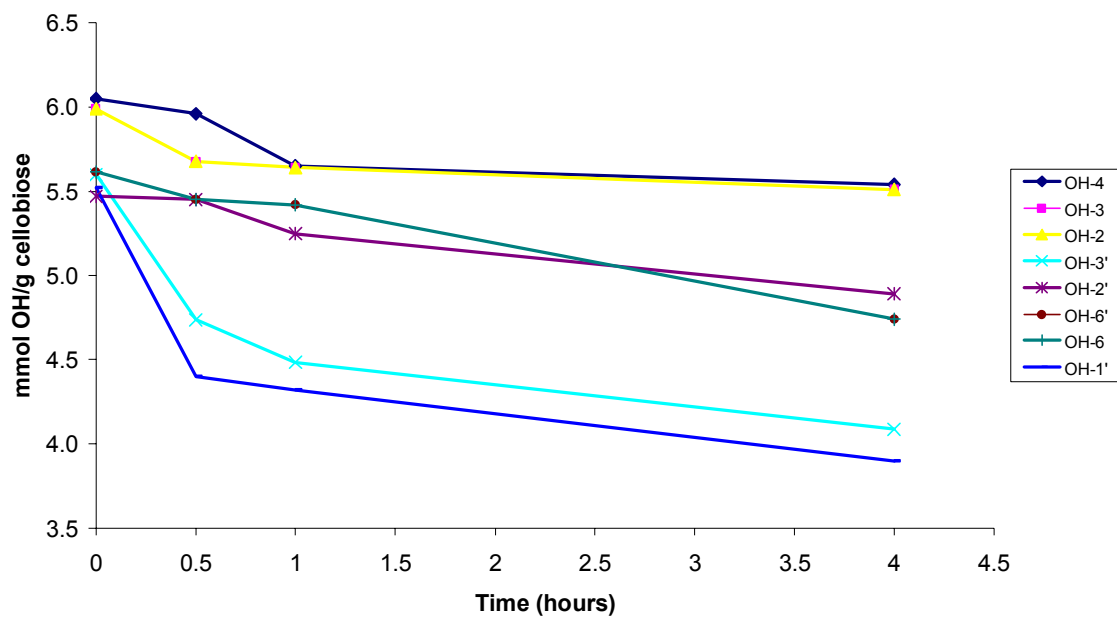


Hydroxyl from fructose as a function of fatty acid

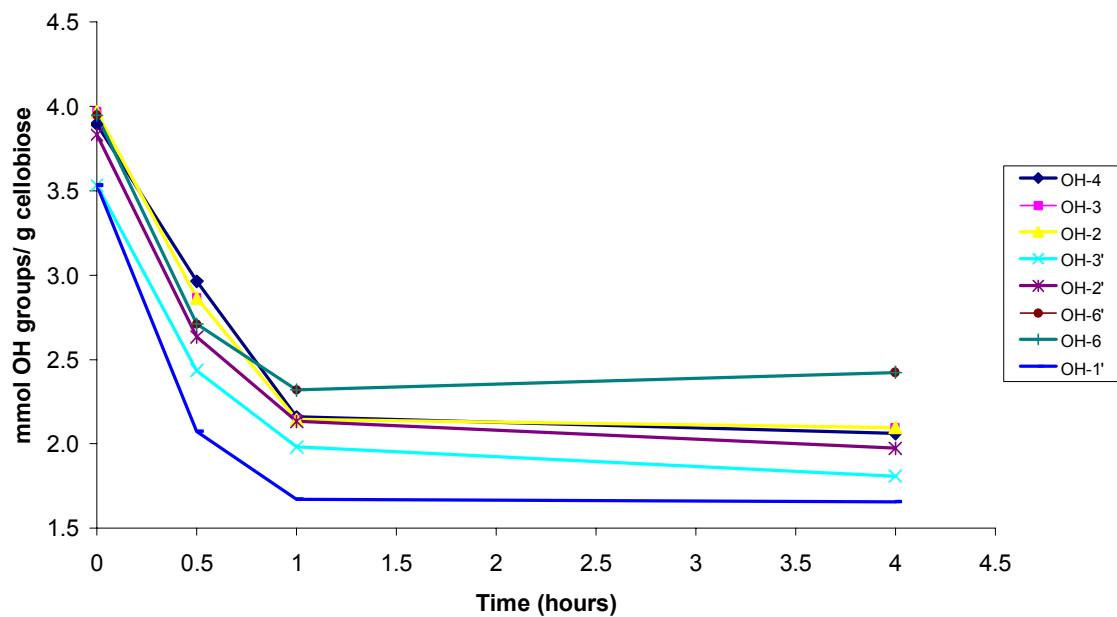


Appendix 9.3: Cellobiose ester reaction mixtures

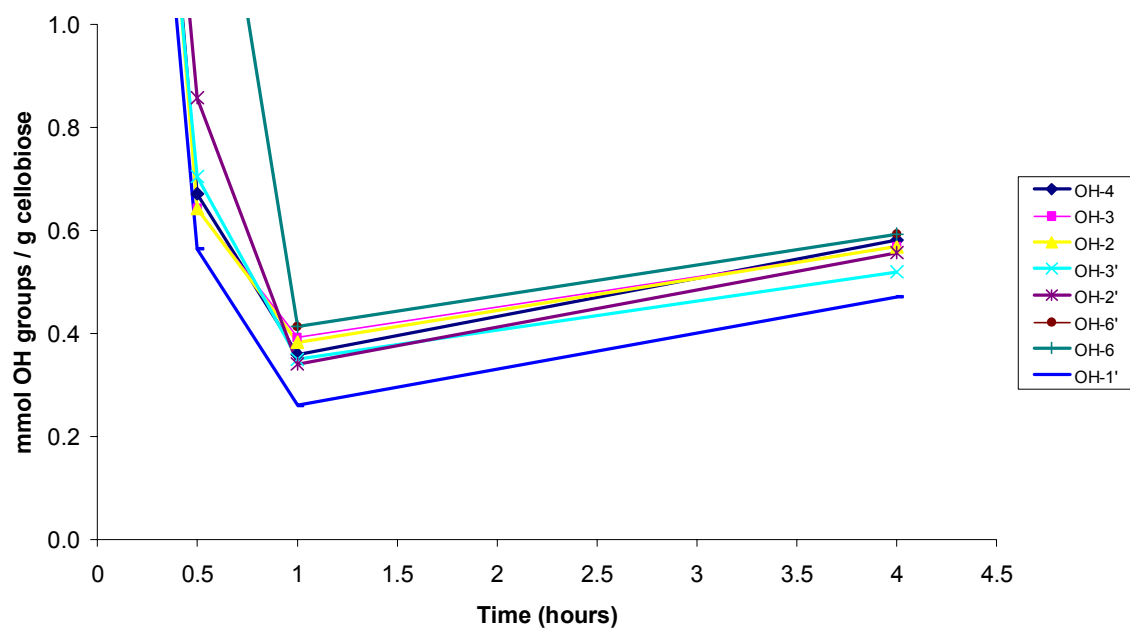
**Hydroxyl Groups in Cellobiose Ester of Butyric Acid (C4)
Reaction Mixture**



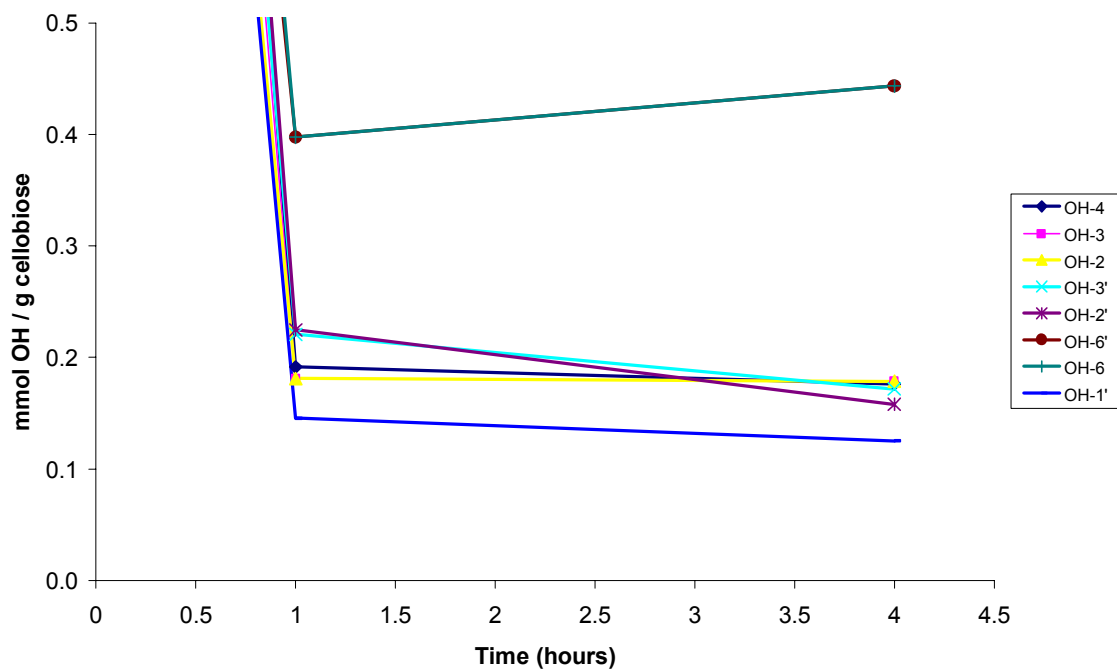
**Hydroxyl Groups in Cellobiose Ester of Caprylic Acid (C8)
Reaction Mixture**



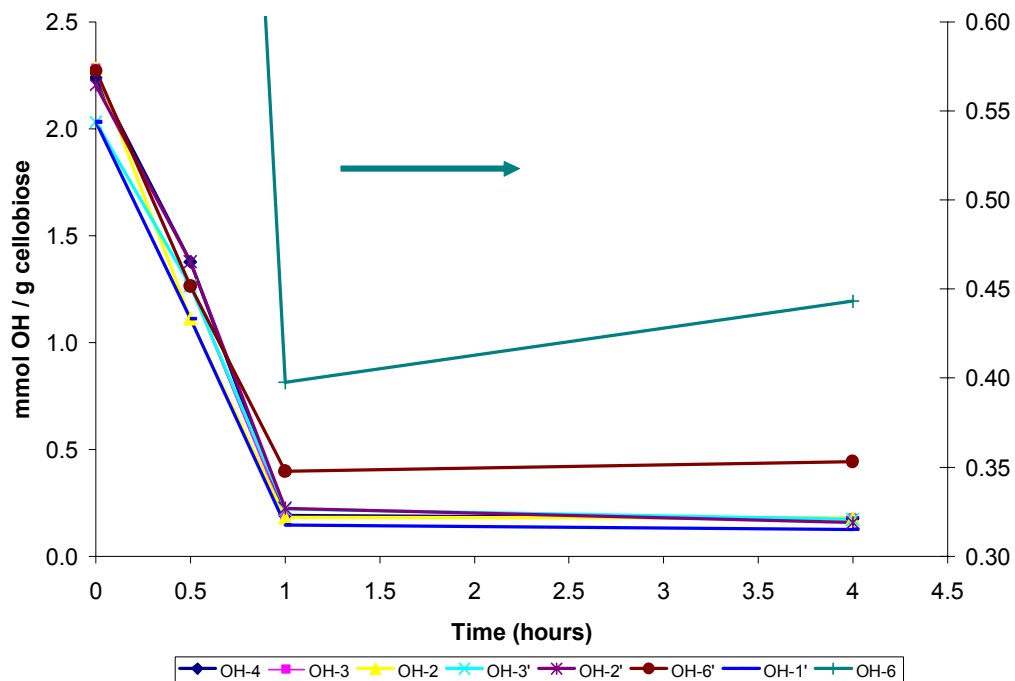
Hydroxyl Groups in Cellobiose Ester of Lauric Acid (C12) Reaction Mixture



Hydroxyl Groups in Cellobiose Ester of Stearic Acid (C18) Reaction Mixture



Hydroxyl Groups in Cellobiose Ester of Stearic Acid (C18) Reaction Mixture



Hydroxyl Groups in Cellobiose Ester of Arachidic Acid (C20) Reaction Mixture

