Universidad de Valladolid

ESCUELA DE INGENIERÍAS INDUSTRIALES

UNIVERSIDAD DE VALLADOLID

ESCUELA DE INGENIERIAS INDUSTRIALES

Grado en Ingeniería Química

ACID HYDROLYSIS OF HEMICELLULOSES FROM HARDWOOD AND SOFTWOOD WITH HOMOGENEOUS AND HETEROGENEOUS CATALYSTS

Autor: Pérez Martínez, Víctor

Responsable de intercambio: Juan García Serna

Universidad de destino: Åbo Akademi, Turku, Finlandia

Valladolid, Junio de 2015.

TFG REALIZADO EN PROGRAMA DE INTERCAMBIO

TÍTULO:	**Acid hydrolysis of hemicelluloses from hardwood and softwood with homogeneous and heterogeneous catalysts**
ALUMNO:	**Víctor Pérez Martínez**
FECHA:	**27 de Mayo de 2015**
CENTRO:	**Åbo Akademi University, Department of industrial chemistry and reaction engineering**
TUTOR:	**Henrik Grénman**

RESUMEN Y PALABRAS CLAVE

Palabras Clave: Hemicelulosa, hidrólisis, degradación de los azúcares, monómeros, oligómeros.

Resumen: En el presente trabajo se ha llevado a cabo la hidrólisis ácida de hemicelulosas previamente extraídas de dos tipos distintos de madera: la encina, perteneciente al grupo de las maderas duras (Hardwood) y el pino piñonero perteneciente al grupo de las maderas blandas (Softwood).

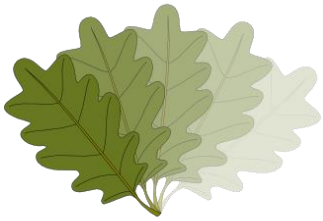
Los experimentos se han llevado a cabo en un reactor batch agitado a temperatura y presión constante. Para cada tipo de madera se ha estudiado la influencia del catalizador utilizando ácido clorhídrico como catalizador homogéneo y Smopex-101 como catalizador heterogéneo. Además, se han realizado experimentos a tres temperaturas distintas (75ºC, 85ºC y 95ºC) y a tres valores de pH (0,5, 1 y 1,5) para determinar la influencia de estos parámetros sobre la hidrólisis.

Para caracterizar el contenido en azúcares y la estructura de las hemicelulosas presentes en las muestras se han llevado a cabo distintos análisis. El contenido total de azúcares se ha determinado mediante metanólisis y el contenido de monómeros y oligómeros (de monómeros a pentámeros) mediante cromatografía de gases. Además, se realizaron distintos análisis con HPLC-Sec para conocer la masa molecular media de las cadenas de hemicelulosa.

ACID HYDROLYSIS OF HEMICELLULOSES FROM HARDWOOD AND SOFTWOOD WITH HOMOGENEOUS AND HETEROGENEOUS CATALYSTS

Master's Thesis

Víctor Pérez Martínez



Johan Gadolin
Process Chemistry Centre

Laboratory of Industrial Chemistry and Reaction Engineering
Faculty of Science and Engineering /Chemical Engineering
Åbo Akademi University

Turku/Åbo 2015

TABLE OF CONTENT

PREFACE

The current work was performed at the Laboratory of Industrial Chemistry and Reaction Engineering, Faculty of Science and Engineering/Chemical Engineering, Åbo Akademi University. The research was a part of the activities at the Johan Gadolin Process Chemistry Centre (PCC), a centre of excellence funded by Åbo Akademi University.

First of all, I would like to express my gratitude to Docent Henrik Grénman for his guidance and kind supervision, also for introducing me into the world of biomass fractionation and teaching me a lot about hemicelluloses, my dearest polymeric friends throughout this year. I am also grateful to Dr. Kari Eränen for his assistance and willingness to help.

I would like to express my gratitude to Juan García Serna in Valladolid and Professor Dr. Tapio Salmi in Turku for enabling my exchange experience in Finland, which I enjoyed both inside and outside the laboratory walls.

I am deeply grateful to M.Sc. Jussi Rissanen and M.Sc. Andrea Perez Nebreda for their help and support with the tedious methanolysis analysis and calculations, as well as to all the professionals at Wood and Paper Chemistry for sharing their knowledge in carbohydrate analysis, especially to B. Sc. Jarl Hemming for his patience and expertise.

I would like to thank to my friends at the laboratory, especially María for her generosity and her eventual lack of patience with me and outside the laboratory to my closest friends: Borja, Carmen, Iñigo and Laia for their non-scientific support. Each of them has contributed to this thesis in some way.

Finally, I would like to take this opportunity to thank my family, mom, dad and my brother Héctor for their trust and support from distance. Also to Alba, we proved that 3000 km is less than it seems.

ABSTRACT

Víctor Pérez Martínez

Acid hydrolysis of hemicelluloses from hardwood and softwood with homogeneous and heterogeneous catalysts.

Diploma Thesis

Diploma Thesis carried out under the supervision of Docent Henrik Grénman, at the Laboratory of Industrial Chemistry and Reaction Engineering, Johan Gadolin Process Chemistry Centre, Faculty of Science and Engineering/Chemical Engineering, Åbo Akademi University, 2015.

Key words

Hemicelluloses, hydrolysis, saccharides, degree of polymerization, degradation, molar mass, kinetics, catalysis, heterogeneous catalysis, homogeneous catalysis.

The biorefinery concept is based on the utilization of a variety of biomass feedstocks and on the production of a large variety of products, including chemicals, biofuels and energy. Hemicelluloses are one of the least studied raw materials in lignocellulosic biomass, however they have a large potential for several applications e.g. in the cosmetics, pharmaceutical and alimentary industries. Hemicelluloses can be extracted from wood by using different methods, the most promising one is the use of pressurized hot water in optimized conditions. Chemically, hemicelluloses are branched heteropolysaccharides and one of their most interesting applications is as a source of rare sugars (xylose, mannose, rhamnose, glucose, etc.) for further valorization. Rare monosaccharides are not usually found isolated in nature and they can be used as platform chemicals in the production of a wide variety of chemical products.

The most efficient way to transform hemicelluloses into smaller molecules, such as monomers or dimers, is through acid hydrolysis. Acid hydrolysis can be performed with different techniques, homogeneous catalysis with mineral and organic acids has typically been used because high yields can be achieved in short periods of time. However, the use of homogeneous catalysts leads to several drawbacks: degradation of the sugars, high equipment costs due to corrosion, the difficulty of separation between catalyst and products and the need to neutralize the reaction mixture. Solid catalysts are being widely studied with the idea to maintain the yield and selectivity of the process while improving its economic and environmental sustainability. The solid catalysts do not have the drawbacks of the homogeneous catalysts because the reaction mixture does not need neutralization, the catalyst can be separated from the products by filtration and the operational costs can be reduced due to low corrosiveness.

Acid hydrolysis of hemicelluloses has been previously studied with purified extracts and model compounds, but not with hemicelluloses recently extracted from the wood. In this work, acid hydrolysis was studied using extracts (PHW) of different concentrations and molar masses. The extraction was performed in temperatures ranging from 130ºC to 170ºC and with extraction times up to 220 minutes. 1,25-2 mm diameter sapwood particles from three different wood species were used in the extraction: Norway Norway Spruce, stone pine and holm oak. The two first species belong to the family of softwoods and the third one to the family of hardwoods. Two different

catalysts were used in the hydrolysis experiments, hydrochloridric acid as a homogeneous catalyst and Smopex-101 as a heterogeneous catalyst. Numerous parallel experiments were performed in order to study the performance of the two catalysts. Moreover, the kinetics were widely studied with Smopex-101 with experiments performed at three different temperatures (75ºC, 85ºC and 95ºC) and at three different pH (0,5, 1 and 1,5) with both stone pine and holm oak.

The experiments at 95ºC and pH 0,5 showed that the hydrolysis of holm oak hemicelluloses can be performed with both homogeneous and heterogeneous catalysts with yields close to 100% towards oligomers (DP<6). However, severe degradation of the sugars was also observed. The hydrolysis of the stone pine hemicelluloses showed much lower yields towards oligomers (DP<6), but no significant degradation occurred.

It was observed that the kinetics of the hydrolysis is influenced strongly by the reaction temperature. In the experiments with holm oak, at 75ºC the reaction barely took place while at 85ºC after 24 hours the yields towards oligomers (DP<6) was over 80% and still increasing. At 95ºC the reaction reached a maximum yield close to 100% yield, however, after 24 hours significant degradation of the sugars was observed. The dependence of the hydrolysis rate on the temperature was found to be even greater with stone pine compared to holm oak. At 95ºC the yields were rather low during the experiments due to severe degradation.

The experiments concerning the influence of pH on the hydrolysis showed that for the holm oak extracts, at low pH (0,5 and 1) significant degradation of the sugars occurred. The degradation was avoided at pH 1,5. However, yield of over 90% towards oligomers were achieved in every pH. With the stone pine extracts, degradation was observed only at pH 0,5. The results showed that the kinetics of stone pine were slower than for holm oak and after 24 hours of reaction the hydrolysis yield was still rather low.

A clear difference in the performance of HCl and Smopex-101 as catalysts was observed in all of the experiments. When the homogeneous catalyst was used, the majority of the formed products were monomers, while with Smopex-101, the concentration of dimers was nearly as high as for the monomers.

RESUMEN

Víctor Pérez Martínez

Hidrólisis ácida de hemicelulosas de maderas duras y blandas mediante catalizadores homogéneos y heterogéneos.

Tesis de Máster

Tesis de máster llevada a cabo bajo la supervisión del Docent Henrik Grénman, en el Laboratory of Industrial Chemistry and Reaction Engineering, Johan Gadolin Process Chemistry Centre, Faculty of Science and Engineering/ Chemical Engineering, Åbo Akademi University, 2015.

Palabras clave

Hemicelulosas, hidrólisis, sacáridos, grado de polimerización, degradación, masa molar, cinética, catálisis, catálisis heterogénea, catálisis homogénea.

El concepto de biorefinería se basa en utilizar todo tipo de biomasa como materia prima y transformarla mediante procesos biológicos y químicos en una gran variedad de productos, principalmente polímeros, biocombustibles y energía. Una de las materias primas menos estudiadas hasta el momento dentro del campo de la biomasa proveniente de la madera son las hemicelulosas, que tienen diversas aplicaciones en las industrias cosmética, farmacéutica o alimentaria. Estas hemicelulosas pueden ser fácilmente extraídas de la madera mediante distintos métodos, siendo el más prometedor el uso de agua caliente presurizada. Químicamente, las hemicelulosas son heteropolisacáridos ramificados y una de sus aplicaciones más interesantes es como fuente de los azucares raros que la componen (xilosa, manosa, ramnosa, glucosa, etc.). Estos monosacáridos no se encuentran fácilmente aislados en la naturaleza y pueden utilizarse como reactivos para la producción de muchos otros compuestos químicos.

La forma más eficiente de convertir las hemicelulosas en moléculas más pequeñas como monómeros y dímeros es mediante la hidrólisis ácida. Existen diferentes maneras de llevar a cabo esta hidrólisis ácida, típicamente se ha recurrido a la catálisis homogénea por medio de ácidos minerales y orgánicos, ya que se obtienen grandes rendimientos en cortos periodos de tiempo, pero existen numerosas desventajas como la degradación de los azúcares, los altos costes de equipos para evitar la corrosión, la dificultad de separación de estos ácidos y la necesidad de neutralizar la mezcla de reacción. Con el objetivo de mantener el rendimiento y la selectividad de estos procesos pero con el fin de mejorar su sostenibilidad económica y ambiental, se ha empezado a estudiar el uso de catalizadores heterogéneos que resolverían los problemas generados por la catálisis homogénea. Esta catálisis heterogénea reduce los costes de los equipos, la mezcla de reacción no necesita neutralización y se puede separar fácilmente el catalizador de la mezcla de productos por filtración.

La hidrólisis ácida de hemicelulosas ha sido estudiada previamente para extractos purificados y compuestos modelo, pero no para hemicelulosas recién extraídas de la madera. En este trabajo se ha estudiado la hidrólisis ácida usando como materia prima extractos de distintas concentraciones y masas molares extraídos mediante agua caliente presurizada en condiciones de temperatura entre 130ºC y 170ºC y variando los tiempos de extracción entre 60 y 220 minutos. Para la extracción se utilizaron astillas comprendidas entre 1,25 mm y 2 mm de diámetro de tres especies de árboles

distintos: abeto, pino piñonero y roble, siendo los dos primeros de la familia de las maderas blandas y la tercera de la familia de las maderas duras. Para los experimentos de hidrólisis se han utilizado dos tipos de catalizadores, ácido hidroclorídrico como catalizador homogéneo y Smopex-101 como catalizador heterogéneo. En los numerosos experimentos se ha estudiado el diferente comportamiento de las maderas con el tradicional catalizador homogéneo y con el catalizador heterogéneo. Además, la cinética con el catalizador heterogéneo ha sido ampliamente estudiada ya que se han realizado experimentos a tres temperaturas distintas (75ºC, 85ºC y 95ºC) y a tres distintos valores de pH (0,5, 1 y 1,5) tanto para pino piñonero como para encina, con la intención de conocer la influencia de estos parámetros en la hidrólisis.

Los experimentos realizados a 95ºC y pH 0,5 han demostrado que la hidrólisis de hemicelulosas de encina es posible tanto con catalizadores homogéneos como heterogéneos con rendimientos de producción de oligómeros (DP<6) cercanos al 100% en ambos casos pero con una importante degradación de los azúcares. Por el contrario, en el caso del pino piñonero, los rendimientos de producción de oligómeros (DP<6) son mucho más bajos, cercanos al 50%, pero sin que aparezca degradación.

Además, se ha comprobado que la cinética de la hidrólisis depende fuertemente de la temperatura, en el caso de la encina, a 75ºC la reacción apenas tiene lugar, mientras que a 85ºC tras 24 horas el rendimiento de formación de oligómeros (DP<6) sigue creciendo más allá del 80%. A 95ºC la reacción alcanza un máximo cercano al 100% pero tras 24 horas existe una gran degradación de los azúcares. En el caso del pino piñonero, la dependencia con la temperatura es incluso más extrema, ya que a 95ºC la degradación es tan grande que no se obtienen en ningún momento rendimientos altos.

Al estudiar la influencia del pH sobre la hidrólisis se ha observado que para el caso de la encina, a niveles muy bajos de pH (0,5 y 1) existe una gran degradación de los azúcares, que es evitada a valores de pH por encima de 1,5, siendo en todos los casos los rendimientos de formación de oligómeros (DP<6) superiores al 90%. En el caso del pino piñonero la degradación aparece solamente en valores de pH 0,5 y los resultados demuestran que la cinética es mucho más lenta que para la encina ya que tras 24 horas la reacción apenas ha tenido lugar.

Durante todos los experimentos se observa una gran diferencia entre la acción de HCl como catalizador y del Smopex-101. Cuando se utiliza el catalizador homogéneo, la mayor parte de los oligómeros formados son monómeros, mientras que cuando se utiliza Smopex-101 la concentración de dímeros es tan alta como la de monómeros.

REFERAT

Víctor Pérez Martínez

Syrahydrolys av hemicellulosor från lövträd och barrträd med homogen och heterogen katalys.

Diplomarbete

Diplomarbete handlett av docent Henrik Grénman vid Laboratoriet för teknisk kemi och reaktionsteknik, Processkemiska center, Fakulteten för naturvetenskaper och teknik, Åbo Akademi, 2015.

Nyckelord

Hemicellulosa, hydrolys, sockrar, polymeriseringsgraden, degredring, molmassa, kinetic, katalys, heterogen katalys, homogeny katalys.

Bioraffinaderikonceptet baserar sig på utnyttjande av olika biomassor och på produktion av ett stort sortiment produkter inklusive kemikalier, biobränslen och energi. Hemicellulosor hör till de minst undersökta komponenterna i lignocellulosa, fast de har stor potential för ett antal applikationer till exempel inom kosmetika, läkemedel och matvaror. Hemicellulosa kan extraheras ur ved med hjälp av olika metoder varav hetvattenextraktion är ett av de mest lovande. Kemiskt sätt är hemicellulosor förgrenade heteropolysackarider och en av de intressantaste användningsmöjligheterna är att direkt förädla de sällsynta sockrarna (xylos, mannos, rhamnos, glukos osv.). De sällsynta sockrarna förekommer oftast inte i naturen som rena komponenter och de kan användas som plattformkemikalier för ett antal kemiska produkter.

Det mest effektiva sättet att konvertera hemicellulosor till mindre molekyler så som monomerer eller dimerer är via syrahydrolys. Syrahydrolys kan åstadkommas med hjälp av olika tekniker. Homogen katalys med mineral eller organiska syror har oftast använts för att hög omsättningsgrader kan åstadkommas inom kort tid. Användning av de homogena katalysatorerna har sina nackdelar: söderfall av sockrar, höga utrustningskostnader p.g.a. korrosion, utmaningar i att separera produkten och katalysatorn samt behovet av neutralisering. Fasta katalysatorer utvecklas kontinuerligt för att kunna bibehålla den höga omsättningsgraden och selektiviteten och för att samtidigt förbättra ekonomin och hållbarheten av processen. Fasta katalysatorer har inte de nackdelarna som homogena katalysatorer har för att ingen neutralisering behövs, produkten kan separeras från katalysatorn med enkel filtrering och produktionskostnaderna är lägre p.g.a. den låga korrosionen.

Syrahydrolys av hemicellulosor har studerats tidigare med renade extrakter och med modellkomponenter, men inte med hemicellulosor nyligen extraherade från ved. I detta arbete undersöktes syrahydrolys med hjälp av extrakter (extraherade med trycksatt hetvatten) av olika koncentrationer och molmassor. Extraheringen utfördes i temperaturområdet 130°C-170°C och med extraktionstider upp till 220 minuter. Splintved

av 1,25-2 mm storlek från tre olika trädslag användes i extraktionen: gran, pinje och stenek. De två förstnämnda hör till barrträden och den sistnämnda till lövträden.

Två olika katalysatorer undersöktes i hydrolysexperimenten, saltsyra som homogen katalysator och Smopex 101 som heterogen katalysator. Ett antal parallellexperiment utfördes för att kunna jämföra funktionen av de två katalysatorerna. Utöver det, undersöktes kinetiken med Smopex 101 i ett vidare temperaturområde mellan 75°C och 95°C och ett pH område mellan 0,5 och 1,5 med både pinje och stenek.

Experimenten vid 95°C och pH 0,5 visade att hydrolys av stenek hemicellulosor kan utföras med båda katalysatorerna med en omsättningsgrad nära 100 % i oligomerer (DP<6). Betydande sönderfall av sockrarna observerades dock i samma experiment. Hydrolys av pinje hemicellulosorna resulterade i mycket lägre utbyte i oligomerer (DP<6), men ingen betydande sönderfall observerades.

Hydrolyskinetiken observerades vara starkt beroende på reaktionstemperaturen. I experimenten med stenek vid 75°C skedde knappast ingen reaktion medan vid 85°C, en omsättningsgrad på över 85 % observerades efter 24 timmar. Vid 95°C gick omsättningsgraden upp till nära 100 % men efter 24 timmar upptäcktes betydande nedbrytning av sockrarna. Inverkan av temperaturen på kinetiken observerades vara ännu starkare för pinje jämfört med steneken. Vid 95°C var omsättningsgraden väldigt låg på grund av stark sönderfall av sockrarna.

Experimenten där inverkan av pH undersöktes visade att det skedde kraftigt sönderfall av monomererna med stenek vid låga pH (0,5 och 1). Sockrarna hölls hela vid pH 1,5. En omsättningsgrad på över 90 % upptäcktes ändå vid varje pH. Med stenek extrakterna upptäcktes nedbrytning endast vid pH 0,5. Resultaten visade att kinetiken med pinje var långsammare än med stenek och efter 24 timmars reaktion var omsättningsgraden ännu relativt låg.

En tydlig skillnad upptäcktes vid varje experiment mellan katalysatorerna HCl och Smopex 101. När den homogena katalysatorn användes, produkten bestod nästan enbart av monomerer medan med Smopex, var koncentrationen dimerer nästan lika hög som för monomerer.

LIST OF FIGURES

LIST OF TABLES

NOTATIONS

Ara	Arabinose
Gal	Galactose
GalA	Galacturonic Acid
GC	Gas Chromatography
Glc	Glucose
GlcA	Glucuronic Acid
HC	Hemicellulose
HO	Quercux Ilex (Holm Oak)
HPLC	High Performance Liquid Chromatography
HPLC-Sec	High Performance Liquid Chromatography Size Exclusion Column
LDPE	Low Density Poly Ethylene
MALLS	Multi Angle Light Laser Scattering
Man	Mannose
PA	Picea Abies (Norway Spruce)
PP	Pinus Pinea (Stone Pine)
Rha	Rhamnose
RI	Refractive Index
Xyl	Xylose

1. INTRODUCTION

Biomass is defined as the biological material derived from living or recently living organisms. The most abundant class of biomass is lignocellulosic biomass which accounts for around 50% of the total biomass in the world [1] [2].

Despite the huge diversity in these renewable resources, just a few chemicals are obtained from them. In the past few years, the big concern about the diminishing of fossil fuel reservoirs has developed a growing interest in creating new ways of using biomass as a raw material, not just for the production of biofuels but also as a source of high-value-added chemicals and new materials [1] [3].

Chemically, wood is divided in three main different fractions: cellulose (40-45%), hemicellulose (20-30%) and lignin (20-30%). All of them can be utilized for many purposes because of their different nature and reactivities [2] [4].

Almost all kind of biomass could be processed in a purpose built facility known as biorefinery [4] [5]. This refining would imply several steps such as pretreatment of the biomass feedstock, separation of the different fractions, and a biological and chemical treatment in order to produce both chemical products and energy [2] [3] [6] [7]. A scheme of an integrated biorefinery is presented in Figure 1:

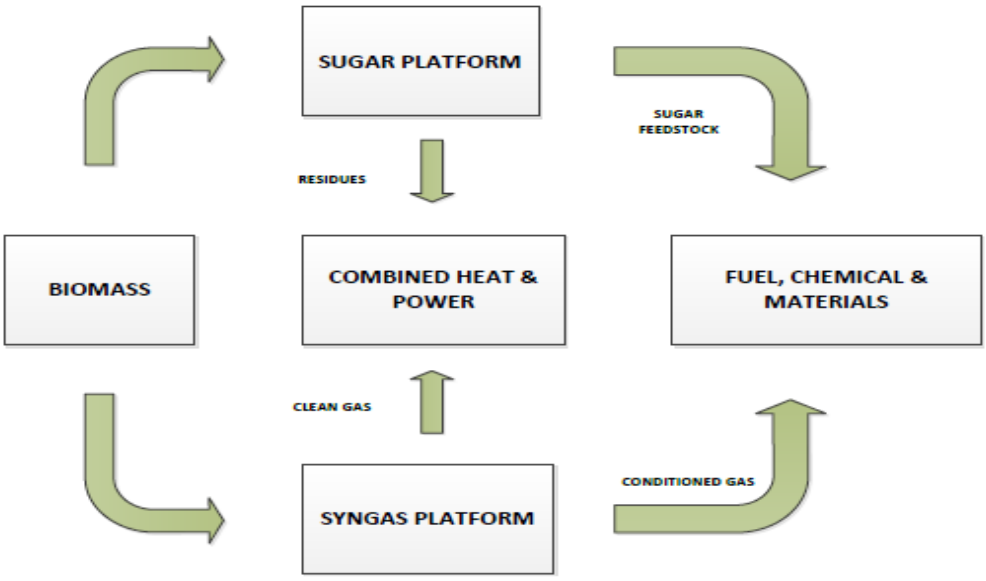


Figure 1-Biorefinery Scheme

This facility is based on the same philosophy as the conventional petroleum oil refineries: many material and heat streams connecting complex separation and reaction units, all coming from a common feedstock in order to produce a huge amount of refined products [3] [7].

The main goal of this industry is to replace the conventional petro-based chemicals and products with bio-based ones. This kind of industry will reduce the dependence from the fossil resources and it will be both economically and environmentally sustainable [4] [7].

The chemistry of wood is very complicated, usually there are many parallel reactions involving macromolecules which will be cleaved into smaller ones [6] [8]. The main reactions happening in the biomass conversion process are hydrolysis, dehydration, isomerization, reforming, hydrogenation and oxidation [3].

Most of them require the presence of a catalyst so it is of great importance to go gain knowledge and study the kinetics of these carbohydrate transformation processes [3].

Conventionally, cellulose has drawn all the attention in this field but in the past few years, the study of hemicelluloses has gained an increasing interest because it could provide an easy source of biopolymers and some rare sugars that are not commonly found in nature, and which could be very useful and versatile as platform chemicals [4] [8].

Hemicelluloses are very different from cellulose. While cellulose has a linear structure formed from glucose monomers, the hemicelluloses are heteropolymers with a branched structure formed by monosugars such as xylose, mannose, arabinose, rhamnose and galactose. These sugars can also be found in their acidic forms, called uronic acids [4] [6]. A schematic representation of the different fractions of wood can be observed in Figure 2:

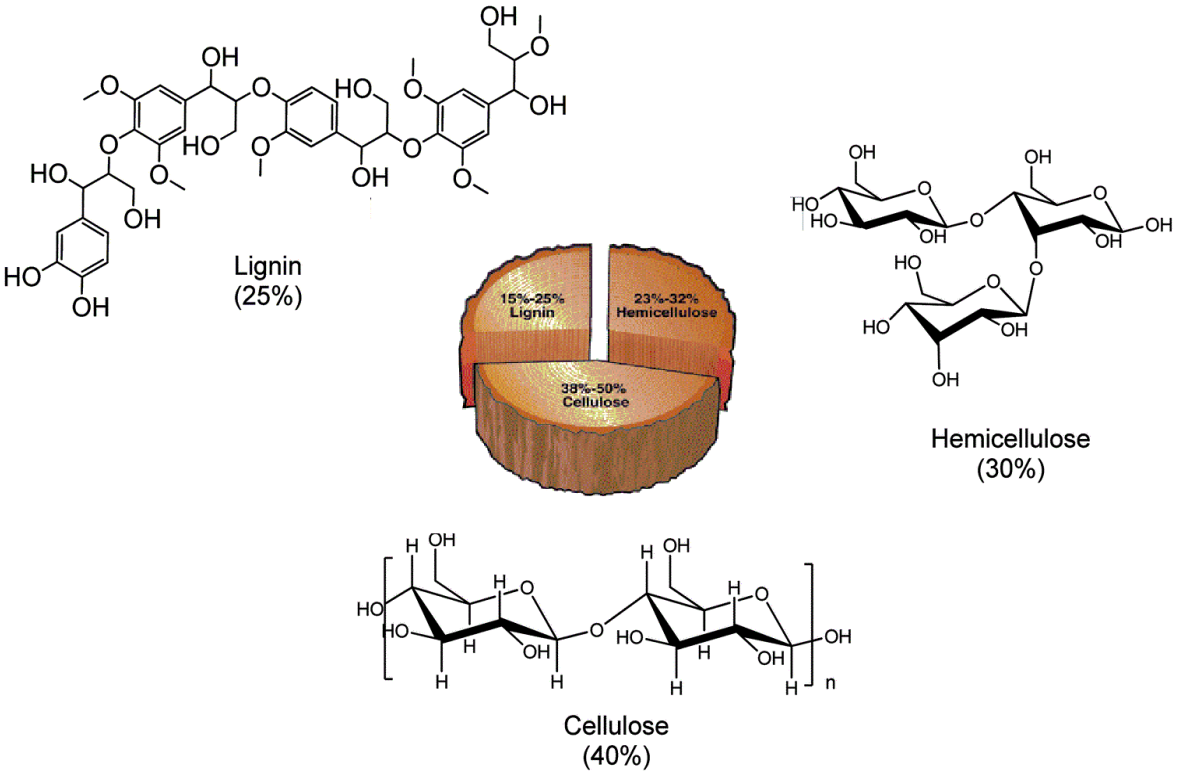


Figure 2-Different fractions of the lignocellulosic biomass

An extra interest lays on the fact that there exists different kinds of hemicelluloses, depending on the type of wood but also on the specie of tree. There are two main groups of wood: softwoods and hardwoods. The main hemicelluloses in softwoods are galactoglucomannans and arabinoglucoronoxylans while in hardwoods the most common hemicelluloses are the glucuronoxylans [6].

The first step in the refining of biomass is the separation of the different fractions [9]. The hemicelluloses can be separated from the rest of the wood material because of one peculiarity, their solubility. While cellulose and pectins remain in the solid phase, the hemicelluloses can be extracted by different methods. Organic solvents have been typically used, but there are also some more environmental-friendly techniques like the Pressurized Hot Water Extraction (PHWE) [5].

Multiple studies have shown that this method is feasible at large scale for extracting the heteropolysaccharides from the wood with high yields [5]. The cellulose and pectins remain intact and the obtained extract is pure and concentrated enough for further processing.

These large hemicelluloses have several applications in the pharmaceutical and food industries, and they can also be an important feedstock for the production of bioethanol [10]. But the most promising application is as a source of rare sugars, which can be obtained by cleaving these larger molecules [4] [6] [11] [12].

The sugar monomers have a high potential for use as platform chemicals that can be used as intermediates for the synthesis of several health-related products [11] [13]. These monomers can be transformed into value-added chemicals through different reactions such as hydrogenation, dehydrogenation and oxidation. The most common commercially used monomers are: xylose, mannose, galactose, arabinose and rhamnose [6] [13]. The structure of these sugars is presented in Figure 3:

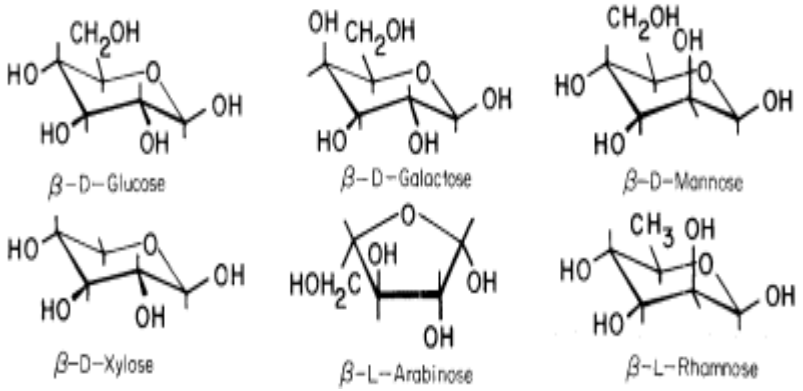


Figure 3-Most commonly used monomers

There exist some sugars which have a potential value by themselves like D-mannose or L-arabinose, which are commonly used in the production of pharmaceuticals and drugs. But in most of the applications, the oxidation or hydrogenation of these sugars is necessary [13].

The hydrogenation of xylose produces xylitol, which is a well-known sweetener, often present in chewing gums and candies because of its tooth decay preventing properties. Glucose can be hydrogenated into a compound called sorbitol, which is a high value added chemical intermediate because of its different reactivities [13].

Such versatile monosugars are crucial for the future feasibility of a bio-based refinery and that is the reason why the hydrolysis of lignocellulosic materials has been studied for more than 100 years, even though not continuously. [14]

The hydrolysis of biomass consists of breaking down the glycosidic bonds present in the different kinds of polysaccharides that conform the wood material [3]. As a result of this cleavage, shorter sugar chains are obtained, ranging from sugar monomers, to dimers, trimers and short oligomers.

It is known that the length of these chains depends strongly on the pH and the temperature. The main advantage of hemicelluloses is that their chains are more easily cleaved than cellulose because of its different structures. Due to this, monosugars can be obtained under milder conditions and undesirable reactions like the degradation of these sugars is minimized [3] [13].

This particular characteristic creates new challenges in the research of lignocellulosic hemicelluloses because it would be very interesting to be able to produce selectively for example either monomers or dimers from the same sugar solution, just by changing the reaction conditions.

The acid hydrolysis of hemicelluloses has been studied with several kind of catalysts, but traditionally it has been performed with mineral acids and enzymes [15].

These kind of homogeneous catalysts can give high yields and conversions but they have serious drawbacks including the high cost of the catalyst recovery and separation [6]. Concretely, the enzymes often suffer from high selectivity cleaving only one specific bond. Moreover, they are not very convenient because it is difficult to control and avoid the further degradation of the monomers [13].

Recent studies have shown that these problems can be avoided with solid catalysts. Heterogeneous catalysts are more suitable for these kind of applications because they can easily be removed from the product and in some cases they can even be reused, which implies an economic benefit in industrial use [14] [15].

Heterogeneous catalysis is flexible and many different types of catalyst can be produced for different applications, some of them have improved the selectivity in the cleavage of the glycosidic bonds giving even higher yields than the homogeneous catalysts [13] [14].

The concept of biorefinery has a very ambitious goal: replacing the petro-based industry that has been developed for more than 50 years and transforming the production of chemicals into a sustainable industry [3] [7] [15].

For this concept to succeed, it is very important to go deeper in the understanding of the green transformation of biomass, such as the extraction and hydrolysis of the hemicelluloses. It is also important to investigate different renewable resources which can be used in a broad raw materials basis [5].

2. THEORETICAL FRAMEWORK

2.1 Basics of Wood Chemistry

As every living or former living organism, wood is formed by cells. As a result of the long evolution period of the plants, these cells conform a very complex, heterogeneous and anisotropic raw material.

Plants require very little input of soil nutrients to be capable of synthesizing many different kind of polymers and forming very advanced structures, they just need a sufficient amount of rain (water) and air (carbon dioxide).

These bio-synthesized polymers can be divided in two major groups: carbohydrates, forming 65 to 75% of the lignocellulosic biomass, and lignin, accounting for the remaining 20 to 35 %. Other minor compounds such as extractives or ashes can be found in wood between 5 and 10%.

The diversity of the wood material is based on in the multiple ways of combining the basic elements carbon, hydrogen and oxygen obtained from the air. The composition and proportion of the different polymers depend on many factors.

The biggest differences exist between different species of trees and types of wood but even in the same family of trees, the chemical composition may depending on the tree part (root, stem or branch), with the geographic location, with the climate or with the soil conditions [16] [17] [18].

This factor complicates studying of wood properties, as raw material for the chemical industry because the composition may vary somewhat.

2.2 Wood Composition

The elemental composition of wood depends on the previously discussed external factors, but in general the percentages are close to the ones shown in Table 1-Elemental Composition of Wood:

Table 1-Elemental Composition of Wood

Element	Dry basis weight (% w/w)
Carbon	45-50
Hydrogen	6-6,5
Oxygen	38-42
Nitrogen	0,1-0,5
Sulphur	0,05 (max)
Metallic Ions	traces

The combinations of these elements are responsible for the variety of molecules and structures that can be found in trees.

Wood as a whole is often referred to in literature as lignocellulosic biomass and it is eventually divided in three different groups: carbohydrates, lignin and extractives.

2.2.1 Carbohydrates

Carbohydrates or sugars are the main constituents of the lignocellulosic biomass, accounting for 65-75 % of the total on dry weight basis. Carbohydrates are polyhydroxy compounds that contain at least one aldehyde or ketone group.

The most common sugar molecules contain five or six carbons and they have a structure. These cyclic structures can form covalent bonds with each other, this kind of bond is known as a glycosidic bond and as a result of this linkage long chains of polysaccharides are created. In these chains, monosugars act as monomers to build up polysaccharides considered as natural polymers.

Thus, carbohydrates are divided in three groups depending of the length of their chains:

- Monosaccharides: Single sugars.
- Disaccharides and Oligosaccharides: Biopolymers formed for a few sugars, two (disaccharides) or up to ten (oligosaccharides).
- Polysaccharides: Polymers formed for more than ten monomeric units.

Monosaccharides

Monosaccharides are the smallest and simplest carbohydrates and they are usually known as single sugars. They are the building blocks from which all bigger carbohydrates are made [19]. There are several ways to classify the monosaccharides.

The most common one is according to the number of carbons in the molecule, conventionally between 3 and 7. They all have the same general elemental composition (CH2O)n, where n is the number of carbons [20]. The prevailing sugar monomers in nature have six or seven carbons and they are called hexoses and heptoses, respectively. The structure of monomers with different number of carbons are presented in Figure 4:

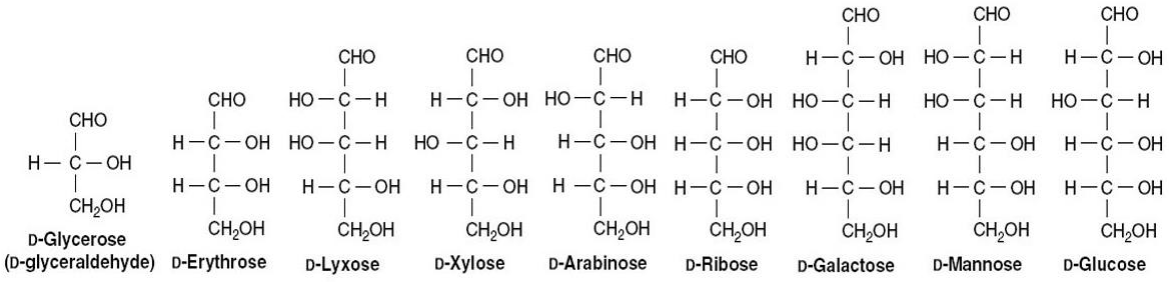


Figure 4-Classification of Monosaccharides according to the number of carbons

These monosugars tend to be stabilized in their ring form, depending if the rings are formed of five or of six carbons, the molecules are known as furanoses or pyranoses. A representation of the furanosic and pyranosic structures of β-D-Galactose can be observed in Figure 5:

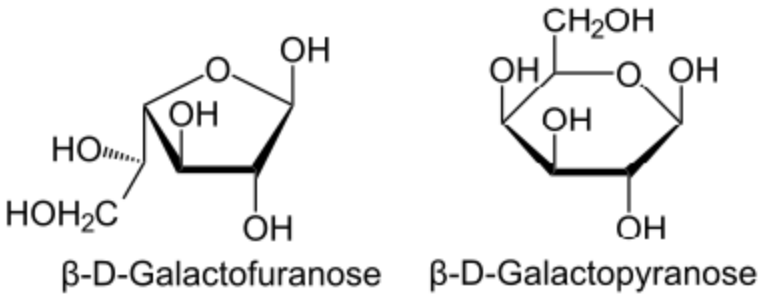


Figure 5-Furanosic and pyranosic forms of β-D-Galactose

All these molecules have either an aldehyde or a ketone group, these two different groups are referred to as aldoses and ketoses. D-glucose is a common aldose and D-fructose is a common ketose. The structures of an aldose and a ketose are presented in Figure 6:

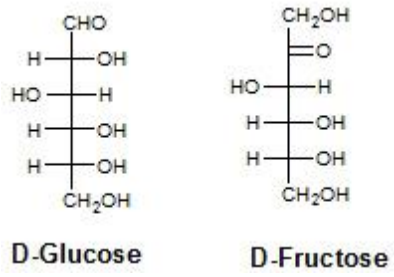


Figure 6-Classification of Monosaccharides according to the main functional group (aldehyde or ketone)

When a primary alcohol group in a hexose is oxidized to a carboxylic acid, a hexuronic acid is produced [21]. These kind of acids are present in pectins and they are important in the study of the extraction and hydrolysis of the hemicelluloses. It is believed that these kind of acid, for example, decrease the enzymatic activity in the hydrolysis of hemicelluloses [14].

Disaccharides and oligomers

Disaccharides or dimers are molecules formed by the attachment of two monosaccharides linked by a glycosidic bond. There are different types of glycosidic bonds, i.e. alfa and beta, and they can be located in different positions (carbons) in the furanosic and glycosidic rings. In many cases, these disaccharides are part of longer chains of polysaccharides.

The presence of the different kinds of glycosidic bonds can be crucial, for example, the cellobiose and the maltose are both disaccharides formed of two units of glucose but they have beta and alfa glycosidic bonds respectively. These dimers are part of two of the most important polysaccharides

in wood chemistry: cellobiose is the repeating unit in cellulose and maltose forms starch. The structures of cellobiose and maltose are presented in Figure 7:

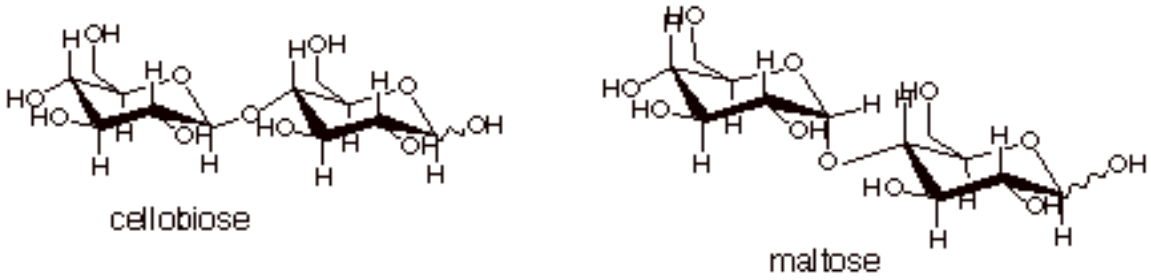


Figure 7-Two example of disaccharides: cellobiose (left) and maltose (right)

These bonds can also be formed between different monosaccharides. The best example is sucrose, table sugar which is a dimer encountered in the lignocellulosic biomass and it is formed from a glucopyranose and a fructofuranose [21]. The structure of the sucrose can be observed in Figure 8:

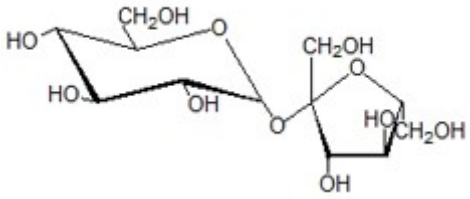


Figure 8-Structure of the sucrose

Short polysaccharides formed from between three and ten monosaccharides are called oligomers [18]. The properties of the hydrocarbons often depend on the chain length. Due to the length of its chain, the oligomers are often soluble in water and they can also be easily hydrolyzed into smaller units. This step, going from poly and oligosaccharides to monomers is the main focus of the current research.

Polysaccharides

Sugars in nature are commonly found in numerous forms of polysaccharides, just a few mono and disaccharides are found isolated in wood material. These polysaccharides have several structural and metabolic functions as they are part of the cell wall.

Polysaccharides are often classified depending on the monomers they contain. If the chain is built up of a single repeating sugar they are called homopolysaccharides, in contrary, if they chain is formed with different types of monosaccharides, they are called heteropolysaccharides.

The composition of these long and complex carbohydrates varies with the specie of the tree and the type of wood, but they are always classified in three different groups: cellulose, hemicelluloses and pectins.

Cellulose

Cellulose is the major constituent in the wood material, ranging from 40 to 50% of the total dry wood weight. It is the trees most important polysaccharide because its fibers are responsible for the structure and shape of the plant.

Chemically, it is a crystalline polymer made up of glucose units. More precisely it is a homopolymer formed of long linear chains of 1-4-β-bonded D-glucopyranoses.

The cellulose composition is the same in every species of tree but the structure and degree of polymerization may vary. The general element composition of cellulose is given by (C6H10O5) n, where n is the degree of polymerization.

The molecular weight and DP of the cellulose have a wide range of distribution because it depends strongly on the feedstock and the environmental conditions. As an example, cellulose presents a DP of 1.000 in newsprint but it is up to 10.000 in cotton [21].

This characteristic structure with long and stable chains and high molecular weight is a result of the multiple intra and intermolecular bonds existing between the different units [22].

Cellulose is the most abundant and widespread biogenic polymer in the world, it is estimated that there exists 3.24E11 m^3 in the world [23].

Numerous studies have focused on cellulose for many years, in its different transformations and its many applications, and yet it is still studied because the economic sustainability of a biorefineries based on the optimal use of all the fractions.

Hemicelluloses

Hemicelluloses are a group of compounds present in wood and they account for about 35% in dry weight. They are branched and amorphous heteropolysaccharides which have been synthesized in different metabolic routes compared to cellulose.

Hemicelluloses are not easy to classify because they are a very heterogeneous group and they are very close in structure to pectins. Hemicelluloses and pectins have similar properties such as lower molecular weights and degrees of polymerization compared to cellulose, a branched and amorphous structure and unlike cellulose they are partially soluble in water.

The lower molecular weight and degree of polymerization explains the fact that these compounds are easier to hydrolyze, which is one of their attractive properties. While cellulose presents a DP of thousands in certain feedstocks, the DP of hemicelluloses ranges between 50 and 300 [24].

These compounds have a very important biological mission, they contribute with cellulose and lignin to the structural and support tasks in the cell wall [17]. A schematic representation of the microscopic structure of the wood material is presented in Figure 9:

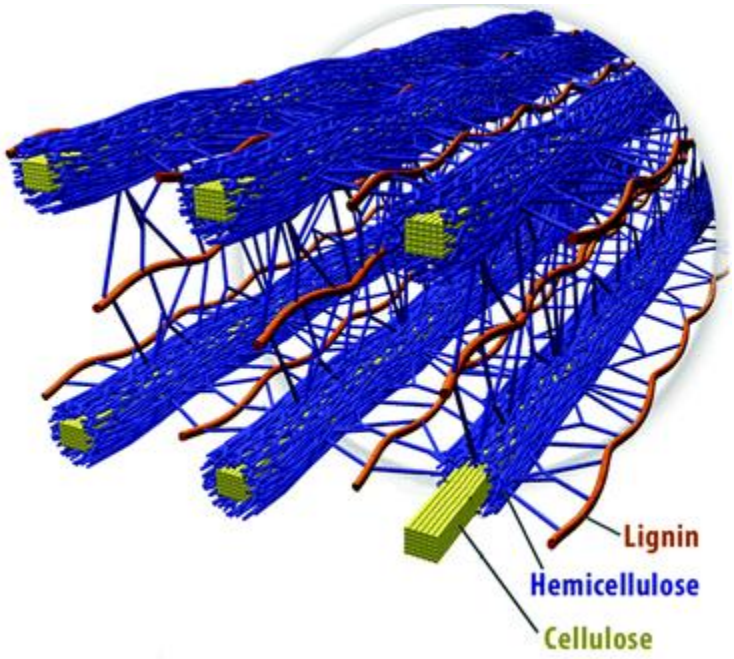


Figure 9-Wood Material Microscopic Structure

The hemicelluloses composition depends on many different factors, the major one is the type of wood (hardwood or softwood) but it also varies with the tree species and with environmental conditions [16] [17] [18].

Hemicelluloses and pectins are composed of a mixture of pentoses (e.g. xylose and arabinose), hexoses (e.g. glucose, galactose and mannose) and hexuronic acids (galacturonic acid and 4-O-methyl-D-glucuronic acid).

The common characteristic in every hemicellulose is the glucose, mannose or xylose backbone. Every monomer is connected with β-1-4 bonds and they adopt an equatorial configuration. The backbone of these sugar polymer can be either a homopolysaccharide or a heteropolysaccharide, and the side groups are usually monomers or short oligomers.

Looking at the monomer composition, hemicelluloses can be divided in four groups [25]:

- Xylans
- Mannans and Glucomannans
- Glucans
- Arabinogalactans

Xylans

The xylans are the most abundant group of hemicelluloses in biomass. Xylans are homopolysaccharides that can be either hemicelluloses or pectins with a common denominator: a backbone of β-1-4 linked D-xylopiranoses [21].

Apart from the backbone, the xylans have different kind of side chains linked to the carbons 2 and 3 varying with the specie of tree. Monomers, oligomers or hexuronic acids can be found as side groups but the most common ones are: glucuronic acid, arabinose, mannose and rhamnose [26].

The most common xylans are the glucuronoxylans (GX), which side chain is 4-O-methyl-D-glucuronic acid linked to the carbon 2 of the xyloses through a α-1-2 bond. The frequency of these side chains depends on the type of wood [27].

In hardwoods, seven over ten xyloses are linked to an O-acetyl group and one over five have a 4-O-methyl-D-glucuronic acid group. The GX are very abundant in hardwoods and grasses and they have

a degree of polymeration varying between 100 and 200. A representation of the structure of glucuronoxylan is presented in Figure 10:

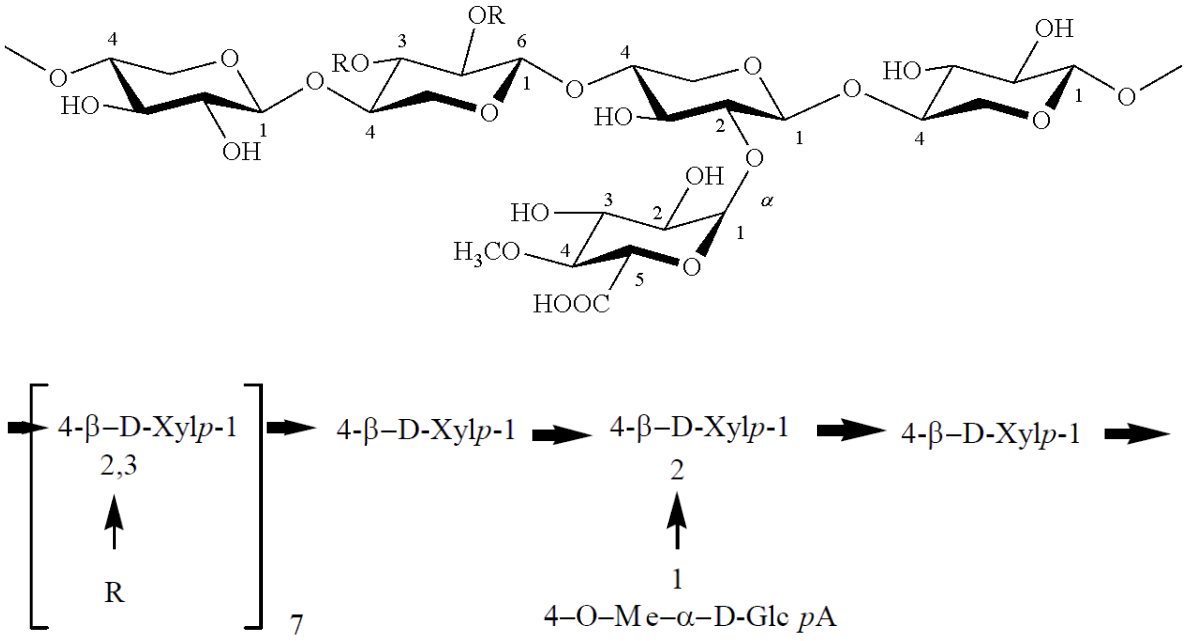


Figure 10-Structure of Glucuronoxylan (GX)

In softwoods, the glucuronoxylans also have some L-arabinose linked to the carbon 3 of the xyloses thanks to a α-1-3 bond. The arabinose group is present in one over eight xylose units. This group of hemicelluloses is called Arabinoglucuronoxylans (AGX) and it constitutes between the 5 and 10% in weight in some species. The degree of polymerization in this case ranges from 90 to 120.

Xylans are interesting for the hydrolysis of hemicelluloses because the bonds linking the xylose units are easily hydrolyzed by acids while the side chains are more resistant. It is possible to cleave also the acetyl groups but an alkali treatment is needed.

Mannans and Glucomannans

Different hemicelluloses have mannans in their backbones, some of them are homopolysaccharides (pure mannans) but they are not predominant. The most abundant hemicelluloses in this group are glucomannans.

Glucomannans are heteropolysaccharides formed by a backbone of β-1-4-linked D-Glucopyranose and D-Mannopyranose units. The carbons 2 and 3 in the mannose and the glucose are linked to acetyl groups in three over four hexose units.

There exists different kinds of glucomannans but the most relevant ones are the Galactoglucomannans. In this type of hemicelluloses, D-galactopyranose acts as the side chain through α-1-6 bonds. A representation of the structure of galactoglucomannan is presented in Figure 11:

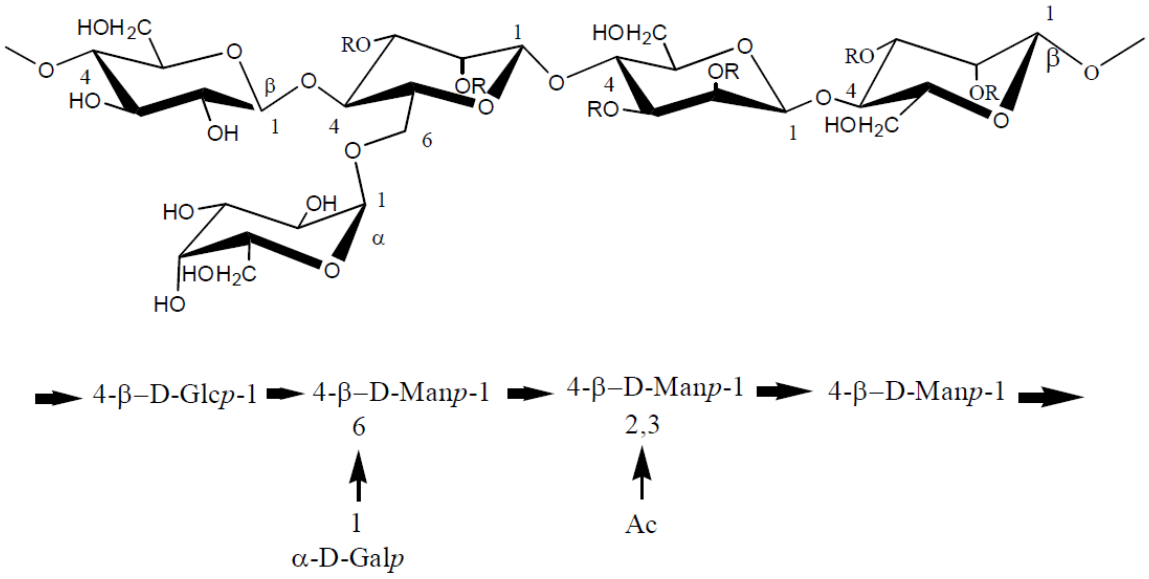


Figure 11-Structure of Galactoglucomannan (GGM)

These hemicelluloses are more precisely called galactoglucomannans (GGM) and they constitute between the 5 and the 15% of the dry weight in some softwood species.

Acetylgalactoglucomannans can be classified according to the galactose content:

- Glucomannans: They have a low content in galactose and they are partially soluble in water, the ratio galactose:glucose:mannose is approximately 0.1:1:4 [27].
- Galactoglucomannans: They present a high level of galactose and they are not soluble in water, the galactose:glucose:mannose ratio is 1:1:3 [18].

The degree of polymerization of the GGM is estimated to be between 118 and 132 units, however also larger units have been reported [11].

Like xylans, galactoglucomannans are a potential source of sugars and a promising raw material for the controlled hydrolysis. The main chain can be hydrolyzed under acid conditions but also the galactose in the side chains is easily cleaved from the backbone [28].

Xyloglucans

Xyloglucans (XG) are another type of hemicelluloses which are not that common but are very important for the cell wall in most of the high plants.

They are heteropolysaccharydes based on a backbone similar to cellulose, and consists of glucose monomers linked by β-1-4 bonds.

This family of hemicelluloses is very complex and the sidechains are usually made of different oligomers connected to the carbon number 6. The most common sequence of this oligomer is:

- Xylopiranoses attached to the position 6 of the glucose with a α-1-6 bond.
- Galactopyranoses linked to the position 2 of the Xylopiranoses through a β-1-2 bond.
- Fucopyranoses molecules appears at the end of this chain thanks to α-1-2 linked to the Galactopyranoses.

Also, galactose residues are often O-Acetylated [29].

The ratios and frequency of the different monomers varies with the different plant families. A representation of the structure of xyloglucan is presented in Figure 12:

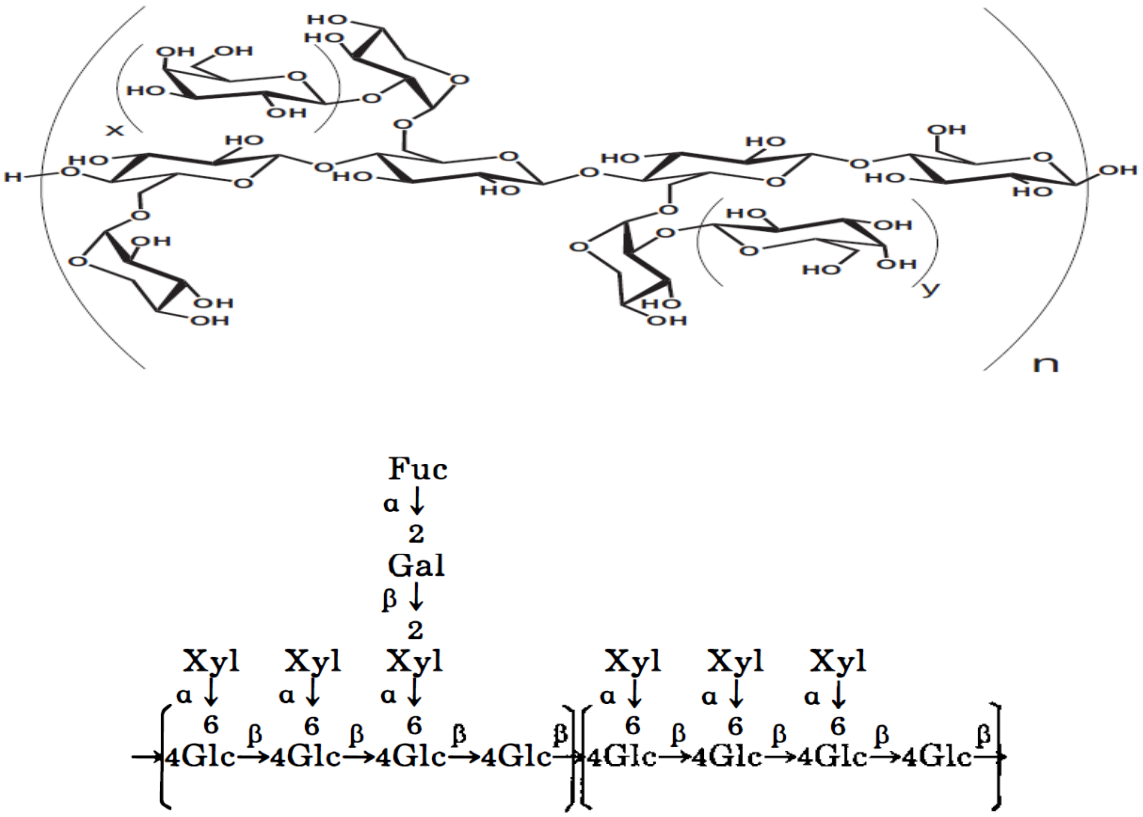


Figure 12-Structure of Xyloglucan (XG)

Arabinogalactans

Arabinogalactans are significant components in just a few species but they are often found in minor amounts in all kinds of lignocellulosic biomass [30].

This group of hemicelluloses is composed of highly branched polysaccharides and their structure has not been entirely studied yet. The branched structure results in very particular properties of high solubility and low viscosity and they are responsible for the conventional classification of arabinogalactans as extractives.

The main chain of the arabinogalactans is constructed of 1-3-β-galactopyranose units and different side chains are frequently attached to the carbon 6.

Different kinds of side chains have been found in arabinogalactans, from monomers and dimers to hexuronic acids. The most typical side chains are monomers like arabinopyranoses and dimers like 3-O-β-L-Arabinopyranosyl-L-Arabinofuranose [18] [27].

Pectins

Pectins are the most highly branched carbohydrates in lignocellulosic biomass, they are a very heterogeneous group of heteropolysaccharides characteristic in their capacity to be easily extracted from wood.

They are very similar to some hemicelluloses but pectins contain large amounts of uronic acids in their backbones, thus sometimes both polysaccharides are classified together even if they are very different chemically.

Three different families of pectins are known: Homogalacturonans, Rhamnogalacturonans and substituted galacturonans, all of them have in common a backbone of partially methylated α-1-4-Galacturonic acid units. A representation of the structure of a pectin molecule can be observed in Figure 13:

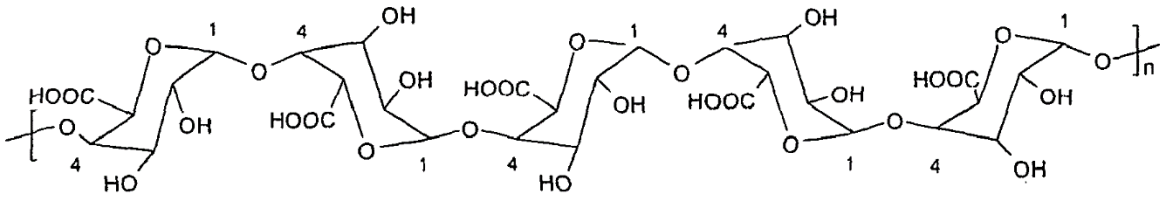


Figure 13-Structure of a pectin

Pectins are part of the primary cell walls but they are not very abundant in the lignocellulosic biomass suitable for biorefining, this is the reason why they have not received as much attention as hemicelluloses.

2.2.2 Lignin

Lignin is not just a compound but a variety of them. All are complex, amorphous and three-dimensional molecules formed by a group pseudo-monomer units called phenylpropane. This phenylpropane consists in a benzene ring attached to a three carbon group.

There exists three kinds of phenylpropane units conforming the lignin. The amount of each of these compounds depends strongly on the different species of wood. The structure of the different phenylpropane units is represented in Figure 14:

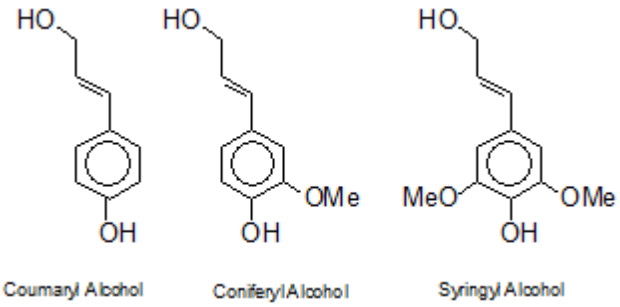


Figure 14-Phenylpropane Units

Coniferyl alcohol occurs in all species and is the dominant monomer in conifers (softwoods).

Coniferyl alcohol is the main monomer in softwoods while syringyl alcohol is more present in hardwoods. Coumaryl alcohol is dominant in other kinds of lignocellulosic biomass such as grass and agricultural crops.

Phenylpropanes molecules tend to create internal bonds in many different ways: directly between the rings, between the propane units and through ether linkages via the hydroxyl groups. This tendency is responsible for the three-dimension structure that gives interesting properties to the lignin molecules.

The lignin has mainly a structural function, it cements the fibers of cellulose together, giving strength to the plants. This rigidity allows the tree to grow tall and firm and also facilitates the flow of water and nutrients through the vascular tissues of the plant, this is the main evolving role of lignin and it is the reason why it is only present in the group known as vascular plants.

But apart from this, lignin has several other biological functions in the plants like being a protective barrier from foreign attacks and preventing the absorption of water from the rest of the structural polysaccharides (cellulose and hemicellulose).

Lignin can also be an interesting polymer from an industrial point of view thanks to its hydrophobic and aromatic properties. It is, after cellulose, the most abundant natural polymer in the world and it is the only one having an aromatic functionality and that is not composed of carbohydrate monomers.

As it is the second most widespread carbon source on Earth, a lot of research has been done with lignin but there are still some gaps in the characterization of this polymer, for example the degree of polymerization.

The DP of lignin is difficult to calculate precisely because it is cleaved during the extraction and the molecule consists in many different substructures.

What it is known is that lignin molecules are much smaller than cellulose and that each polymer is composed approximately of 25 aromatic rings. An example of the lignin structure is presented in Figure 15:

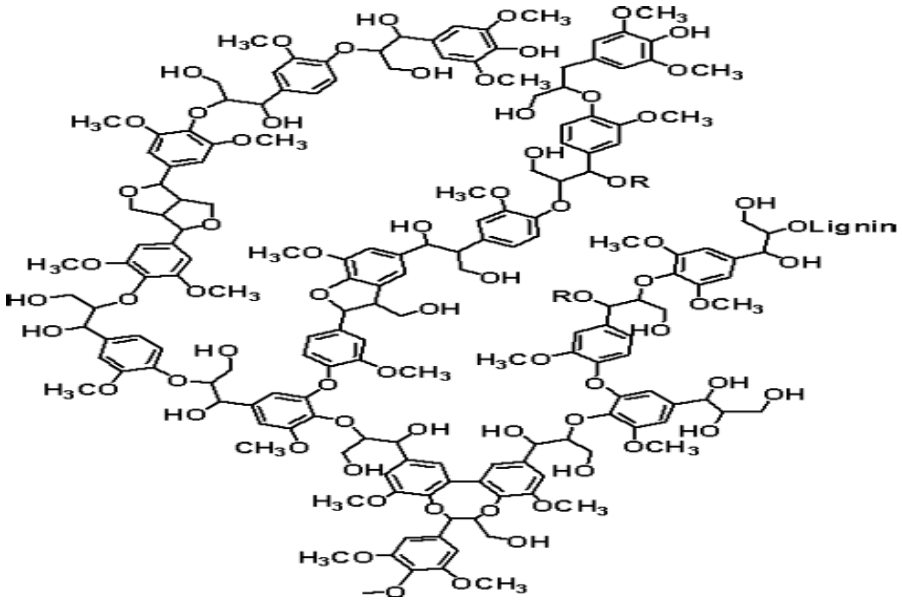


Figure 15-Lignin Structure

2.2.3 Extractives

Beside the lignocellulosic material, there exist another small fraction which constitutes between 4 and the 10% of the dry weight in normal wood species. This fraction is a mix of low molecular weight organic compounds, known as extractives. Compounds like fat, waxes, proteins, resins, terpenes and essential oils can be found among this mixture.

These extractives do not contribute to the cell wall structure but they have important function as intermediates in tree metabolism, as energy reserves and as a part of the defense system against microbial attacks.

As they can be easily extracted with water or with neutral solvents, they could also have an important role in the biorefineries. For example, in the case of some species of Stone Pine, some interesting chemical compounds like rosin and turpentine can be extracted.

Polysaccharides may also be linked to other polysaccharides by direct glycosidic cross-links or, ether cross-linkages or ester cross-linkages [31]. The nature of polysaccharides - including their monosaccharide substituents, glycosidic linkages, intra- and inter-molecular associations and distribution within the cell walls - are all crucial factors in the determination of the hydrolysis dynamics in biorefining technologies.

2.3 Wood Classification

The classification of wood is a really diverse issue, it can be done according to several standards. Two different classifications are presented here for obtaining a better understanding in the subject. A physical classification separates the different parts of the tree in sapwood and heartwood and a biochemical classification between softwood and hardwood.

2.3.1 Physical classification

From a physical point of view, the wood material from one single tree can be classified in different fractions: heartwood, sapwood and bark [32]. The different parts of a tree can be observed in Figure 16:

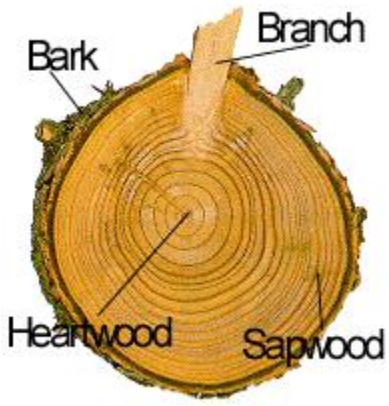


Figure 16-Different parts in a tree section

The bark provides important protection for the tree, isolating the trunk from the climate and from external agents. The composition of bark is completely different from the rest of the tree so it will not be considered in this work as it has not been used in the experiments.

The inner part of the trunk can be divided in two: sapwood and heartwood. Sapwood is considered the “living” part of the tree in which the sap flows and brings nutrients and water to the different cells. It presents mild color and its mechanical properties are reduced, this is why it needs the heartwood to keep growing during the lifespan of the tree.

The heartwood can be found in the very center of the trunk and it is the oldest wood in the tree. There is a moment in the life of a tree when it doesn’t need the whole trunk to transport sap, at this moment, the central part of the trunk starts to accumulate different compounds called extractives and it becomes more rigid and hard, so it is considered to be the “dead” part of the tree. The extractives are substances of different nature which give the tree its characteristic color. The heartwood helps the tree to grow taller because supports the growing sapwood.

Chemically speaking, the composition of sapwood and heartwood is very similar, having just slight differences in the cellulose and lignin concentrations which are lower in the heartwood. The only major difference is the presence of extractives in the heartwood. These extractives can complicate the analysis of the wood and this is one reason why only sapwood was used in this study. Moreover, the biorefinery industry uses mainly younger trees from thinning which contain mainly sapwood.

The chemical composition of both fractions is presented in Table 2:

Table 2-Main component groups in the different wood zones in %(w/w) of dry wood

Component (Technique)	**Heartwood**	**Sapwood**
Lignin		
(Acetyl bromide, UV)	32,4 ± 1,6	30,9 ± 0,8
(Klason)	28,3 ± 0,3	27,7 ± 0,1
Hemicelluloses		
(Methanolysis, GC)	25,7 ± 1,4	24,3 ± 1,4
Lipophilic extractives		
(Micro-Soxhlet, GC)	0,51 ± 0,03	0,88 ± 0,05
(Ultrasonic, GC)	0,57 ± 0,8	0,91 ± 0,7
Cellulose		
(By difference)	45,5 ± 1,7	47,1 ± 1,6

2.3.2 Chemical classification

From the chemical point of view, the wood material can be classified in two general groups: softwood and hardwood. Apart from the chemical differences, they present several biological contrasts.

The classification of a wood species is not a trivial issue, and it can lead to misunderstandings; the lignocellulosic material forming the softwood is not weak or fragile and the material in the hardwood is not always so strong and rigid. But in general, softwoods are less robust than hardwoods.

Softwood and hardwood are just familiar surnames for the correct classification which is done between gymnosperms or coniferous (softwoods) and angiosperms (hardwoods), whose difference resides in the reproduction method.

The first chemical difference in their composition is that softwoods have a higher lignin content than hardwoods and in contrary a lower polysaccharide content (cellulose and hemicellulose). The different component fractions in softwoods and hardwoods are presented in Table 3:

Table 3- Different component fractions in softwoods and hardwoods in % (w/w)

Component	Softwoods	Hardwoods
Lignin	25-30	18-25
Polysaccharides	66-72	74-80
Extractives	2-9	2-5
Ash	0,2-0,6	0,2-0,6

Large differences can also be found in the lignin and hemicellulose compositions which determine the different properties of the wood materials.

As previously mentioned lignin is formed by three different monomers: p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol.

While softwood lignin is mainly formed by p-coumaryl alcohol and conyferil alcohol, in the hardwoods lignin the sinapyl alcohol is also present.

One of the main and most important differences for the current work is the composition of the hemicellulose fraction. The compositions for both hardwood and softwood are presented in Table 4:

Table 4-Hemicellulose Composition of hardwood and softwood in %(w/w)

Hemicellulose	Hardwood	Softwood
Methylglucuronoxylans	80-90	5-15
Arabinomethylglucuronoxylans	0,1-1	15-30
Glucomannans	1-5	1-5
Galactoglucomannans	0,1-1	60-70
Arabinogalactans	0,1-1	1-15
Other Galactans	0,1-1	0,1-1
Pectins	1-5	1-5

Softwood

Softwoods is are generally considered to belong to the family of trees called gymnosperms or coniferous, which means that their seeds do not grow in a flower but in a cone. They are known for having needle-shape leaves and for being evergreen, their leaves do not fall during the winter [33].

Softwood is generally used as building material and its value is lower than the hardwoods because they grow faster and they are typical from cold climates. Two pictures of different softwood trees can be observed in Figure 17:

Figure 17-Two examples of softwood: (Left) Pinus Stone Pinea, Stone Pine, (Right) Picea Abies, Norway Spruce

The most important sugars forming the hemicelluloses of the softwoods are mannose, xylose and glucose. The main hemicelluloses are galactoglucomannan (20%) and arabinoglucuronoxylans (10%). Moreover, softwoods also contain arabinogalactans, xyloglucans and other species of glucans [34].

The softwood species studied in this work are Stone Pine and Picea Abies, Stone Pine and Norway Spruce respectively. Their total sugar composition has been analyzed in a previous study and the results are presented in Table 5:

Table 5- Sugar Composition (%) of PP and PA and total hemicellulose fraction (%)

	4-O-MeGlcA	**Ara**	**Gal**	**GalA**	**Glc**	**GlcA**	**Man**	**Rha**	**Xyl**	**Total HC in wood**
Stone Pine	0,70%	7,10%	15,00%	5,60%	**13,50%**	0,40%	**36,40%**	1,20%	**20,20%**	27,00%
Norway Spruce	2,03%	5,28%	7,06%	5,41%	**16,44%**	0,00%	**39,51%**	0,97%	**23,28%**	23,11%

Hardwood

Hardwood belongs to the family of trees called angiosperms, which means that their seeds grow protected by flowers and fruits. They have usually broad leaves and these leaves fall during the cold seasons [33].

Hardwoods are common in warm climates and they grow slower than softwoods. Their trunks are not straight and they are highly branched, this is why they are not used as building material but as a high quality and resistance material in furniture and other applications. Two pictures of different hardwood trees can be observed in Figure 18:

Figure 18-Two examples of hardwood: (Left) Quercux Ilex, Holm Oak, (Right) Betula, Birch

The predominant sugars in the hemicelluloses of the hardwoods are xylose, glucose and 4-O-Methyl-D-glucuronic acid. The most important hemicelluloses are xylans, especially O-acetyl-4-O-methylglucuronoxylan, abbreviated as glucuronoxylan (GX) which accounts for almost 90% of the total.

The hardwood species studied in this work is Quercux Ilex, Holm Oak, from which the total sugar composition has been analyzed in previous studies. The results of the analysis are presented in Table 6:

Table 6- Sugar Composition (%) of HO and total hemicellulose fraction (%)

	4-O-MeGlcA	**Ara**	**Gal**	**GalA**	**Glc**	**GlcA**	**Man**	**Rha**	**Xyl**	**Total HC in wood**
Holm Oak	6,00%	4,70%	6,20%	6,00%	**9,30%**	0,70%	3,60%	2,20%	**61,40%**	30,20%

2.5 Reactions of hemicelluloses

Hemicelluloses have unique chemical properties which make them suitable for a wide range of transformations.

2.5.1 Hydrolysis

The structure and composition of hemicelluloses make them suitable for many industrially interesting reactions. One of the most promising transformations of hemicellulose is the hydrolysis of the polymeric chains in order to obtain monomers and short oligomers for further valorization.

The heterogeneity of hemicelluloses is a very important aspect because a huge amount of rare sugars are present in their structures. Following the definition of the international society of rare sugars, these monomers rarely occur isolated in nature, so the hemicelluloses are expected to be one of the biggest sources of these monosaccharides [35].

The production of rare sugars in high yields is going to be a vital aspect for the economic viability of the biorefineries because of their different applications as intermediates and platform chemicals in the synthesis of higher added-value products.

Hydrolysis has been studied for years with other lignocellulosic materials with different goals, for example, hydrolyzing cellulose to obtain glucose. The hydrolysis of hemicelluloses has not been as widely studied however, some very interesting results have been found. Due to their branched structures and lower DP compared to cellulose, the hydrolysis can be performed under milder conditions and according to rather environmental-friendly procedures. Moreover, the milder conditions help in avoiding monomer degradation.

The hydrolysis of biomass is based on the cleavage of the glycosidic bond linking two consecutive units in a polymeric chain. Cellulose presents a very crystalline and regular structure that requires high temperatures and highly concentrated acids to be cleaved. These drastic conditions lead to the instability of the sugars and the degradation of the products.

In contrast, hemicelluloses can be easily cleaved under modest temperature and acid concentrations, minimizing the degradation of the products and increasing the yield to the desired product, in this case, monomers [3]. Hydrolysis can also be achieved by alkaline hydrolysis but it is problematic because it causes several side reactions and thus the yields are not that high [36].

Mechanism of Acid Hydrolysis

The mechanism of the acid hydrolysis is well known due to numerous detailed studies and it is based on the cleavage of the C-O-C bonds linking two sugar molecules [36]. It starts with the protonation of the glycosidic or pyranic oxygen, which is the oxygen present between two different monomeric units. The bond is broken due to the protonation and to the action of the electronegative effect and a carbocation is formed. This carbocation is unstable and it reacts with water, regenerating the proton. As a result, the polymeric chain splits in two fractions that can be monomers, dimers or longer oligomers.

The protonation may happen at any positions in the chain and thus two different options are contemplated. If the protonation takes place in an intermediate point of the chain, two low weight oligomers will be released, but if the protonation occurs at the end of the chain (end biting process), an oligomer and a monomer will be produced [37]. A schematic representation of the mechanism of hydrolysis is presented in Figure 19:

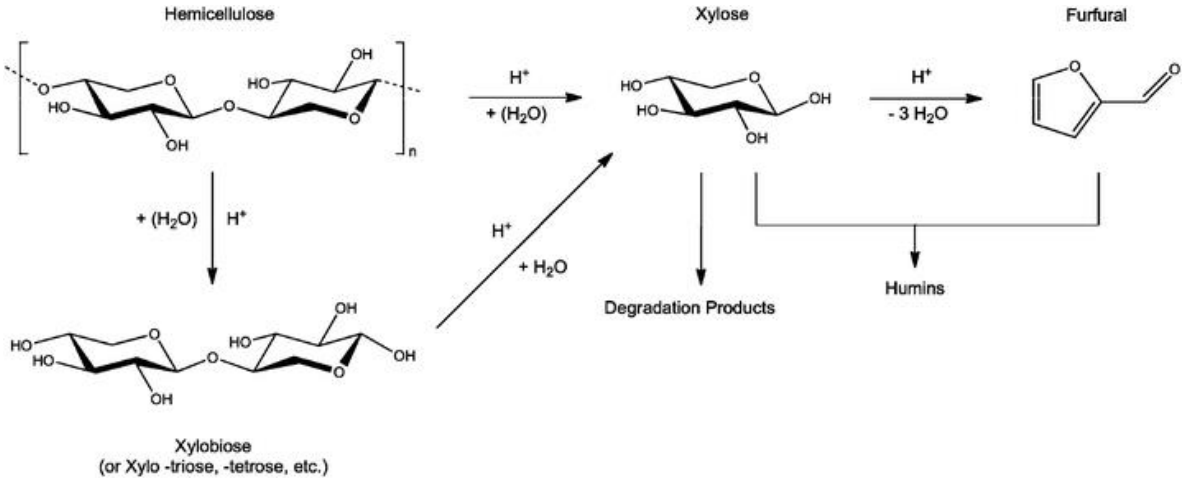


Figure 19-Mechanism of Hemicellulose Hydrolysis

After the breaking of the glycosidic bonds and the consequent release of shorter oligomers, the reaction can continue to the degradation of these molecules into undesired products. At high temperatures and low pH pentoses are degraded into furfural and hexoses into 5-hydroximetilfurfural. This degradation leads to a serious decrease in the yields towards monomers.

Conditions affecting the hydrolysis

The process of depolymerization and degradation of hemicelluloses goes through several often practically irreversible consecutive reactions leading to the desired product. It is very important to understand the kinetics of every reaction step in order to be able to optimize the reaction conditions and maximize the yields. A schematic representation of the kinetics of hemicellulose hydrolysis is presented in Figure 20:

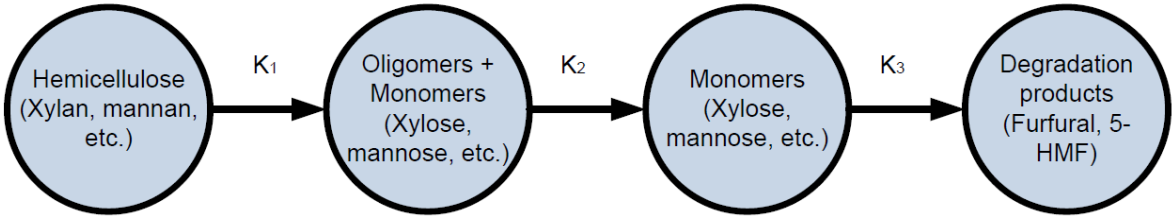


Figure 20-Schematic representation of the reaction sequence of hemicellulose hydrolysis

The adequate control of the depolymerization process is crucial for the future development of the biorefineries. The main goal of this industry is to be able to produce selectively different products from different biomass sources. The most important factors that must be studied are: pH, temperature, acid concentration and the type of catalyst.

Furthermore, the hydrolysis of a hemicellulose is influenced by its structure in many different aspects.

- **Side chains:** The inductive and steric effects caused by the interaction between the main chain and the side chains are important for the stability and reactivity of the different glycosidic bonds. This could explain at which point of the chain the hydrolysis will take place.

- **Uronic acids:** The presence of uronic acids in the side chains stabilizes the molecule, counteracting hydrolysis in this specific area of the hemicellulose.

- **Number of carbons in the cycles:** Furanoses (5 carbons) are much easier to hydrolyze than pyranoses (6 carbons) because the angular strain present in the furanosic rings is not present in the pyranoses. This could explain why arabinose based hemicelluloses (softwoods) are easier to hydrolyze than xylose based (hardwoods) [6] [38].

- **Anomers:** The kinetics of the hydrolysis is different also depending on the anhydrosugar structure. Beta anomers are hydrolyzed faster than alfa anomers.

2.6 Acid Catalysis

In acid catalysis the chemical reaction is catalyzed by an acid and the mechanism is often explained by the Brønsted–Lowry theory of acid-base interaction. The acid, which is usually a proton, is transferred from the catalyst to the reactant, this process is called protonation and it is the rate-determining (slow) step of the process. After the reaction, the catalyst is regenerated. A schematic representation of the reaction mechanism is displayed in Equation 1:

$$HA + R + H_2O \xrightarrow{Slow} HR(H_2O)^+ + A^- \xrightarrow{Fast} P + HA \quad (1)$$

$$HA = Acid\ Catalyst$$

$$R = Reactant$$

$$P = Product$$

The acid hydrolysis of hemicelluloses has been studied with different catalysts: enzymes, solid catalysts, and organic and inorganic diluted acids. The conversion and yields depend strongly on the structure of the hemicellulose and on the experimental conditions namely of acidity and temperature [39].

For many years, the most common technique has been the homogeneous catalysis through strong acids like sulfuric acid, hydrofluoric acid or phosphoric acid but recently, a numerous sort of solid catalyst, such as zeolites or ion-exchange resins, have proved their efficiency.

2.6.1 Homogeneous Acid Catalysis

The acid catalysis using homogeneous catalysts has been widely studied with both organic and inorganic acids. Concretely, the hydrolysis of hemicelluloses has been performed with different many different kind of acids including sulfuric acid, phosphoric acid, nitric acid, acetic acid, oxalic acid, trifluoroacetic acid, and maleic acid [6].

The optimum pH and temperature have demonstrated high conversion and yields towards the desired products. Studies have shown that the kinetics of hydrolysis is very fast and the conversion towards monomers goes rapidly through a maximum after which degradation occurs [40].

These homogeneous catalysts have several drawbacks:

- **Sugar degradation:** The harsh conditions needed to achieve high conversions also lead to a high dehydration of the released sugars [39]. The formation of these by-products reduces dramatically the yields towards the desired product. The optimization of temperature and pH is a demanding task.

- **Catalyst separation:** When inorganic acids are used and the reaction has finished, the solution has to be neutralized and the precipitated salts must be removed from the mixture. If organic acids are used, the separation is not that problematic because they can be easily evaporated from the mixture. However, separation is often a costly process with homogeneous catalysts.

- **Corrosivity of materials:** The homogeneous catalysts are not usually expensive but the strength of the acids and the high temperatures reached during the process call for extra investments in anticorrosive materials.

Enzymatic catalysis avoids the problem of corrosion and partly also separation but it has demonstrated to be less practical, because often a cocktail of enzymes has to be used and the control of the process becomes complicated. Also the sensitivity to high temperature and the rather slow reaction rates limit the use of enzymes.

All of these drawbacks are trying to be overcome with the use of new solid catalysts.

2.6.2 Heterogeneous Acid Catalysis

The heterogeneous acid catalysis fulfils the same goal as the homogeneous catalysis. It is meant to provide a source of protons to the reactant in order to achieve the protonation that will help the reaction to take place [41].

By using a solid catalyst, the separation problems are overcome because the catalyst can be easily separated by filtration and no further neutralization is needed [6]. Also, the easy recyclability is a clear advantage. Even though the solid catalysts can be more expensive than the homogeneous acids, the material costs would be minimized because there is no need for anticorrosive materials in the same extent.

Apart from the operational costs, an advantage of heterogeneous catalysts is their selectivity towards sugars. Many different catalysts are being developed for different applications, depending on the target product of the hydrolysis. With the use of solid catalyst, the cleavage of glycosidic bonds is controlled and secondary reactions like the degradation of the monomers are avoided optimally [39].

In brief, the kinetics are not as fast as with homogeneous catalysts, but the conversion and yields are higher due to the specificity of the catalysts.

The specificity of the solid catalyst also permits to achieve higher yields because undesired reactions are avoided and the degradation of the products is minimized.

But the heterogeneous catalysis have also some problems and uncertainties. The mass transfer (both external and internal), the shape and structure of the matrix, the size of the pores or the acidity and the effectiveness of the acidic groups are parameters of great concern. All of these different variables belonging to the catalyst design have a big influence on the kinetics of the reaction [40] [42] [43].

The characteristics of the solid particles have to be adapted to the necessities of the reactant molecule in order to make sure that the molecules can reach the active site and the reaction can take place. In solid catalysts the acid groups are not dissolved in the reaction medium, they are embedded in a solid matrix that can present different morphologies. The molecules of reactant have to be transferred from the liquid to the surface of the catalyst (external mass transfer step) molecule and then, they have to be transferred to the place where the acidic groups are linked (internal mass transfer step), the active site. In this place, the reaction takes places and then, the products must be desorbed from the particle and transferred again to the reaction medium [41].

The heterogeneous acid catalysis is considered a green process because it avoids the problems with the separation and the continuous production of waste because the solid catalyst can be easily removed by filtration and it can be often reused. The corrosion problems are also minimized because it allows to decrease the rough temperatures needed in homogeneous catalysis.

Heterogeneous catalysis is a hot topic in the organic chemistry field, this is why many different kinds of solid catalyst are being used and investigated. The most interesting ones are zeolites, ion-exchange resins and fibrous catalysts. The discussed acid catalysts can be observed in Figure 21:

Figure 21-Different acid catalyst (from left to right): Zeolite, Ion exchange resin and super acid fibrous catalyst

Just a few studies have been performed in the field of heterogeneous hydrolysis of hemicelluloses, but it has been shown that due to the big size of the hemicelluloses, catalysts with big pores or with a large surface area must be used. Most of the studies have, thus, focused on the use of ion-exchange type of acid catalysts [40]. These catalysts have several acidic groups in their structures. These acidic groups provide the hemicelluloses present in the liquid with the protons needed to break the glycosidic bonds [39] [44].

Higher yields have been achieved using ion-exchange resins with big pores or fibrous catalysts that are not porous materials but have a large surface area.

Ion Exchange Resins

Ion Exchange resins were the first synthetic molecules used for catalysis that simulated the action of the soluble acids [45]. There exist many different types but the most commonly used are based on a matrix of cross-linked polystyrene.

They may possess numerous kinds of acidic groups which it determine the strength of the acidity. For example, if the anchored group is a sulfonic acid (-SO3H) the catalyst will act like a strong acid while if the group is a carboxylic group (-COOH) the catalyst will be similar to a weak acid. A schematic representation of an ion-exchange resin is presented in Figure 22:

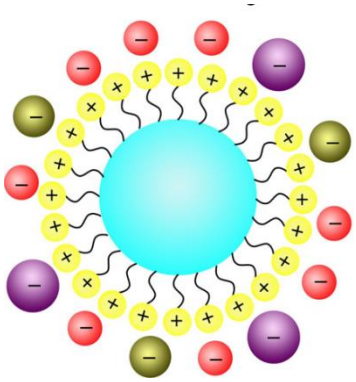


Figure 22-Cation Exchange Ionic Resin

Ion exchange resins are widely used in the industry because of their versatility, they can be produced in many different ways and they are suitable for a wide range of applications. They are able to exchange cations or anions, depending on the structure of the matrix and the embedded groups.

Their porous structure can be designed to accommodate different molecular sizes, from the gel-type that presents microporous and microreticular pores to the mesoporous or macroporous structures that are suitable for the transformation of hemicelluloses

The acidity of the catalyst gives an idea on the strength of the acidic groups and the capacity is related to the amount of available active sites.

Zeolites

Zeolites are micro or mesoporous aluminosilicate minerals that are commercially used for many applications as catalysts and sorbents. Their structure is composed of two components that define their unique properties:

- **Matrix:** It works like the support or body of the acidic groups. It is made of covalently linked aluminum, silica and oxygen. The aluminum groups gives the zeolite structure a negative charge that has to be balanced.

- **Counter-Cation:** Small positive charged molecules accommodated in the pores of the zeolite that balance the electronegativity of the matrix. They are usually Na+, K+, Ca2+, Mg2+ or protons.

The counter-cation groups are responsible for the acidity of the zeolites and due to the weak bonds linking the counter-cation and the structure, zeolites are perfectly designed to work as ion exchange materials [46]. A schematic representation of the structure of zeolites is presented in Figure 23:

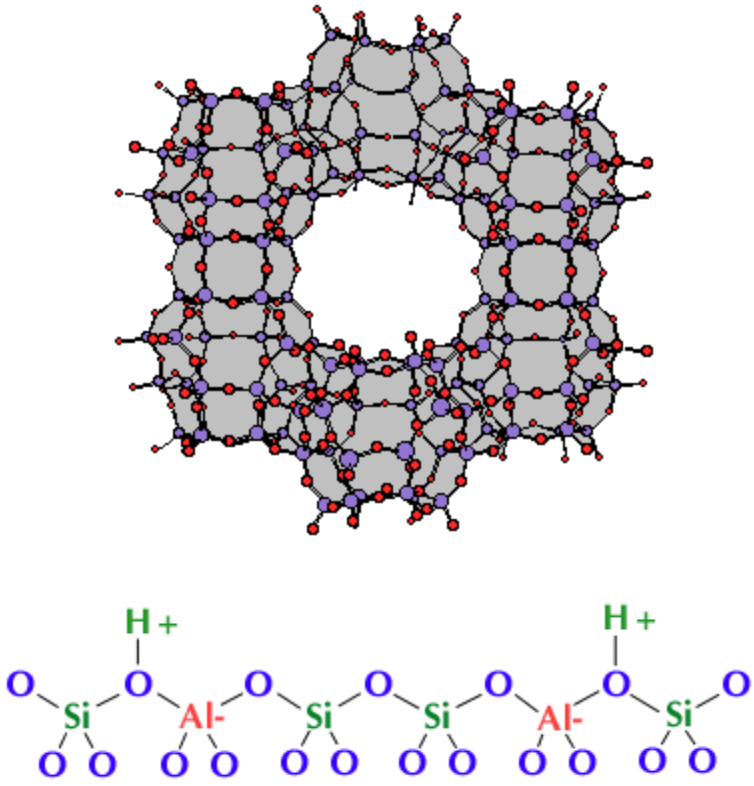


Figure 23-Zeolite Structure

It is possible to adjust their acidity by changing the acidic groups, but unfortunately, due to their rather small pore size, they are not ideally suitable for the reactions involving large molecules like hemicelluloses.

Fibrous Catalysts

There exist a special kind of ion exchange catalysts which is very suitable for the hydrolysis of hemicelluloses. This kind of catalyst is known as superacid fibrous catalyst and it has a non-porous structure. It is formed of small fibers with embedded acidic groups on the surface. Due to the small size of the fibers it has a large surface area. An example of the catalyst is presented in Figure 24:

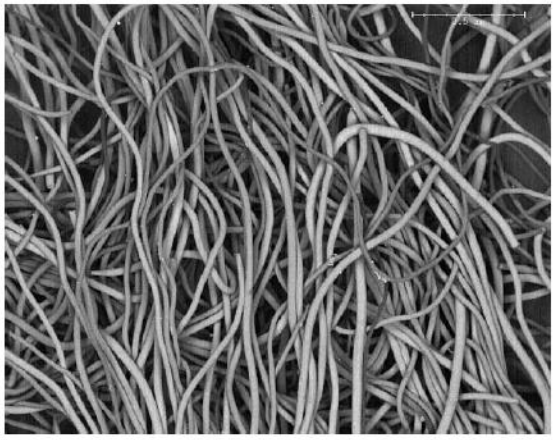


Figure 24-Sem micrographs of the catalyst fibers; the fiber diameter=10 μm, fiber length=4 mm

As the external surface area is high, the acidic sites are well accessible for the bulky hemicellulose molecules. This facilitates the reaction rate as internal mass transfer limitations do not defer the kinetics as with porous catalysts.

The fibrous catalyst used in the present study for the hydrolysis of hemicelluloses is called Smopex-101 and it is manufactured by linking sulphonic groups to a structure of poly (ethylene-graft-polystyrene). The structure of Smopex-101 can be observed in Figure 25:

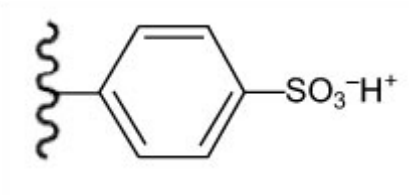


Figure 25-Smopex-101 structure

2.7 Analysis Methods

Chromatography is a field of analytical methods used to separate components from a complex mixture. The column used in the separation provides: an immobile phase called stationary phase and the substance to be fractionated moves through the stationary bed in a definite direction called the mobile phase [47] [48].

Chromatography includes a wide range of different methods of analysis but all of them present the same four different parts: the injection system, the mobile phase, the stationary phase and the detector. [49]

- **Injection System**: The injection system is in charge of introducing the sample in the system. For this purpose, the sample must be diluted in the mobile phase which can be a liquid, a gas or even a supercritical fluid, thus there are many different types of injectors. The main requirements of an injector are not to overload the chromatographic system (often a column) and also keeping the ratio between sample and mobile phase in a constant value.

- **Mobile Phase**: The mobile phase is the media in which the separation takes places. In the injection system, the sample mixture is diluted in the mobile phase and then pumped into the column where it interacts with the stationary phase. When the mobile phase abandons the column, it is called eluate. Many different kinds of chromatography are used, depending on the fluid used as a mobile phase: Gas Chromatography, Liquid Chromatography or even Supercritical Chromatography.

- **Stationary Phase**: The stationary phase is an immobile and immiscible substance which interacts with the components of the mixture being carried by the mobile phase. These interactions can have different natures (acidity, solubility, polarity, size, etc.) and they cause the separations of the different compounds. The stationary phase can be a solid or a liquid and it must be chosen carefully according to the mobile phase in order to have a proper separation.

- **Detector**: The detector is the instrument situated at the end of the chromatographic system and it is the responsible of measuring and recording the signal of each component. These signals are displayed in time graphs called chromatograms where each different compound can be identified depending on its retentions times. Some types of detectors allow the separation of the mixture but others can be used only for analyzing because they destroy the sample.

Each method of separation in chemistry exploits a unique property of the compounds in the mixture. Distillation uses the boiling point and decantation uses the density. In chromatography, the different components are separated because of the difference in their retention or elution time.

The retention time is defined as the time that each molecule spends in the chromatographic column, from the injection system to the detection device. Both the mobile phase and the stationary phase are chosen to produce different affinities between the sample and the stationary phase, some compounds are more attracted to the stationary phase due to different forces such as polarity, solubility or size. These weak interactions create differences in the migration times which produce the separation of the molecules. The mechanism of a chromatographic column can be observed in Figure 26:

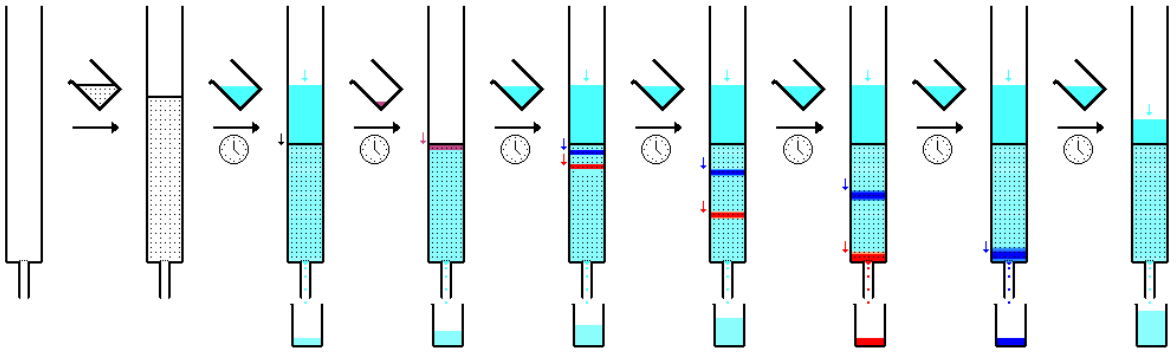


Figure 26-Separation in a chromatographic column system

The retention time of a particular compound depends on many factors, the chosen mobile and stationary phase, the length of the column, the temperature of operation and the sample mixture nature. Therefore a chromatographic analysis needs different standards in order to link each retention time with a given compound.

All the information collected from the detector is displayed in a chromatogram. The chromatogram is in fact, a plot of Area versus Time, where it is possible to find different peaks at different retention times, each one of them corresponding to a molecule. It isn't the peak height that matters, but the total area under the peak because one compound can emerge from the column in small amounts during a long time [50].

Due to the patterns and internal standards, the areas can be translated into concentrations following Equation 2:

$$C_i = \left(\frac{A_i}{A_{in-std}}\right) \cdot \left(\frac{V_{in-std} \cdot C_{in-std}}{V_i}\right) \cdot \left(\frac{1}{\frac{A_{i-cal}}{A_{in-std-cal}}}\right) \quad (2)$$

$$R_f = \frac{1}{\frac{A_{i-cal}}{A_{in-std-cal}}} \quad (3)$$

Where:

$$A_i = Specific\ component\ area$$

$$A_{in-std} = Area\ of\ internal\ standard$$

$$V_{in-std} = Volume\ of\ internal\ standard$$

$$C_{in-std} = Concentration\ of\ the\ internal\ standard$$

$$V_i = Volume\ of\ the\ sample$$

$$A_{i-cal} = Specific\ component\ area\ from\ the\ calibration\ sample$$

$$A_{in-std-cal} = Area\ of\ internal\ standard\ from\ calibration\ sample$$

$$R_f = Response\ factor$$

Chromatographic techniques are the most commonly used analysis methods in the analysis of sugars. Different types of methods are used to obtain different information about the sugar mixture [51].

In the current study, High Pressure Liquid Chromatography with Size Exclusion Detector (HPLC-sec) was used to obtain information about the molar masses of the hemicelluloses and Gas Chromatography (GC) was used to study the amount and the chemical composition of the samples [52].

Three different analysis were used:

- **Total Sugar Content Analysis**: This analysis was used to measure the total concentration of sugars present in the sample. It enables the quantification of each specific sugar by cleaving the hemicelluloses chains into its monomeric units [51] [53].

- **Monomer Analysis**: The monomer analysis was used to quantify the amount of each monosugar present in the sample without cleaving the hemicelluloses chains [51] [53].

- **Oligomer Analysis**: The oligomer analysis gives information on the monomer-pentamer concentration in the solution [51] [53].

In the Total Sugar Content analysis the hemicelluloses chains must be cleaved to monomers. There are several ways of doing this, but acid methanolysis has proven to be the most efficient method [53].

Acid methanolysis has two main advantages over the acid hydrolysis. The first one is that acid methanolysis is not as harsh so it does not degrade the crystalline structures of cellulose, thus the glucose monomers present in the solution can be attributed to hemicelluloses only. The other reason is that acid methanolysis permits the analysis simultaneously of both neutral and acidic sugar, so the uronic acids are not left outside the scope of the hemicellulose analysis [52] [54] [55].

2.7.1 Liquid chromatography

Liquid chromatography (LC) refers to the type of chromatography that uses a liquid fluid as the mobile phase, and it is usually carried out in a column or a plane. Nowadays, the most common Liquid Chromatography technique is called HPLC or High Performance Liquid Chromatography. In HPLC the liquid is pumped at high pressure into a pressure resistant column packed with a stationary phase usually composed of spherically shaped particles, porous particles, gels or even a porous membrane [56].

As described before, chromatography techniques in general and HPLC in particular are mass transfer processes which involve weak interactions between the mobile phase and the stationary phase, these different interactions can be grouped as adsorption/desorption relations. The strength or weakness of these relations determine the speed of every molecule inside the column.

One of the most important applications of the HPLC technique is analyzing the average molar mass of a sample. Molar mass is a crucial factor in the study of polymers and proteins. This type of chromatography is referred to as HPLC-Sec or Gel Permeation Chromatography, where SEC stands for Size Exclusion Chromatography, and it utilizes packed microporous particles or gels as the stationary phase [56].

The retention time in this method depends on the size of the particles. Smaller molecules get trapped into the pores of the particles while big molecules cannot go inside these pores and take a straighter and shorter path. The fractionations and the average molar mass can be quantified. The mechanism of a SEC column is represented in Figure 27:

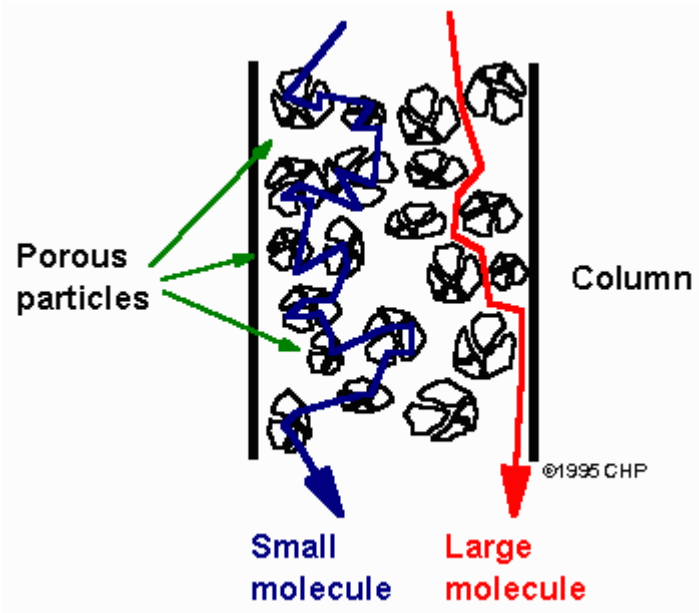


Figure 27-Separation process in a SEC column

The procedure is the same when the stationary phase is a porous gel instead of a bed of microporous particles, smaller particles go inside the pores of the gel and they are slowed down. This is a very

versatile technique because different range of sizes for the gel and the microporous particles can be chosen in order to fractionate the sizes as wished [56].

In order to identify the molecular size of the compounds and match it with the corresponding retention time, different optical properties of the substances are used. The most common detectors are: UV detectors, Refractive index detectors and MALS (Multi Angle Laser Scattering).

2.7.2 Gas chromatography

In the Chromatography (GC), also referred to as Gas Liquid Chromatography (GLC), an inert gas is used as mobile phase. In this type of chromatography, the mobile phase is used only has a transportation fluid and it does not interact with the sample [57].

This technique is very common to analyze organic compounds which have to be volatilized in order to be analyzed in the column. Often, these compounds are not volatile enough and they need a pretreatment that also improves the separation because the silyl derivatives are less polar and more thermally stables than their corresponding organic molecules. [58]

This pretreatment is called derivatization and there exist three different methods: silylation, methylation and acetylation [59]. The most commonly used method in the study of saccharides is the silylation and it consists of replacing of the polar groups of a molecule (OH) with a silyl group (R_3Si). The derivatization process begins with the deprotonation of the molecule using a strong base, and then silylation agents HDMS and TMCS are added. The structure of the silylation reagents is presented in Figure 28:

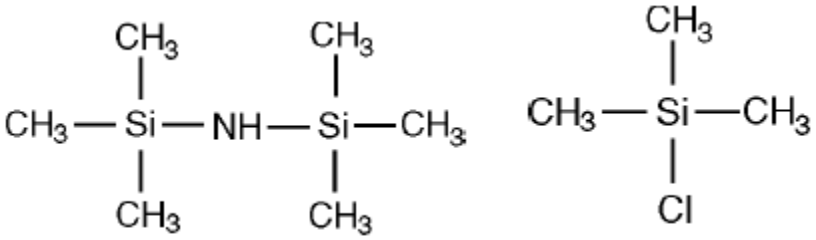


Figure 28-Silylation reactants, (left) HDMS, (right) TMCS

There are two main kinds of columns for gas chromatography:

- **Capillary columns**: Very long and thin tubes with high resolution. The stationary phase is a thin layer of liquid coated in the walls of the tube.

- **Packed columns**: The stationary phase is adsorbed over small particles of siloxane polymer gums.

The procedure is simple: the sample is vaporized and diluted in the inert gas which is pumped through the column. The retention times will depend on the solubility of each compound in the liquid coating the walls of the tube or the support particles [60].

3. EXPERIMENTAL SECTION

3.1. Equipment

The hydrolysis experiments were performed in an isothermal batch reactor made from glass. In order regulate the temperature, the reactor was equipped with a double jacket with circulating silicone oil. A schematic representation of the reactor is presented in Figure 29:

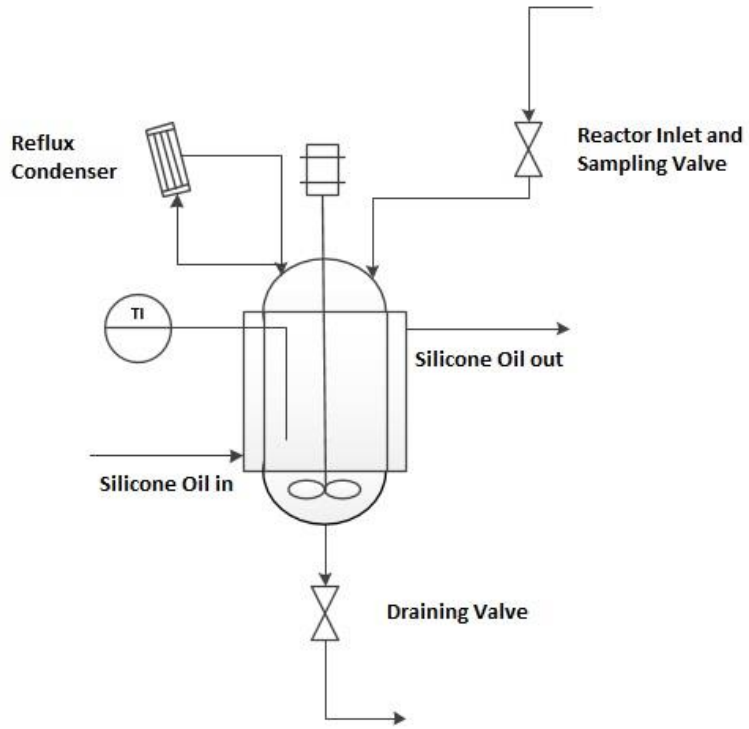


Figure 29-Reactor Diagram

The temperature of the silicone oil bath was controlled manually based on the temperature inside the reactor. The temperature was measured online with an electrode (thermocouple) in direct contact with the bulk which was connected to a computer. The temperature was recorded using the software Picolog© with 20 second intervals. The temperature inside the reactor stayed within ±1ºC during the experiments.

The experiments were performed under air at atmospheric pressure. In order to avoid the loss of liquid by evaporation, a reflux condenser with circulating cooling water was connected to the reactor. A water lock was installed to avoid external contamination of the liquid. Pictures of the reactor and the silicone oil bath are presented in Figure 30:

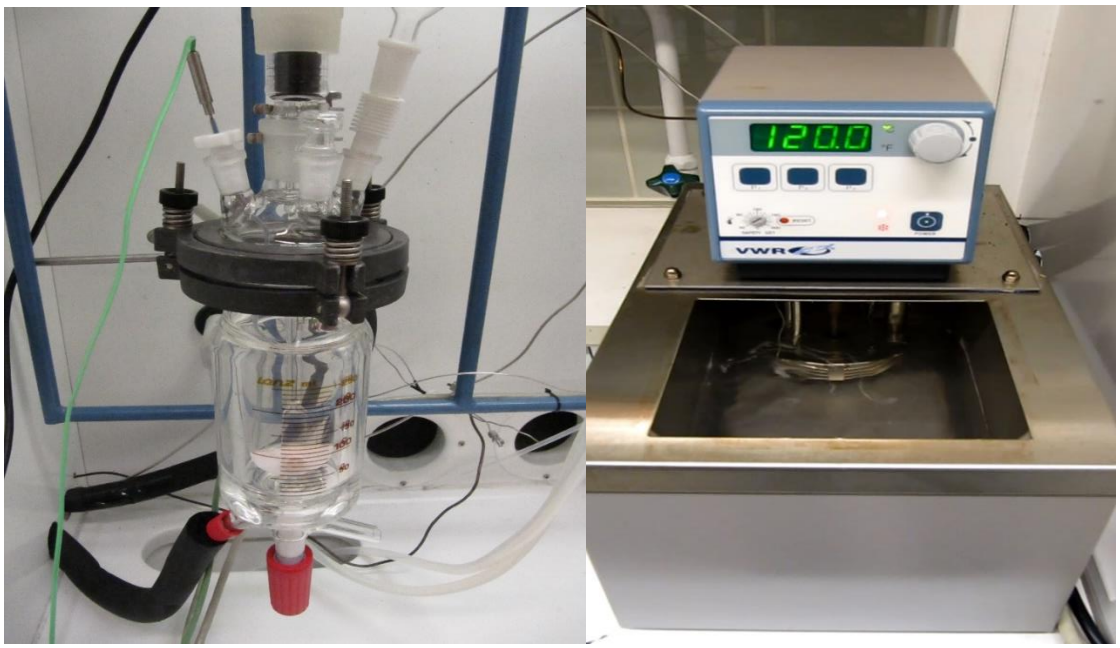


Figure 30-Reactor set up and Silicone oil bath

Moreover, the reactor was equipped with a polypropylene blade impeller rotating at 200 rpm in order to avoiding external mass transfer limitations and guarantee the homogeneity of the bulk. It has been shown in previous studies with similar reagents that the agitation was sufficient to overcome mass transfer limitations also considering the rather slow reaction rates involved.

The samples were extracted from the top of the reactor with the help of a 10 mL plastic syringe attached to a silicone tube. The diameter of the silicone tube allowed the simultaneous extraction of the solid catalyst with the liquid in order to maintain the solid liquid ratio constant.

The samples were then vacuum filtered with a paper filter with large pores to allow also the largest molecules to pass the filter and stored in 25 mL LEDP bottles in a freezer (-18ºC).

The laboratory equipment and materials used in the experiments are described below:

Storage and defreezing of the extracts:

1- ELECTROLUX® freezer at -18ºC.

2- 50 mL plastic (LDPE) recipients LAMAPLAST ®.

3- ASEA SKANDIA CYLINDA® fridge at 10ºC.

4- Plastic buckets.

Preparation of the reaction mixture:

1- 100 mL volumetric flask.

2- 100 mL measuring cylinder.

3- 5 mL plastic pipettes.

4- 1, 10 and 25 mL glass pipettes.

5- HUHTAMAKI® Plastic spoons.

6- Electronic scale METTLER TOLEDO ® AB204-S/PH MODEL.

7- Glass funnel.

8- VWR Phenomenal pH-meter.

Sampling, filtering and storage:

1- Erlenmeyer flask.

2- 10 mL polypropylene syringes.

3- 20 cm long silicone tubes.

4- Buchner funnel.

5- WHATMAN® ashless filter paper 589/1 diameter 90 mm with a particle retention size between 12-25 µm.

6- 10 mL plastic recipients LAMAPLAST ® LDPE.

7- 50 mL plastic recipients LAMAPLAST ® LDPE.

8- MEMMERT® Modell 400 oven with a constant temperature of 100 ºC

9- ELECTROLUX® freezer at -18ºC.

Pictures of the main instruments and materials used in the experiments are presented in Figure 31:

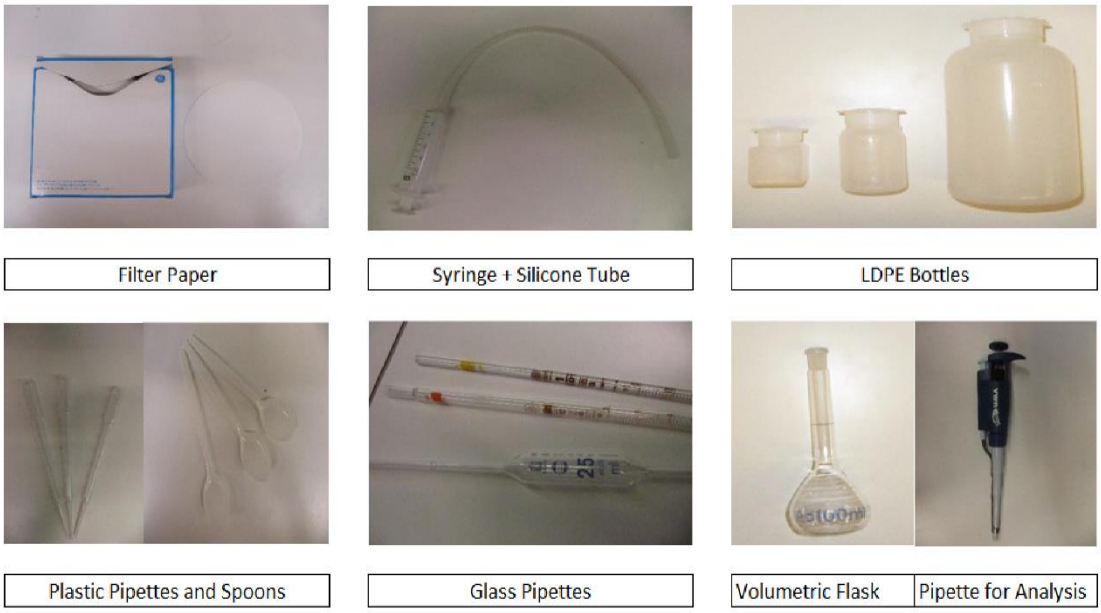


Figure 31-Main tools used in the sampling and storage

3.2. Materials

3.2.1 Raw materials

The extracts used in the hydrolysis experiments originated from a previous work in the extraction of hemicelluloses in a batch reactor. The main idea was to utilize the carefully chosen extract which were obtained in precisely controlled conditions from selected raw materials. Moreover, the extracts were well characterized in advance. Some studies have been made on the hydrolysis of hemicelluloses with heterogeneous catalysts. However, in the majority of cases model compounds or purified extracts were used. The current work focuses on the hydrolysis of actual extracts of rather high concentration.

The hemicelluloses were extracted with hot water from 1,25-2 mm diameter wood chips of the following wood species: Stone Pine, Quercux Ilex and Picea Abies in a batch reactor. Pictures of the wood chips and the extracts can be observed in Figure 32:

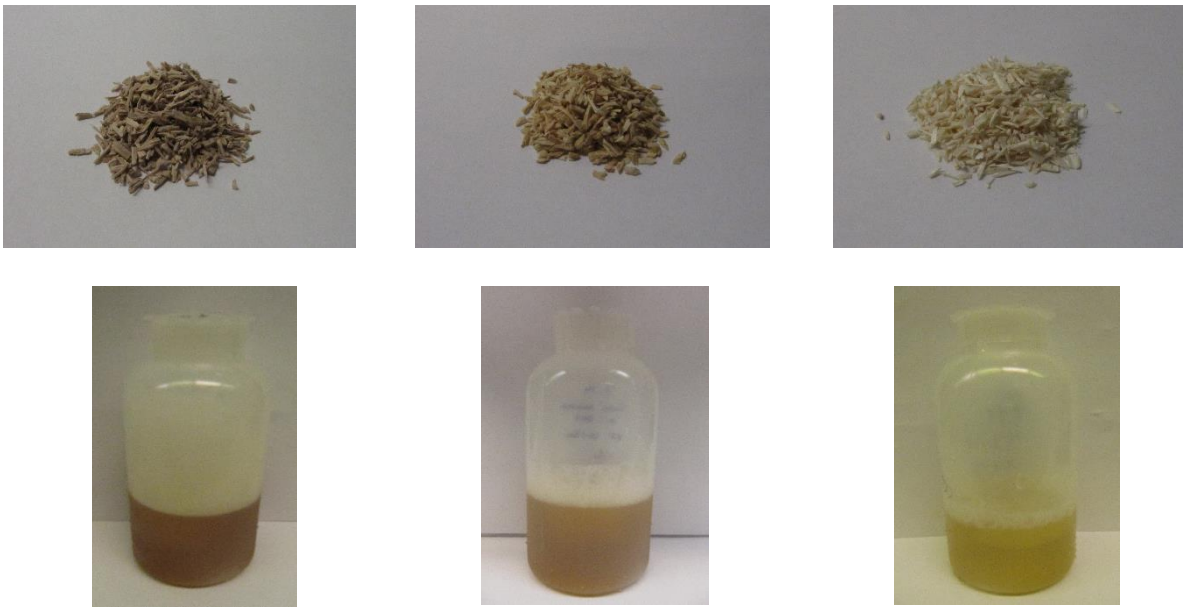


Figure 32-Wood chips (1,25-2mm) and extract for hydrolysis. From left to right: Quercux Ilex, Pinus Stone Pinea, Picea Abies

The extracts were extracted at constants temperatures ranging from 130ºC to 170ºC using different extraction times between 60 minutes and 220 minutes. The substrates were vacuum filtered with a Buchner funnel in order to remove part of the solids.

A few milliliters of each sample were evaporated in order to measure the solid content of the extracts. Methanolysis and molar mass analysis were performed on the samples in order to determine the specific sugar content and the size of the molecules. The substrates used in the hydrolysis experiments are presented in Table 7:

Table 7-Properties of the selected extracts used in the current work

Extract Nº	Wood	Extraction Temperature (ºC)	Extraction Time (min)	HC conc. (g/L)	Solid conc. (g/L)	Mw (g/mol)
1	Holm Oak	130	220	4,06	6,75	10650
2	Stone Pine	130	220	5,7	5,76	5702
3	Norway Spruce	130	220	2,8	4,81	5900
4	Holm Oak	130	140	2,02	6,23	13750
5	Holm Oak	140	160	8,43	10,89	5370
6	Stone Pine	150	120	9,22	10,46	3649
7	Stone Pine	160	80	6,36	11,9	2254

3.2.2 Catalysts

The selection of the catalyst in this research has been based on the experience of previous work and on the specific characteristics of the catalysts.

HCl was chosen as the reference homogeneous catalysts to compare with the results obtained with the heterogeneous one. A 4 M solution of HCl in water was prepared. The reaction was stopped by neutralizing with NaOH 0,1 N to a pH of approximately 4. No precipitation of salts was observed during the neutralization.

Smopex-101® was chosen as the solid catalyst in the current work for the hydrolysis of model compounds and extracts of the hemicelluloses from Holm Oak and Stone Pine. This fibrous catalyst has shown high efficiency in hydrolysis in previous works. The functional group of Smopex-101 and its microscopic structure are presented in Figure 33:

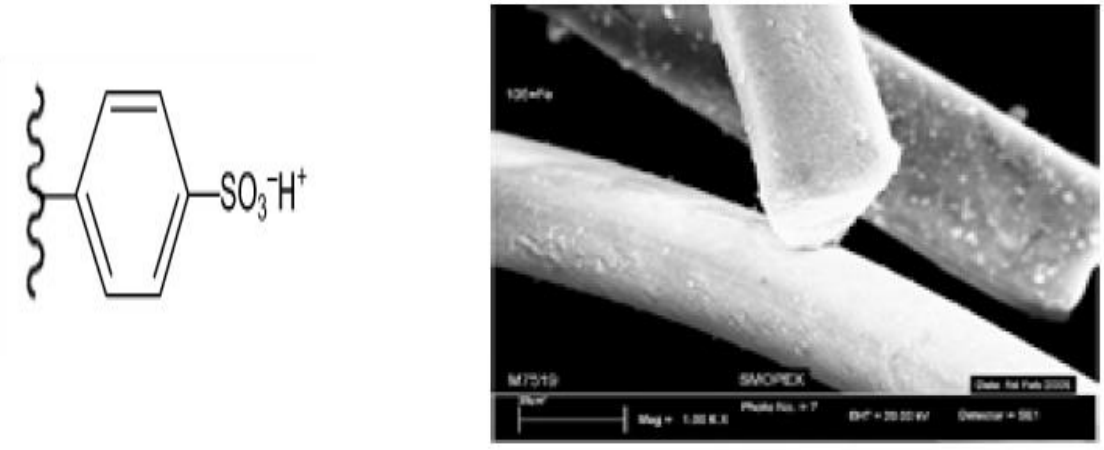


Figure 33-Sulfonic functional group of Smopex-101 (left) and SEM picture of the fibers of Smopex-101 (right)

Mass transfer limitations are one of the biggest issues in the use of solid catalysts. The molecule must be transferred from the bulk to the surface of the catalyst and then the molecule needs to absorb on the active site where the catalysis takes place. This is especially important in the reactions involving polymers and other kind of large molecules like hemicelluloses because they cannot penetrate small pores.

Fibrous catalyst minimize these problems by having a non-porous structure and a high surface area.

Smopex is a wide family of fibrous catalyst which have a backbone structure made of polyethene-graft-polystyrene with different functional groups, each of them suitable for selected applications.

Smopex-101 was chosen for the current work because it has sulfonic groups (SO_3H) which act as strong acid groups. It presents high capacity which means that it contains a high concentration of the sulfonic groups. This is important as low pH was aimed for in the experiments and low capacity catalysts would lead to unrealistically high solid-liquid ratios.

Also, as the experiments are performed under atmospheric pressure and in liquid phase, the thermal stability of Smopex was sufficient. The characteristic of Smopex-101 are listed in Table 8:

Table 8-The Properties of Smopex-101

Catalyst	**Smopex-101**
Type	Fibrous
Diameter (mm)	0,01
Average Pore Diameter (Å)	-
Length (mm)	4
Maximum Operation Temperature (ºC)	120
Backbone Structure	Polyethene-graft-polystyrene
Acid Functional Group	SO_3H
Humidity Content (%)	48
Surface Area (m^2/g)	-
Capacity (mmol/g)	3,2

3.3. Procedure

The extracts were chosen based on high molar mass and available quantity in order to be able to perform two experiments with the same extract. 100 mL of extract was used in each experiment.

The extracts were taken from the freezer (-18ºC) and placed in a fridge (6ºC) overnight in order to melt them. The extracts were shaken vigorously in order to guarantee the homogeneity of the mixture and to make sure that all the sugars were dissolved in case that some of them precipitated because of the consecutive freezing-melting cycles. 100 mL of the extract were measured into a volumetric flask and introduced into the reactor.

In the case that a solid catalyst was used, the exact amount was weighted and placed into the reactor. The humidity content of the catalyst (48%) and the capacity of the catalyst (3,2 mmol/g) as well as the pH of the extract were considered when calculating the amount of catalyst needed to reach the desired pH.

Once the catalyst and the extract were in the reactor the stirring was set to 200 rpm and the heating was turned on. The heating time was typically 30 min. Previous experiments have shown that the hydrolysis is very temperature sensitive and rather slow. Thus, the heating time can be considered reasonable compared to the kinetics.

When the desired temperature was reached the timer was switched on and a 0 sample was taken. The experiments lasted 24 hours and 12 samples of between 6 and 8 mL were taken during each experiment

The sampling times were chosen according to previous studies so that the sampling intervals were smaller in the early stages of the experiment in order not to miss the kinetics.

The last sample was taken at 24 hours in order to study the degradation of the sugars. A first sample was usually taken in the beginning in order to verify the reagent properties. The sampling times of the experiments are listed in Table 9:

Table 9-Sampling times during the experiments

Sample Nº	Time (h)
S 0	0
S 1	0,33
S 2	0,66
S 3	1
S 4	1,5
S 5	2
S 6	3
S 7	4
S 8	6
S 9	8
S 10	10
S 11	24

The sampling was performed by stopping the stirring momentarily and introducing a silicone tube attached to a 10 mL syringe in the reaction mixture. Between 6 and 8 mL of sample were withdrawn containing both the solid and the liquid. The samples were vacuum filtered and stored in 25 mL PELD and placed in a freezer in order to stop the reaction completely.

After 24 hours, the last sample was taken and the silicone oil bath and the stirring were switched off and the remaining reaction mixture was removed from the reactor by opening the bottom valve.

The liquid was filtered under vacuum and stored in a polypropylene bottle. The solid catalyst was recovered from the filter paper and dried in an oven at 60ºC.

The procedure was slightly different when the experiments were performed with the homogeneous catalyst. The extract was introduced in the reactor in the same way, but the catalyst was not injected until the desired temperature was reached. At this moment, the temperature decreased slightly during a few minutes-

The sampling was performed as previously, the only difference was that the samples were rapidly quickly neutralized by adding a determined amount of NaOH. The neutralized samples were shaken vigorously until the mixture was homogeneous and stored in the freezer at -18ºC.

3.3.1 Temperature

Three different temperatures were chosen to study its influence on the kinetics. The maximum reaction temperature that could be used in the experiment was 95ºC in order to avoid the boiling of the reaction mixture, 95ºC was chosen in order to study the maximum obtainable reaction rate. Two additional temperatures were used: 85ºC and 75ºC to study the activation energy. In the experiments with HCl, a temperature of 95ºC was used.

3.3.2 pH

The pH values of the experiments were carefully chosen in order to study the dependence of the hydrolysis on the acidity. With solid catalysts it is not always possible to operate at the desired pH due to the possible mass transfer limitations and stirring problems if the liquid to solid ratio is too low. Based on previous findings pH 0,5, pH 1 and pH 1,5 were chosen for the experiments.

The initial pH of each extract, as well as the reaction pH and the amount of catalyst used in both dry and wet basis are shown in Table 10:

Table 10-Amount of solid catalyst needed

Experiment Nº	Wood	Extract Nº	Initial pH	Reaction pH	Wet catalyst (g)	Dry catalyst (g)
4	Holm Oak	4	4,61	1	6,5088	3,1242
5	Holm Oak	4	4,61	1,5	2,0572	0,9874
6	Holm Oak	1	4,33	0,5	20,5847	9,8807
7	Holm Oak	5	3,88	0,5	20,5792	9,8780
8	Holm Oak	5	3,88	0,5	20,5792	9,8780
9	Stone Pine	6	3,62	1	6,4948	3,1175
10	Stone Pine	6	3,62	1,5	2,0432	0,9807
11	Stone Pine	2	3,77	0,5	20,5767	9,8768
12	Stone Pine	7	3,62	0,5	20,5721	9,8746
13	Stone Pine	7	3,62	0,5	20,5721	9,8746

In the experiments performed with HCl it would have been possible to go under pH 0,5 but they would not have been comparable with the rest of the experiments which is why this pH was chosen.

A solution of HCl 4M was prepared and a predetermined amount was injected into the reactor when the reaction mixture had reached the desired temperature. Each sample was neutralized with NaOH 0,1 N to pH 4. At this pH, the reaction is stopped and the precipitation of sugars is still avoided.

The amounts of acid and base used in the hydrolysis experiments are shown in Table 11:

Table 11-Added HCl and NaOH

Experiment Nº	Wood	Extract Nº	pH Initial	pH Reaction	pH Neutralization	HCl 4 M (mL)	NaOH 0,1 N (mL)
1	HO	1	4,33	0,5	4,02	8,58	18,96
2	PP	2	3,77	0,5	4,30	8,58	18,95
3	PA	3	4,5	0,5	4,02	8,58	18,95

3.4. Experimental Matrix

The experimental matrix is presented in Table 12:

Table 12-Experimental matrix

Experiment Nº	Wood	Catalyst	pH	T (ºC)	Extract Nº	HC conc. (g/L)	Solid conc. (g/L)	Mw (g/mol)	Catalyst (g)
1	Holm Oak	HCl	0,5	95	1	4,06	6,75	10650	-
2	Stone Pine	HCl	0,5	95	2	5,7	5,76	5702	-
3	Picea Abies	HCl	0,5	95	3	2,8	4,81	5900	-
4	Holm Oak	Smopex	1	95	4	2,02	6,23	13750	6,5084
5	Holm Oak	Smopex	1,5	95	4	2,02	6,23	13750	2,0570
6	Holm Oak	Smopex	0,5	95	1	4,06	6,75	10650	20,5790
7	Holm Oak	Smopex	0,5	85	5	8,43	10,89	5370	20,4798
8	Holm Oak	Smopex	0,5	75	5	8,43	10,89	5370	20,5661
9	Stone Pine	Smopex	1	95	6	9,22	10,46	3649	6,4940
10	Stone Pine	Smopex	1,5	95	6	7,65	8,68	3649	2,0430
11	Stone Pine	Smopex	0,5	95	2	5,13	5,19	6603	20,5765
12	Stone Pine	Smopex	0,5	85	7	6,36	11,90	2254	20,5713
13	Stone Pine	Smopex	0,5	75	7	6,36	11,90	2254	20,5722

3.5. Analysis

3.5.1 Total Dissolved Solids Content

All of the analysis performed in this research are based on chromatographic methods. Reading a chromatogram is a complex issue and in order to get reliable results, the peaks must be well defined

and large enough. The area of the peaks depends mainly on the amount of sample used in the analysis and on the concentration of the sample. This is why it is crucial to be able to estimate the hemicellulose content in every sample.

In order to estimate the hemicellulose content, few milliliters of the extract (5-10 mL) were placed overnight in an oven at 100ºC, until the solution was fully evaporated. The weight of the dry vessel and of the vessel with the solution was compared and the solid content calculated.

The results of the TDS analysis are presented in Table 13:

Table 13-Total Dissolved Solids Content of the Extracts

Extract Nº	Wood	Vessel (g)	Vessel+Liquid (g)	Vessel+Solid (g)	Liquid (g)	Solid (g)	TDS (g/L)
1	HO	2,9504	12,2685	3,0121	9,3181	0,0617	6,7540
2	PP	3,4431	12,4175	3,4938	8,9744	0,0507	5,7624
3	PA	3,0060	7,7110	3,0351	4,7050	0,0291	6,2900
4	HO	1,6193	11,0785	1,6773	9,4592	0,0580	6,2596
5	HO	2,7931	12,1450	2,8929	9,3519	0,0998	10,8851
6	PP	3,1800	12,5290	3,2759	9,3490	0,0959	10,4629
7	PP	3,5797	13,0820	3,7016	9,5023	0,1219	13,0850

It should be noted that the solid content is not the same as the hemicelluloses content, but it is still a good estimation and enough to obtain a good signal in the chromatograms.

3.5.2 Average Molar Mass

The average molar mass method is a qualitative analysis technique that allows to know the average molar mass of the molecules present in a determined sample. It is a very important parameter, especially in the study of the hemicelluloses, as it gives an idea of the degree of polymerization of the polysaccharides.

The average molar mass was determined for each extract. All the samples were prepared by diluting a small amount of sample with distilled water. 2 mL of this dilution was placed in a vial with a final concentration ranging between 1,5-3 g/L. The samples were also filtered with the help of a 5 mL syringe through a 0,2 µm nylon filter in order to remove some solids that could cause the clogging of the columns

This analysis rely on the HPLC-SEC (High Performance Liquid Chromatography-Size Exclusion Chromatograph) technology in combination with the multiangle laser light scattering (MALLS) equipment with a refractive index (RI) detector. The precise system consists of two Ultrahydrogel TM linear 7,8x300 mm columns in series. The eluent used was a solution of $NaNO_3$ 0,1 M with a flow rate of 0,5 mL/min.

There are different parameters that can be used for measuring the average molar mass, they are defined as:

$$M_w = \sum \frac{M_i^2 \cdot N_i}{M_i . N_i} = Mass\ Average\ Molar\ Mass \quad (4)$$

$$M_n = \sum \frac{M_i \cdot N_i}{N_i} = Number\ Average\ Molar\ Mass \quad (5)$$

$$Pd = \frac{M_w}{M_n} = Polidispersity \quad (6)$$

Being:

$$M_i = Molar\ mass\ of\ a\ determined\ molecule$$

$$N_i = Number\ of\ molecules\ with\ a\ M_i\ mass$$

A lot of information can be extracted from these parameters but it is not as understandable as the DP. An estimation of the DP can be calculated by considering that all of the monomer units have a molar mass similar to glucose (180 g/mol).

$$DP_{stimated} = \frac{M_w}{180 \frac{g}{mol}}$$

The results from the average molar mass analysis are presented in Table 14:

Table 14-The Average Molar Mass of the extracts

Extract Nº	Wood	Mw (g/mol)	Mn (g/mol)	Mw/Mn	~DP
1	Holm Oak	10650	5208	2,045	59
2	Stone Pine	5702	2806	2,032	32
3	Norway Spruce	5900	2692	0,198	33
4	Holm Oak	13750	4398	3,127	76
5	Holm Oak	5370	2562	2,096	30
6	Stone Pine	3649	2097	1,74	20
7	Stone Pine	2254	1371	1,644	13

3.5.3 Monomer Content

The monomer analysis is a quantitative method that gives the amount of sugars that are present in the sample in their monomeric forms. Given that the hemicelluloses are heteropolysaccharides they are formed from different monomeric sugars. The results of this analysis are the concentration of the different sugars in the sample.

This analysis is performed using gas chromatography and as described in a previous section, a pretreatment of the samples known as silylation is needed. Three calibration samples were prepared by adding a solution containing a known amount of different monomers as well as sucrose which gives semi quantitative information about the amount of dimers present in the sample. Xylitol is added in every sample as an internal standard. At the end, the silylation reagents are added transforming the organic compounds into more volatile substances. This improves the reliability of the results by reducing the overlapping of the chromatographic peaks.

The monosaccharides were analyzed by GC-FID on a 25 m x 0,2 mm internal diameter column coated with cross-linked methyl polysiloxane after silylation of the samples. The column parameters were 100ºC raised at 2ºC/min to 170ºC and 12ºC/min to 290ºC and carrier gas H2 with a 40 mL/min flow rate.

The goal of the monomer analysis was to study the change of the concentration as a function of time. The detailed procedure can be found in the Appendix I.

3.5.4 Oligomer Content

The oligomer content analysis is a quantitative method that calculates the amount of short oligomers (from monomers to pentamers) that are present in a determined sample. The information given by this analysis is the ratios of oligomers with a DP under 6, not concentrations, so it has to be coupled with the monomer analysis to obtain quantitative results.

This analysis is also performed using gas chromatography and the pretreatment of the samples is equivalent to the one in the monomer content analysis. The calibration samples are prepared with xylitol and sucrose, in order to have semi quantitative information for both monomer and dimer content. The internal standard depends on the species of wood. For the softwoods such as Stone Pine and Norway Spruce, the internal standard is betulinol but for the hardwoods like Holm Oak, cholesterol must be used. The reason for this is that betulinol can be found in nature in some hardwoods and it could interfere the analysis.

The oligosaccharides were determined by GC on a 7.5 m x 054 mm of internal diameter column coated with cross-linked methyl polysiloxane after the silylation of the samples. The GC was a Perkin-Elmer Autosystem XL instrument and the temperature profile was 100ºC, ramping rate 12ºCmin to 340ºC, the carrier gas was H2 with a flowrate of 7mL/min.

With previous information on the total monomer content and with the analyzed ratios of oligomers with DP<6, the concentration of all of these oligomers were calculated. This information is also very important to study the hydrolysis kinetics and to understand how the hemicelluloses are being cleaved during the reaction. The detailed procedure can be found in the Appendix I.

3.5.5 Total Sugar Content

The total sugar content is a crucial parameter in order to analyze the conversion and the hemicellulose structure present in the reaction mixture. With the help of the monomer and the oligomer analysis is it possible to determine the concentration of monomers, dimers, trimers, tetramers and pentamers in the sample.

In order to study the total content it is necessary to cleave entirely the hemicelluloses and bring all the sugars to their monomeric form, this process is called Acid Methanolysis. The methanolysis analysis is also performed by gas chromatography, just as described in the previous sections, but it requires a different pretreatment. In order to break the hemicelluloses chains 2 mL of HCl in methanol (2 M) is added to every sample and they are introduced in an oven for several hours. The rest of the process is equivalent to the method of the monomer and oligomer analysis, in this case the internal standards are sorbitol and resorcinol. This methanolysis can be performed for both liquid and solid phases.

This analysis has been used for studying the degradation and the evolution of the total sugar content during the whole reaction time. Three samples have been taken in every experiment, the first one before starting the reaction in order to know the raw material and the initial extract, the second one when the monomer yields reach a maximum and where the degradation should not be very high and the third one at the end when the degradation can be important.

The silylated sugars were analyzed by GC with a flame ionization detector (GC-FID). The GC oven parameters were 100ºC raised at 4ºC/min to 175ºC, and at 12ºC/min to 290ºC. The detailed procedure for methanolysis and its whole description is included in Appendix I

3.6. Experimental results

3.6.1 The Influence of the catalyst

The first experiments were performed with both catalysts under the same conditions, in order to compare their performance with both holm oak and Stone Pine extracts.

Holm Oak Hydrolysis (T=95ºC, pH=0,5)

The first experiments were performed using a Holm Oak extract containing 4,06 g/L of hemicelluloses. The same extract was used in the experiment with HCl and with Smopex-101.

The first analysis performed on the samples was the monomer analysis, in order to determine the extraction of the monomers and their degradation during the experiments. The results of the monomer analysis of the experiment with HCl can be seen in Figure 34.

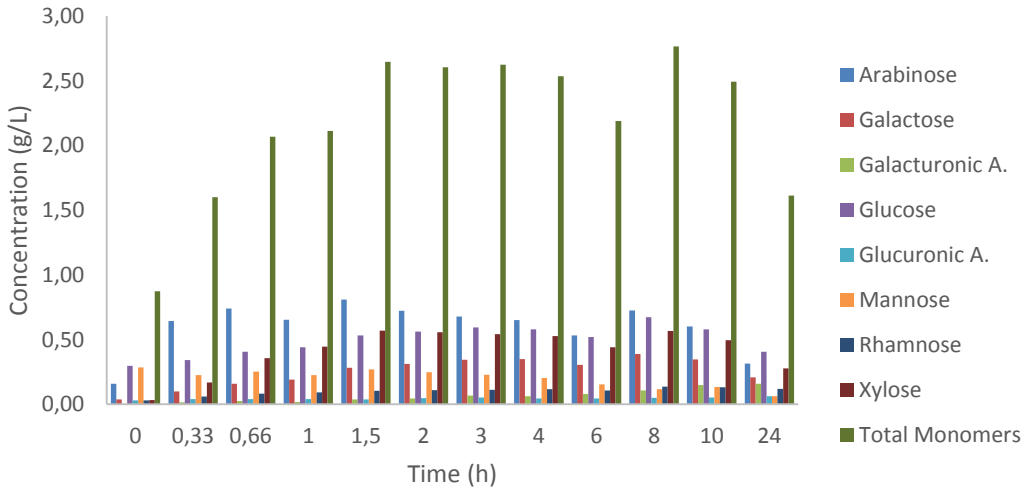


Figure 34-The monomer concentration as a function of time for Holm Oak extract hydrolysis catalyzed by HCl (T=95ºC, pH=0,5)

As shown in Figure 34 the specific sugar concentration, and as a result the total concentration of monomers, increased rapidly during the first hours of reaction and it remained stable during a few hours. The acid catalyst does not cause severe degradation of the monomers during the first 10 hours of reaction. Significant degradation can be observed experimentally, after 24 hours. The highest yield obtained towards monomers was about 65%.

The parallel experiment was performed in identical conditions but using Smopex-101 instead of HCl. The results can be seen in Figure 35.

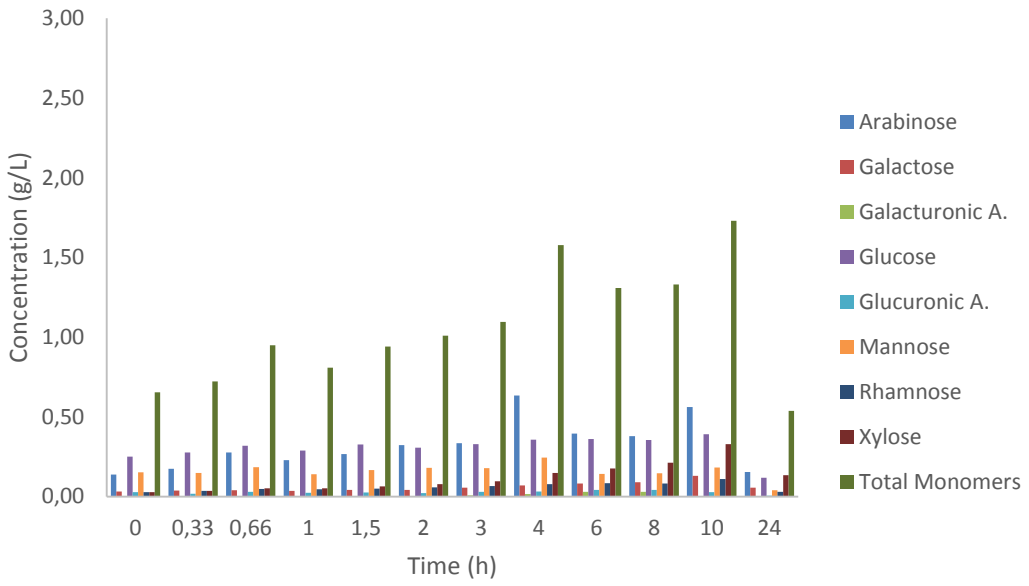


Figure 35-The monomer concentration as a function of time for Holm Oak extract hydrolysis catalyzed by Smopex-101 (T=95ºC, pH=0,5)

The monomer concentration is somewhat lower, compared to the experiment catalyzed by HCl. The monomer concentration increased during the first 10 hours with a rather constant rate but after 24 hours severe degradation was observed. The highest yield obtained in the experiment was about 95% towards monomers. It is possible that even higher values were obtained between the experimental point at 10 h and 24 h.

Already from this first analysis, a difference in the catalysts performance was evident. In order to get a clearer picture of the reaction mechanisms an oligomer analysis was performed on the samples.

The results of the oligomer analysis for the HCl catalyzed experiments can be seen in Figure 36 and Figure 37:

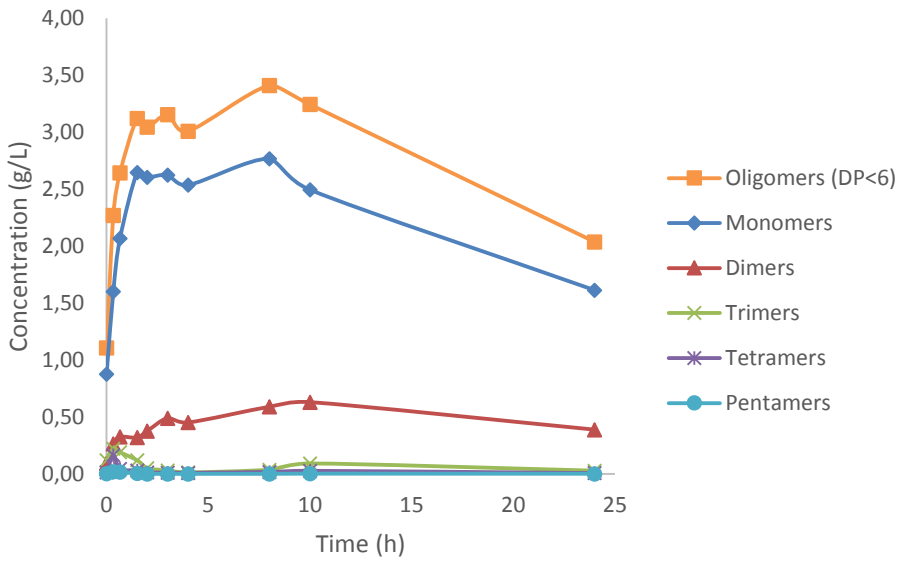


Figure 36-The oligomer (DP<6) concentration as a function of time for Holm Oak extract hydrolysis catalyzed by HCl (T=95ºC, pH=0,5)

A rapid hydrolysis of the polymers can be observed and a maximum for the oligomers (DP<6) concentration was reached in the first few hours of reaction. Significant degradation was observed first after 10 hours of reaction.

Should be noticed that the monomer concentration followed the same trend as the total oligomers (DP<6) concentration and that it was clearly the dominant species present in the solution. Some dimers were produced in the first stages of the reaction but the concentration profile follows the same trend as for monomers.

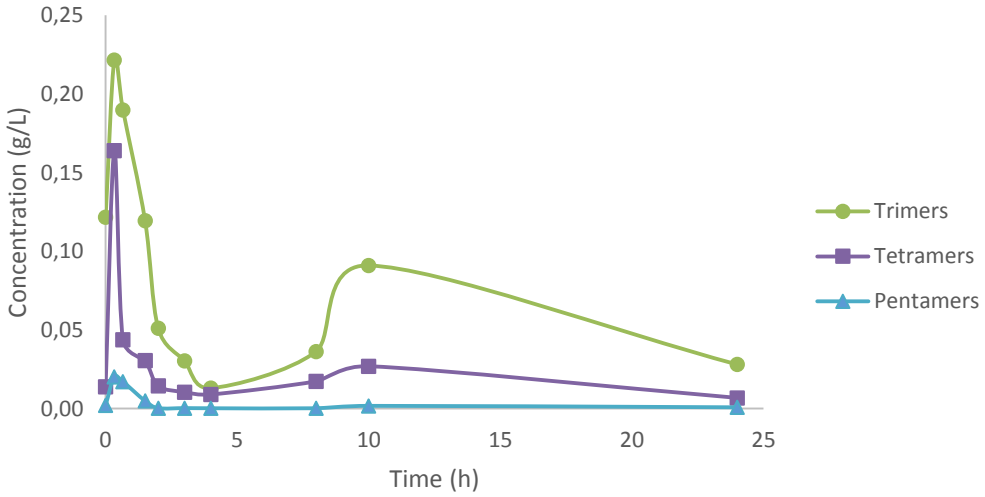


Figure 37-The tri, tetra and pentamers concentration as a function of time for Holm Oak extract hydrolysis catalyzed by HCl (T=95ºC, pH=0,5)

The trimers, tetramers and pentamers were present in the solution in the beginning of the experiment but they were quickly hydrolyzed to dimers and monomers. At the end of the experiment there were just traces of them.

The same analysis were performed for the parallel experiment with Smopex-101, the results are presented in Figure 38 and Figure 39:

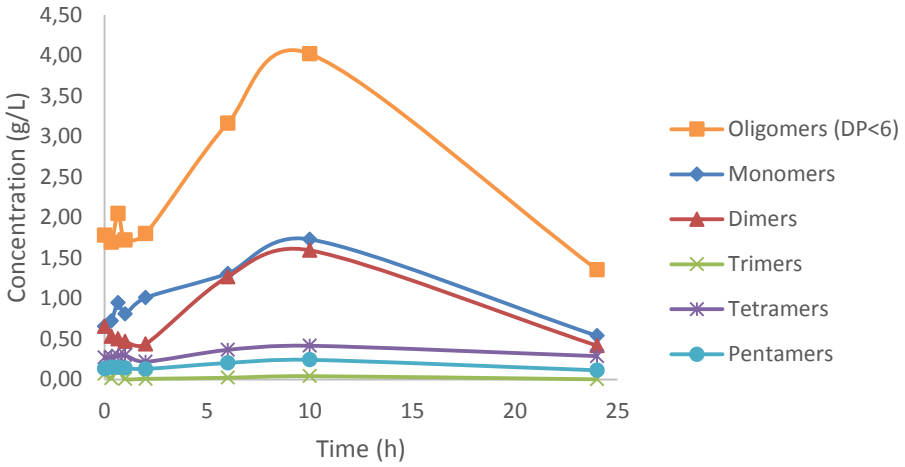


Figure 38-The oligomer (DP<6) concentration as a function of time for Holm Oak extract hydrolysis catalyzed by Smopex-101 (T=95ºC, pH=0,5)

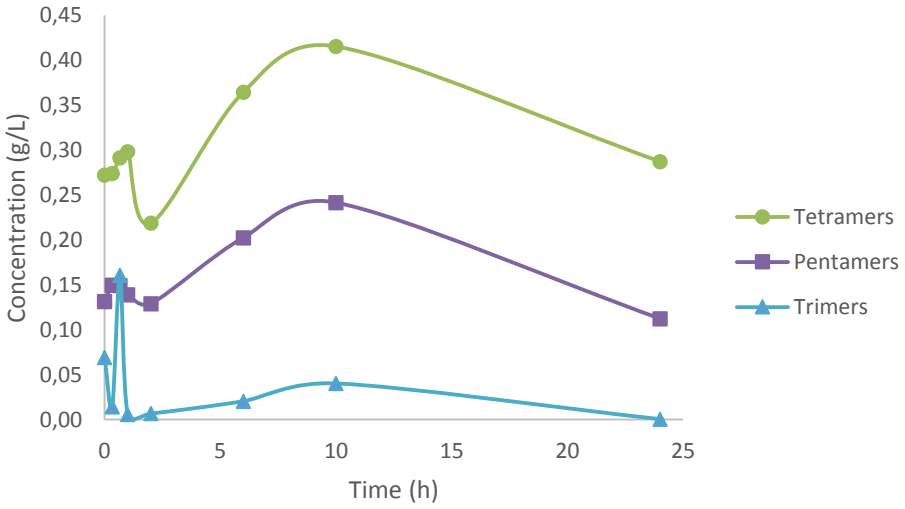


Figure 39-The tri, tetra and pentamers concentration as a function of time for Holm Oak extract hydrolysis catalyzed by Smopex-101 (T=95ºC, pH=0,5)

It can be observed that the reaction was not as fast as with HCl and that a maximum in the oligomer concentration was reached after 10 hours of reaction. It should be noted that the yield towards

monomers was considerably lower compared to the experiments performed with HCl because a large amount of dimers were produced and some part of the larger oligomers (DP<6) were not hydrolyzed.

Significant degradation was observed after 10 hours of experiment and a significant amount of the monomers and dimers were degraded.

When all the concentration are known, the results can be viewed in terms of yield. The yield will is defined as:

$$Yield\ (100\%) = \frac{Specific\ Sugar\ Concentration}{Total\ Hemicellulose\ Concentration} \cdot 100$$

The yields towards monomers, dimers and short oligomers (DP<6) for the parallel experiments were calculated based on the hemicellulose concentration in the extract. The yields of the experiment with HCl are displayed in Figure 40:

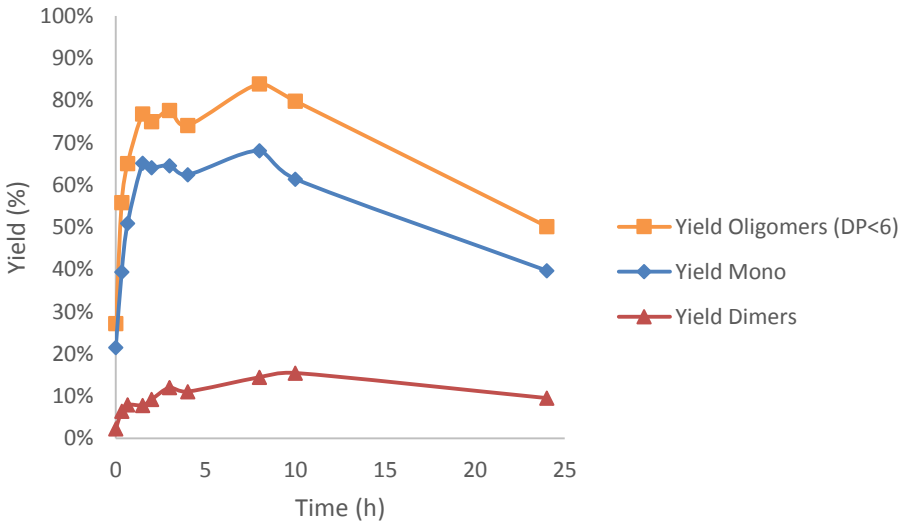


Figure 40-The yield of Holm Oak hemicelluloses to oligomers (DP<6), monomers and dimers as a function of time in a hydrolysis catalyzed by HCl (T=95ºC, pH=0,5)

As displayed in Figure 40, the yields towards short oligomers (DP<6) reached a maximum of nearly 90% after about 10 hours of reaction. The monomers were clearly the largest fraction in the hydrolysis products, the yield towards monomers was about 65%. The amount of dimers increased at the early stages of the reaction and remained rather constant until the end of the experiment, approximately in 10%.

The same calculations have been made in the case of Smopex-101 as catalyst. The yields of the experiment with Smopex-101 are displayed in Figure 41:

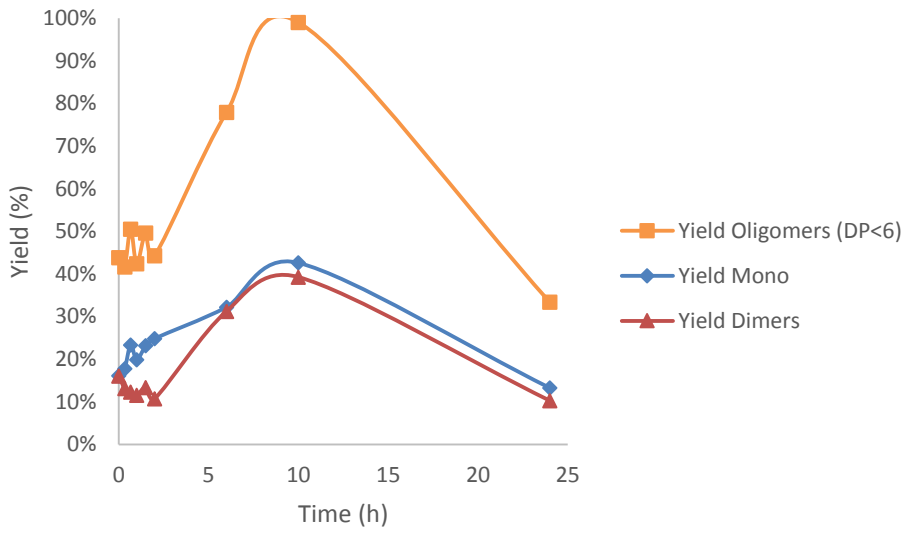


Figure 41-The yield of Holm Oak hemicelluloses to oligomers (DP<6), monomers and dimers as a function of time in a hydrolysis catalyzed by Smopex-101 (T=95ºC, pH=0,5)

In the parallel experiment performed with Smopex-101, the yield towards oligomers (DP<6) reached a maximum of almost 100% which indicates that the degradation is not as rapid compared to the hydrolysis than with HCl. The yield towards monomers and dimers is very similar, which differs significantly from the results obtained with HCl. More monomers were produced in the beginning of the experiment but the amount of dimers increased during the experiment reaching similar values as the monomers. Severe degradation was observed after 24 hours.

The yields of both parallel experiments are compared in Figure 42 and Figure 43:

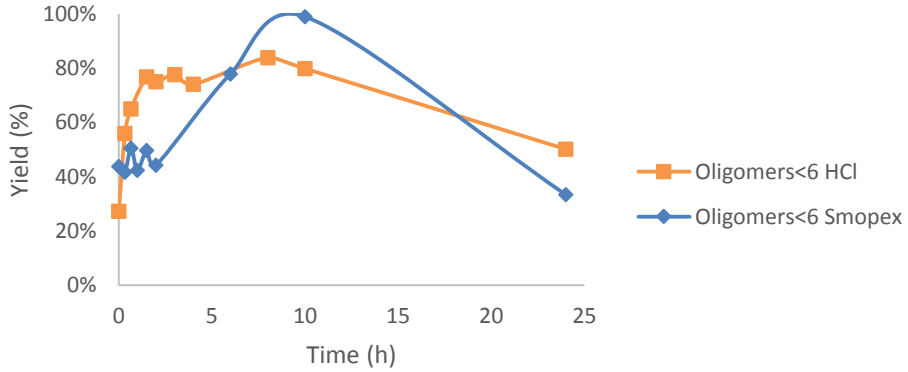


Figure 42-The yield of Holm Oak hemicelluloses to oligomers (DP<6) as a function of time in a hydrolysis catalyzed by HCl and Smopex-101 (T=95ºC, pH=0,5)

As can be seen in Figure 42, the hydrolysis was much faster with HCl and a clear maximum was reached after a few hours of reaction. The experiment with Smopex-101 displayed slower kinetics

and higher overall conversion was reached. The degradation was very similar after 24 hours in both experiments.

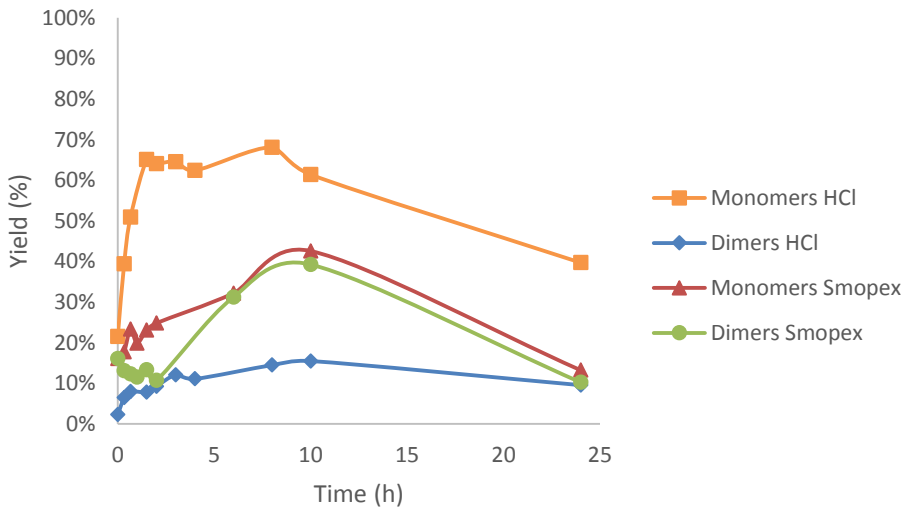


Figure 43-The yield of Holm Oak hemicelluloses to monomers and dimers as a function of time in a hydrolysis catalyzed by HCl and Smopex-101 (T=95ºC, pH=0,5)

The difference in the experiments performed with HCl and Smopex-101 can clearly be seen in Figure 43. With HCl a high amount of monomers were produced but the dimer concentration remained low and constant. Then again with Smopex-101, the amount of monomers and dimers were very similar.

Stone Pine Extract Hydrolysis (T=95ºC, pH=0,5)

Similar experiments were performed using a Stone Pine extract with a hemicellulose concentration of 5,70 g/L. The same extract was used for the parallel experiments with HCl and Smopex-101.

The monomer analysis was performed in order to evaluate the yield to monomers production and the degradation during the experiments. The results of the monomer analysis from the experiment performed with HCl can be seen in Figure 44:

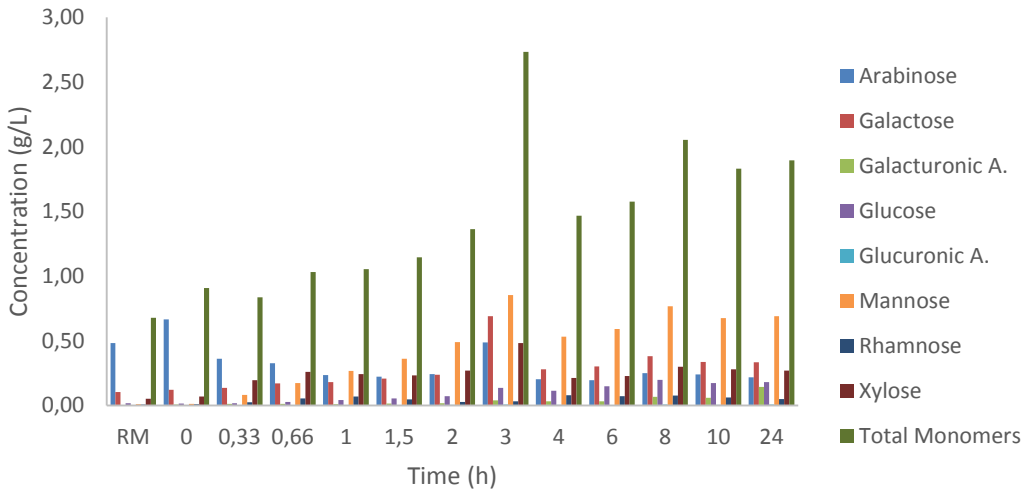


Figure 44-The monomer concentration as a function of time for Stone Pine extract hydrolysis catalyzed by HCl (T=95ºC, pH=0,5)

As can be seen in Figure 44 the specific sugar concentration, and as a result the total monomer content, increased at a constant rate until the end of the experiment. The kinetics of the reaction was as fast as in the experiments with holm oak, however no degradation was observed after 24 hours.

The parallel experiment was performed in the same experimental conditions but using Smopex-101 instead of HCl. The results of the monomer analysis can be seen in Figure 35:

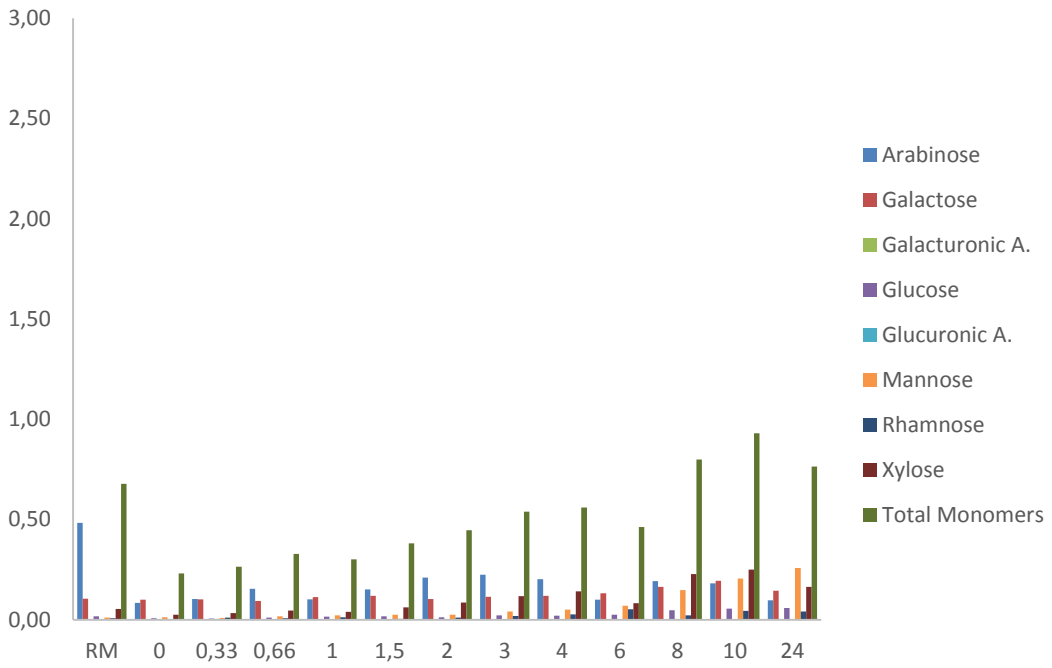


Figure 45-The monomer concentration as a function of time for Stone Pine extract hydrolysis catalyzed by Smopex-101 (T=95ºC, pH=0,5)

As can be observed in Figure 45, the concentration of monomers was significantly lower than in the experiment with HCl, but the trend is very similar. The concentration increased at a steady rate during the experiment and no significant degradation was observed.

Already from this first analysis, a difference in the catalysts performance was evident. In order to get a clearer picture of the reaction mechanisms an oligomer analysis was performed on the samples.

The results of the oligomer analysis for the HCl catalyzed experiments can be seen in Figure 46 and Figure 47:

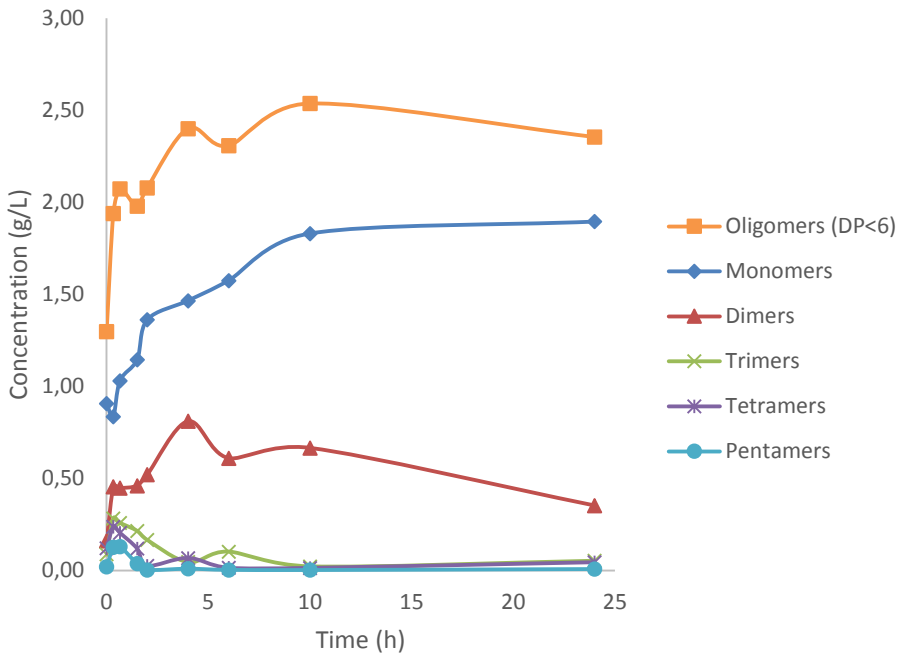


Figure 46-The oligomer (DP<6) concentration as a function of time for Stone Pine extract hydrolysis catalyzed by HCl (T=95ºC, pH=0,5)

As can be seen in Figure 46, rapid kinetics were observed and the maximum concentration for the oligomers (DP<6) was reached in the first few hours of the experiment and no significant degradation was observed.

The monomers are clearly the dominant species in the product distribution. Some dimers were produced in the early stages of the reaction.

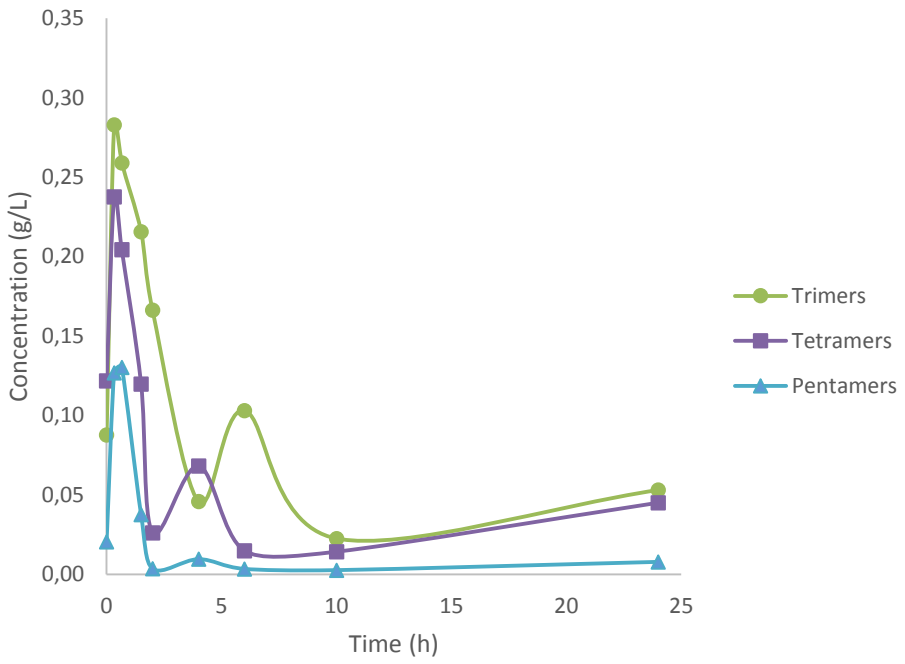


Figure 47-The tri, tetra and pentamers concentration as a function of time for Stone Pine extract hydrolysis catalyzed by HCl (T=95ºC, pH=0,5)

The trimers, tetramers and pentamers were formed in the solution in the beginning of the experiment but they were quickly rapidly into dimers and monomers. The observed increase in the final stages of reaction is most likely due to the formation of polymeric compounds from the sugar degradation products.

The results of the parallel experiment performed with Smopex-101 are presented in Figure 48 and Figure 49:

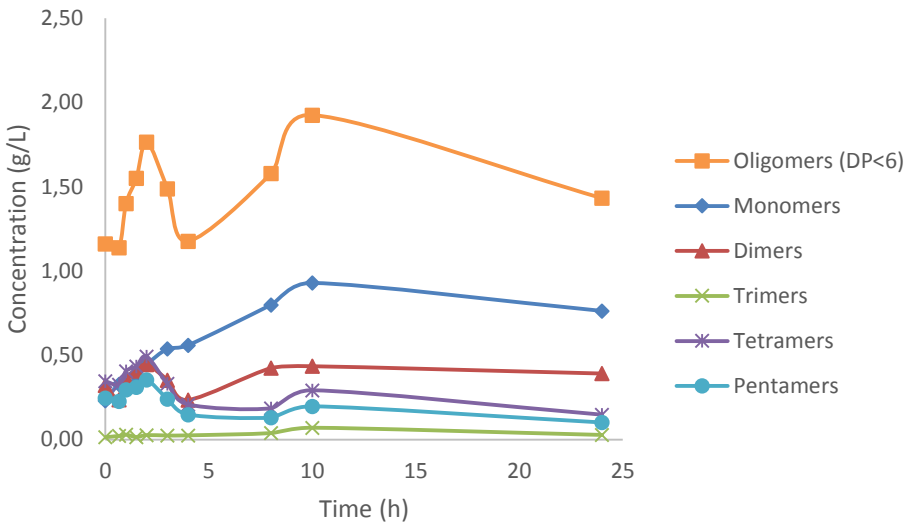


Figure 48-The oligomer (DP<6) concentration as a function of time for Stone Pine extract hydrolysis catalyzed by Smopex-101 (T=95ºC, pH=0,5)

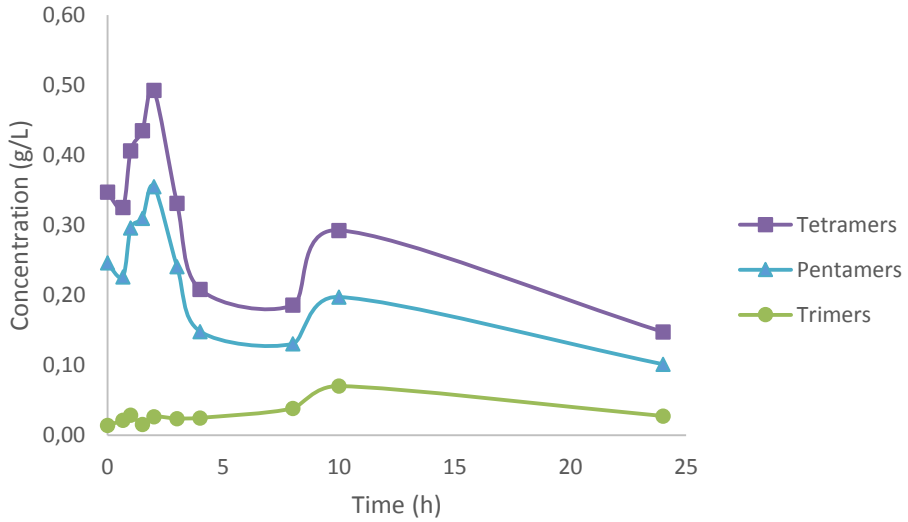


Figure 49-The tri, tetra and pentamers concentration as a function of time for Stone Pine extract hydrolysis catalyzed by Smopex-101 (T=95ºC, pH=0,5)

As seen in Figure 48, the increase of the oligomer concentration was not as fast as with HCl. The large oligomers (trimers, tetramers and pentamers) were clearly hydrolyzed after 2 hours of reaction as can be observed in Figure 49.

Both monomers and dimers reached a maximum after 10 hours of reaction. No severe degradation was observed in this case.

The yields in the experiment performed with HCl can be seen in Figure 50:

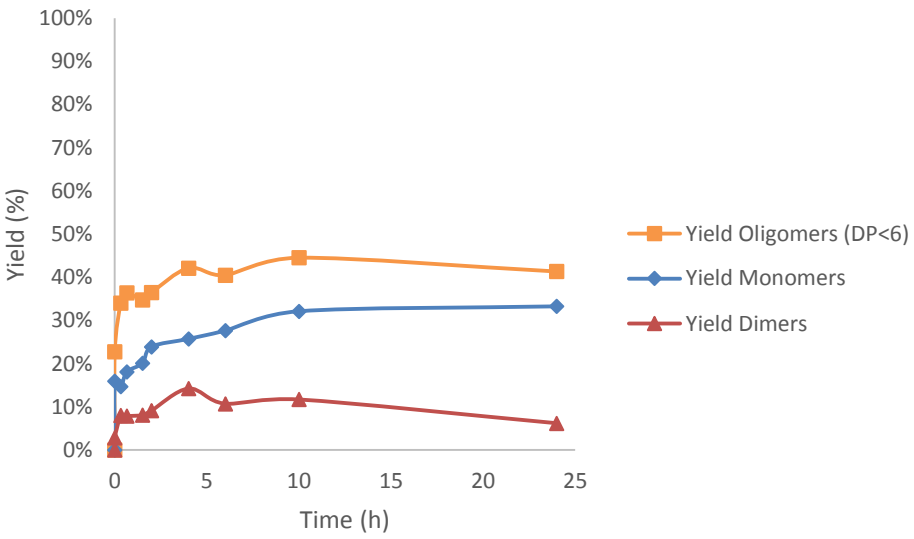


Figure 50-The yield of Stone Pine hemicelluloses to oligomers (DP<6), monomers and dimers as a function of time in a hydrolysis catalyzed by HCl (T=95ºC, pH=0,5)

The results in Figure 50 show very low yields to the oligomers (DP<6) compared to the ones obtained with holm oak, only around 40%. The monomer production accounted for most of the yield, around 30% of the total. The dimer concentration increased at the first stages of the experiment but kept constant for the rest of the reaction.

The results show rapid kinetics in the beginning of the experiment but after approximately 5 hours, the yield reached a maximum and the hydrolysis stopped. No degradation was observed neither.

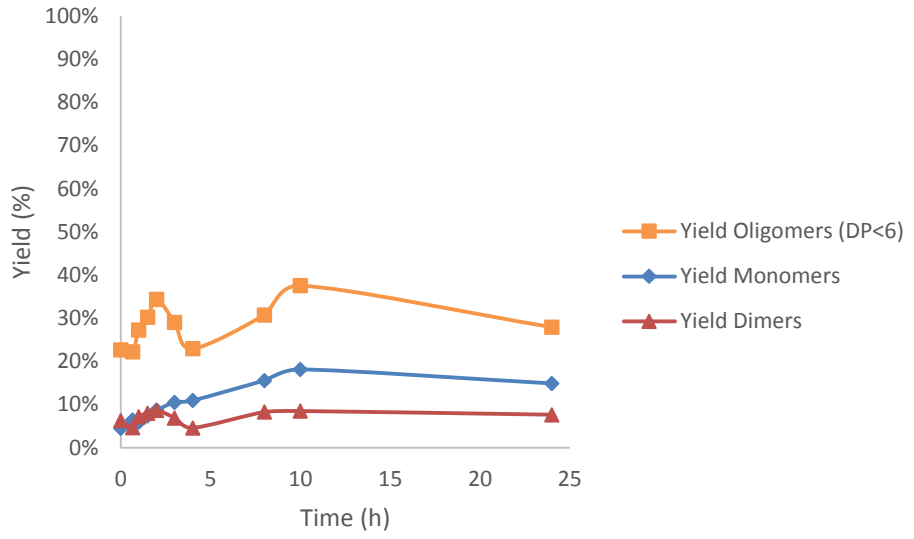


Figure 51-The yield of Stone Pine hemicelluloses to oligomers (DP<6), monomers and dimers as a function of time in a hydrolysis catalyzed by Smopex-101 (T=95ºC, pH=0,5)

The behavior of the hydrolysis using Smopex-101 as a catalyst was very similar to HCl (Figure 50 Figure 51). The yields were even lower, and the maximum yield was about 40%. The yields of the parallel experiments are compared in Figure 52 and Figure 53:

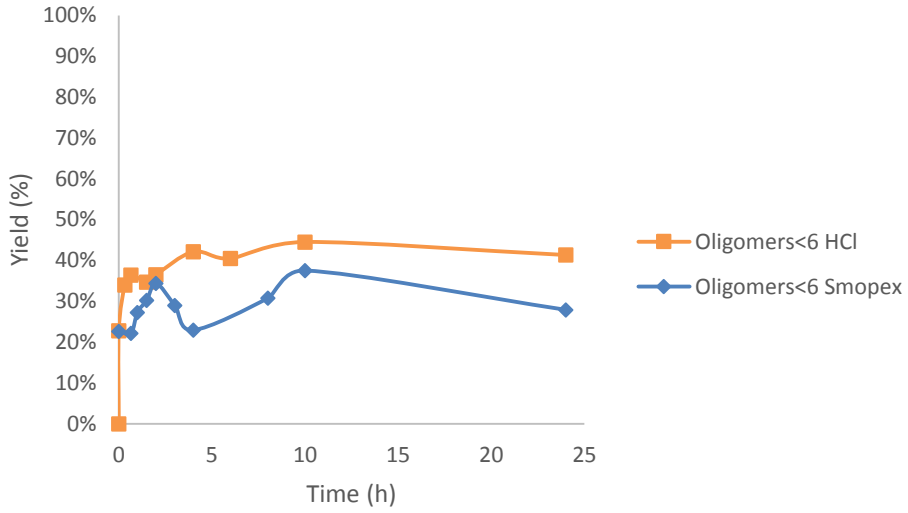


Figure 52-The yield of Stone Pine hemicelluloses to oligomers (DP<6) as a function of time in a hydrolysis catalyzed by HCl and Smopex-101 (T=95ºC, pH=0,5)

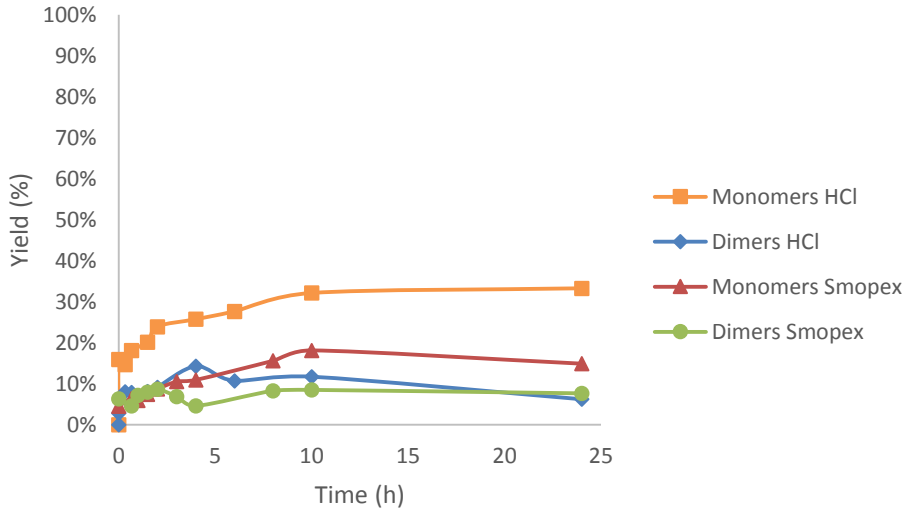


Figure 53-The yield of Stone Pine hemicelluloses to monomers and dimers as a function of time in a hydrolysis catalyzed by HCl and Smopex-101 (T=95ºC, pH=0,5)

The hydrolysis with both catalysts was very fast in the beginning but the production of oligomers (DP<6) stopped after a few hours of reaction.

As with holm oak, Smopex-101 produced a mixture with similar concentrations of monomers and dimers while HCl produced predominantly monomers. No severe degradation was observed in the experiments.

3.6.2 The influence of temperature

In order to study the dependence on the hydrolysis on the temperature, two additional experiments were performed at 85ºC and 75ºC, in pH =0,5.

Holm Oak Hydrolysis (pH=0,5, Smopex-101)

The experiments were performed using a Holm Oak extract, with a hemicellulose concentration of 8,43 g/L.

In order to study the formation of monomers and the degradation during the experiments at 75ºC and 85ºC, monomer analysis was performed. The result of the analysis is depicted in Figure 54 and Figure 55:

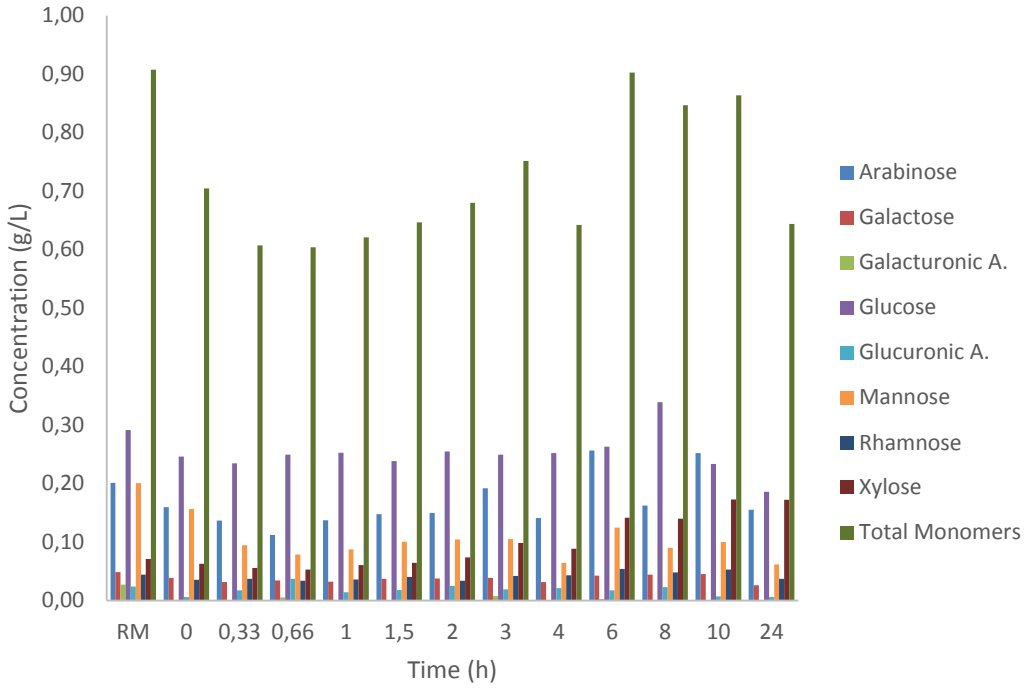


Figure 54-The monomer concentration as a function of time for Holm Oak extract hydrolysis catalyzed by Smopex-101 (T=75ºC, pH=0,5)

The monomer concentration showed a weak increase during the first hours of experiment, however, the reaction rate was very slow. The concentrations were very low, always under 1 g/L. Weak degradation was observed between the two final points of the experiment.

The results of the oligomer analysis are shown in Figure 55 and Figure 56:

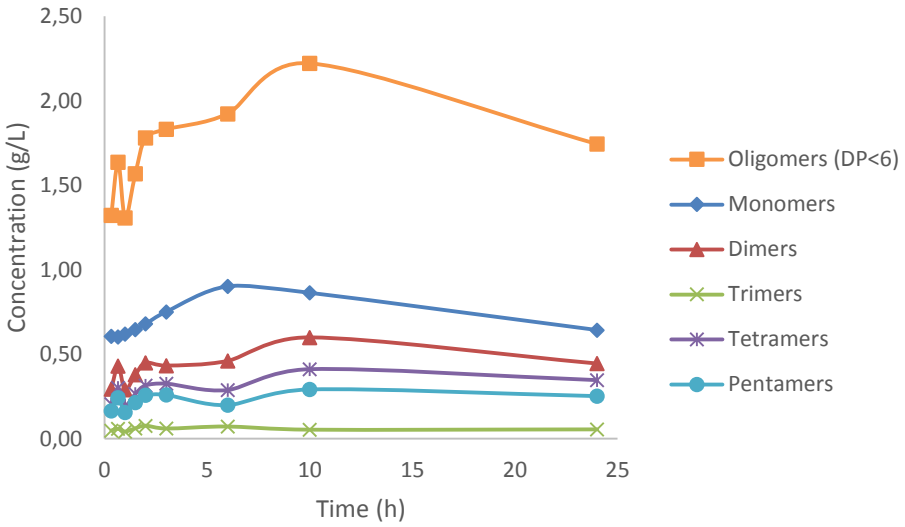


Figure 55-The oligomer (DP<6) concentration as a function of time for Holm Oak extract hydrolysis catalyzed by Smopex-101 (T=75ºC, pH=0,5)

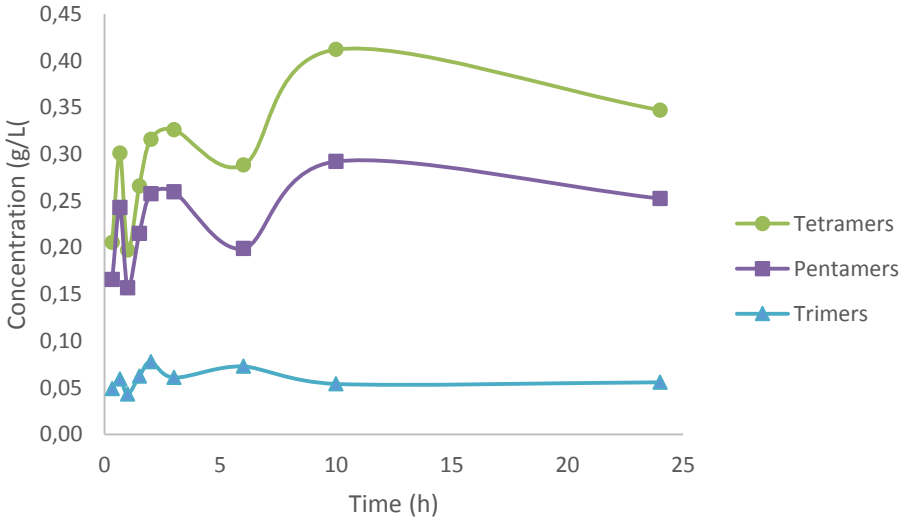


Figure 56-The tri, tetra and pentamers concentration as a function of time for Holm Oak extract hydrolysis catalyzed by Smopex-101 (T=75ºC, pH=0,5)

The oligomer analysis presented in Figure 55 and in Figure 56 confirm the previous observations. At the early stages of the experiment some hydrolysis was observed but, after a few hours the production of oligomers (DP<6). Between 10 and 24 hours of experiment some degradation was observed.

The monomer results from the experiment performed at 85ºC and pH 0,5 using the same extract are presented in Figure 57:

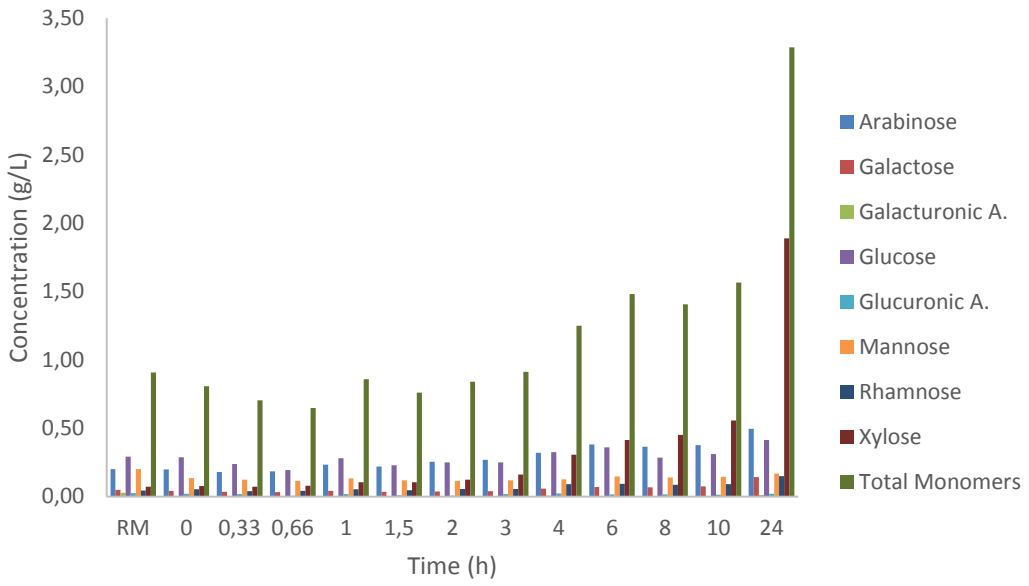


Figure 57-The monomer concentration as a function of time for Holm Oak extract hydrolysis catalyzed by Smopex-101 (T=85ºC, pH=0,5)

The concentration of monomers increased at a constant rate during the experiment. Still after 24 hours, the concentration was increasing and no degradation was observed.

Compared to the results obtained at 75ºC, the concentrations were much higher, most of them over 1 g/L, reaching over 3 g/L at the end of the experiment.

The results from the oligomer analysis are shown in Figure 58 and Figure 59:

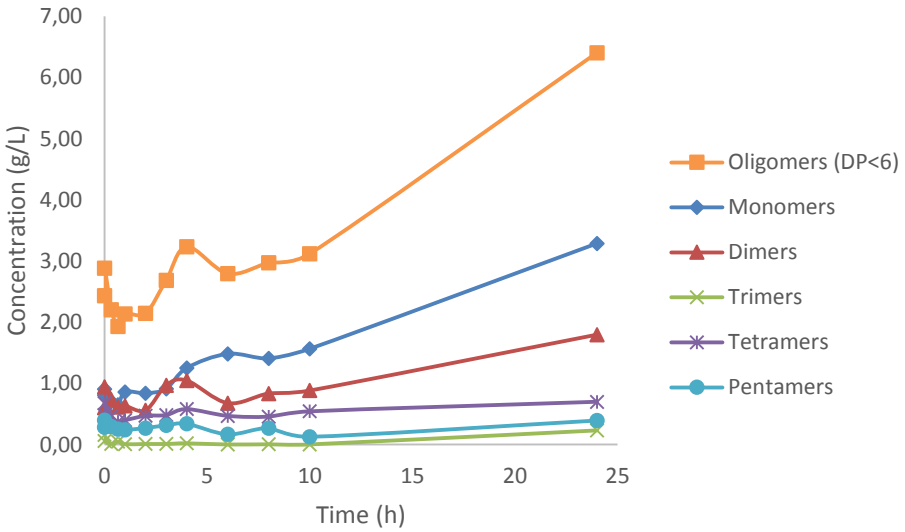


Figure 58-The oligomer (DP<6) concentration as a function of time for Holm Oak extract hydrolysis catalyzed by Smopex-101 (T=85ºC, pH=0,5)

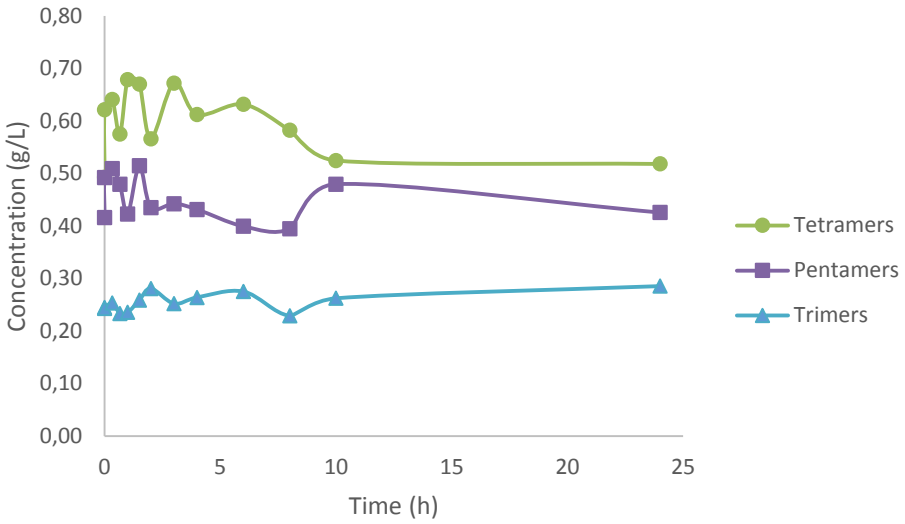


Figure 59-The tri, tetra and pentamers concentration as a function of time for Holm Oak extract hydrolysis catalyzed by Smopex-101 (T=85ºC, pH=0,5)

The oligomer results show that the hydrolysis yield had not reached a maximum when the reaction was stopped. The kinetics was not very fast, but the monomer and the dimer concentrations increased at a constant rate.

The trimers, tetramers and pentamers did not display any degradation, they remained constant from the beginning of the reaction. The concentration of trimers was again lower than for tetramers and pentamers. The observed increase in the final stages of reaction is most likely due to the formation of polymeric compounds from the sugar degradation products.

The yields towards oligomers (DP<6), monomers and dimers in the reactions performed at 75ºC and 85ºC are shown in Figure 60 and Figure 61:

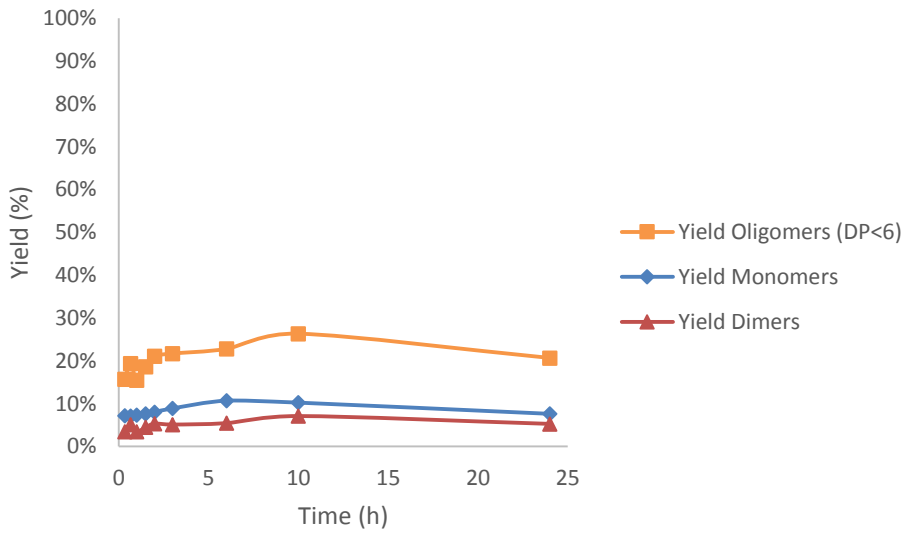


Figure 60-The yield of Holm Oak hemicelluloses to oligomers (DP<6), monomers and dimers as a function of time in a hydrolysis catalyzed by Smopex-101 (T=75ºC, pH=0,5)

The yields at 75ºC show that hydrolysis barely occurred. The oligomer concentration increased slightly but the monomer and dimer concentrations remained constant. No degradation was observed. These results demonstrate that the hydrolysis was strongly influenced by the temperature.

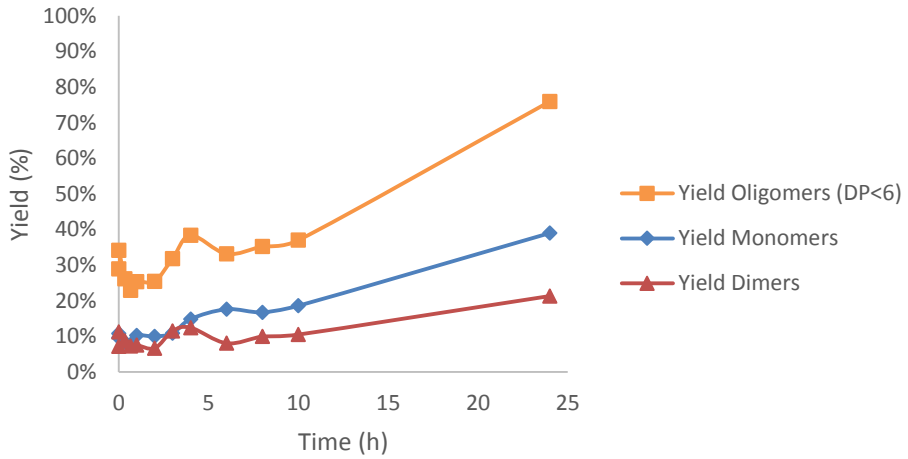


Figure 61-The yield of Holm Oak hemicelluloses to oligomers (DP<6), monomers and dimers as a function of time in a hydrolysis catalyzed by Smopex-101 (T=85ºC, pH=0,5)

At 85ºC, the reaction did not reach its maximum after 24 hours. The results show that the yields were quite high, nearly the 80% for oligomers (DP<6), 40% for monomers and 20% for dimers.

These comparisons between the three different temperatures were made in terms of yield because the extracts had a different initial concentration. The results from the three different experiments at pH=0,5 with Smopex-101 are plotted in Figure 62:

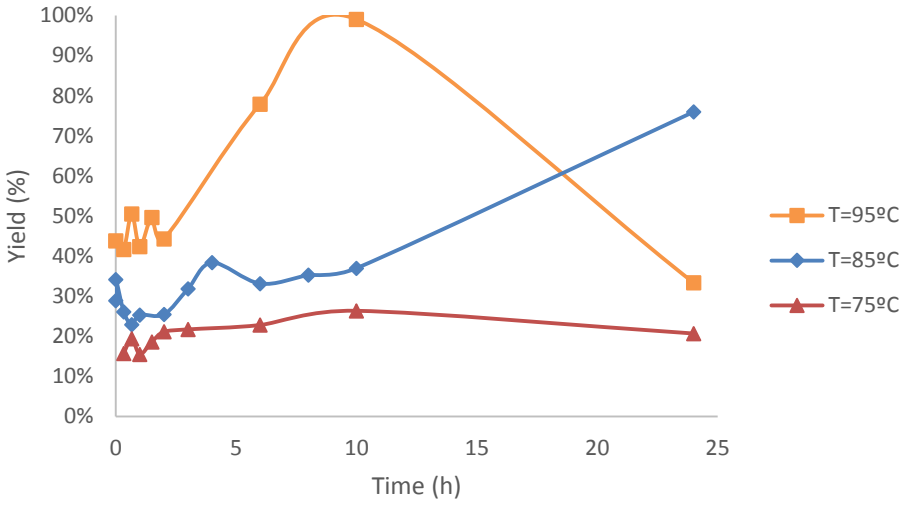


Figure 62-The influence of temperature on the yield of Holm Oak hemicelluloses to oligomers (DP<6) as a function of time in a hydrolysis catalyzed by Smopex-101 (pH=0,5)

After 24 hours at 75ºC the reaction had barely taken place, at 85ºC the concentration of oligomers (DP<6) was still increasing and at 95ºC already severe degradation had taken place. The figure clearly shows that the hydrolysis is very temperature sensitive.

The yields towards monomers and dimers from the three different experiments at pH 0,5 with Smopex-101 are plotted in Figure 63 and Figure 64:

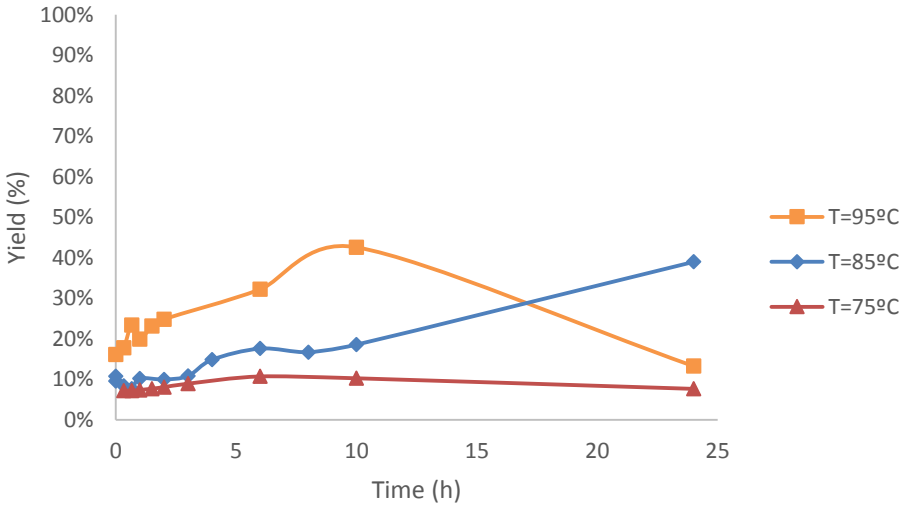


Figure 63- The influence of temperature on the yield of Holm Oak hemicelluloses to monomers as a function of time in a hydrolysis catalyzed by Smopex-101 (pH=0,5)

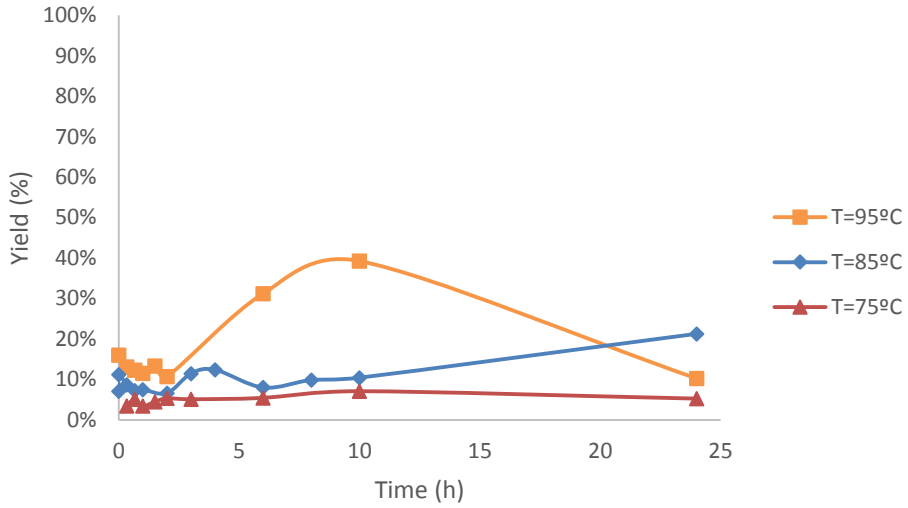


Figure 64-The influence of temperature on the yield of Holm Oak hemicelluloses to dimers as a function of time in a hydrolysis catalyzed by Smopex-101 (pH=0,5)

Looking at the monomers and dimers separately (Figure 63 and Figure 64) the same conclusions can be obtained, the reaction is highly influenced by temperature. The optimum yield was reached at about 10 h of reaction and an even better result could have been obtained at 85ºC by prolonging the reaction times.

It should also be observed that with Smopex-101, both monomers and dimers are obtained almost in the same proportion. Based on these results, Smopex-101 does not seem to be the perfect catalyst for the production of monomers, but it can be very suitable for the production of dimers or a mixture of the two.

Stone Pine Hydrolysis (pH=0,5, Smopex-101)

The new experiments were performed using a Stone Pine extract with a concentration of hemicelluloses of 6,36 g/L. This extract was used for both experiments at 75ºC and 85ºC.

In order to study the production of the monomers and the degradation during the experiments at 75ºC and 85ºC, the monomer analysis was performed. The results of the analysis are shown in Figure 65 and Figure:

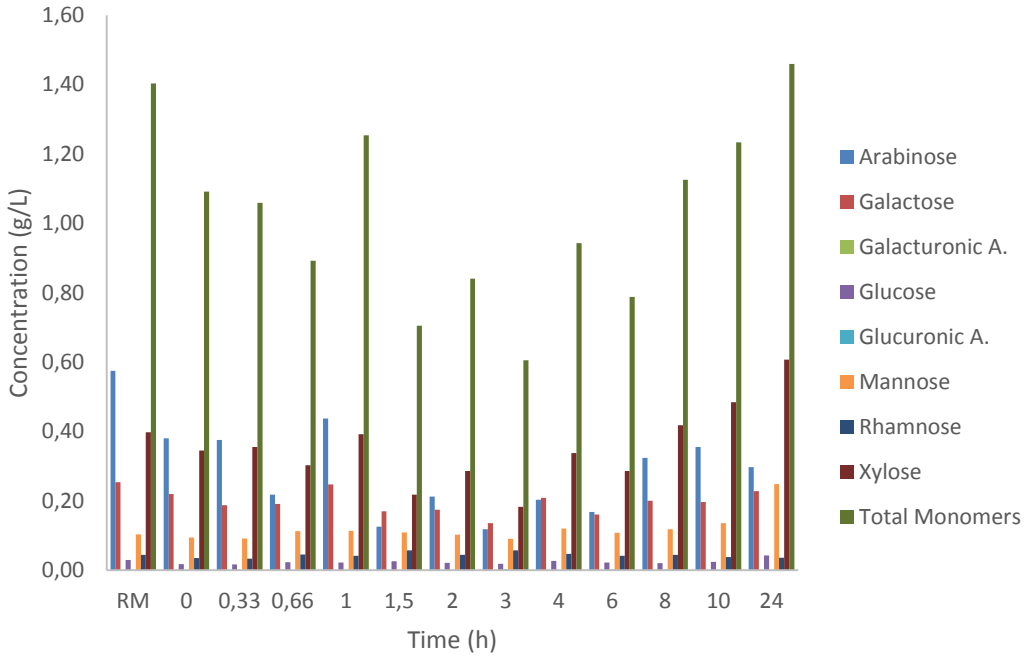


Figure 65-The monomer concentration as a function of time for Stone Pine extract hydrolysis catalyzed by Smopex-101 (T=75ºC, pH=0,5)

Based on the results in Figure 65, it can be concluded that the concentration of monomers was very low during the whole experiment and no significant hydrolysis was observed.

The results of the oligomer analysis shown in Figure 66 and Figure 67:

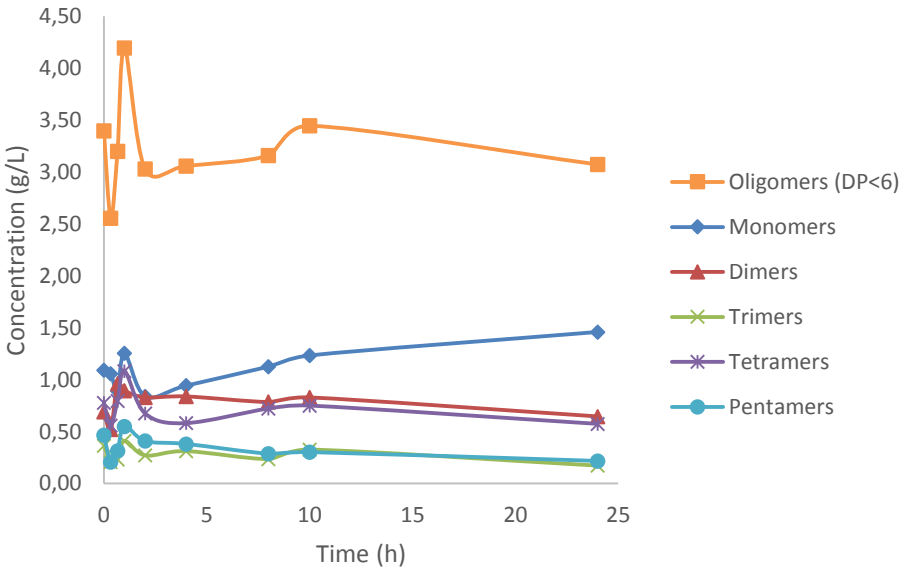


Figure 66-The oligomer (DP<6) concentration as a function of time for Stone Pine extract hydrolysis catalyzed by Smopex-101 (T=75ºC, pH=0,5)

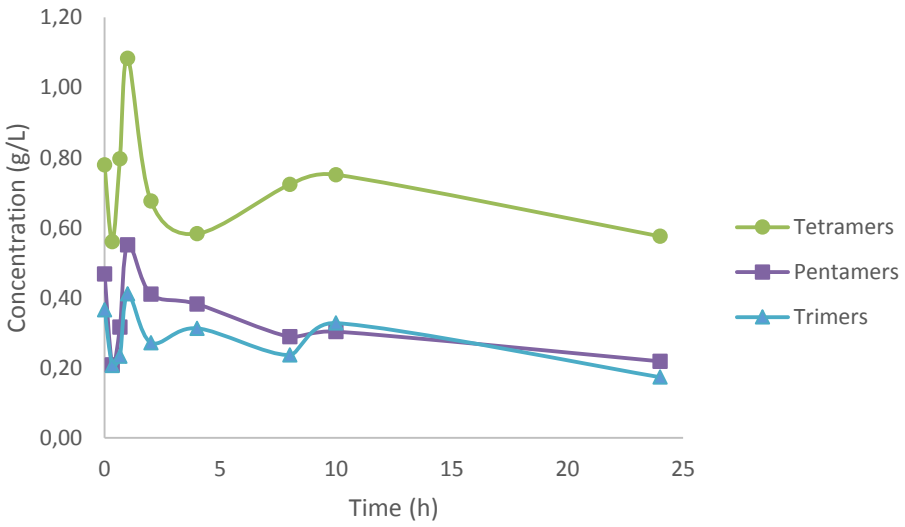


Figure 67-The tri, tetra and pentamers concentration as a function of time for Stone Pine extract hydrolysis catalyzed by Smopex-101 (T=75ºC, pH=0,5)

The oligomer results show that the concentration of different oligomers (DP<6) increased in the beginning of the experiment but after reaching a maximum at around 2 hours, the oligomers (DP<6) started to be hydrolyzed while the monomer concentration kept increasing.

Looking at the larger oligomers, the tetramers concentration was unusually high compared to the trimers and the pentamers from the early stages of the reaction.

The same extract was used for the experiment at pH=0,5 and 85ºC. The monomer results are presented in Figure 68:

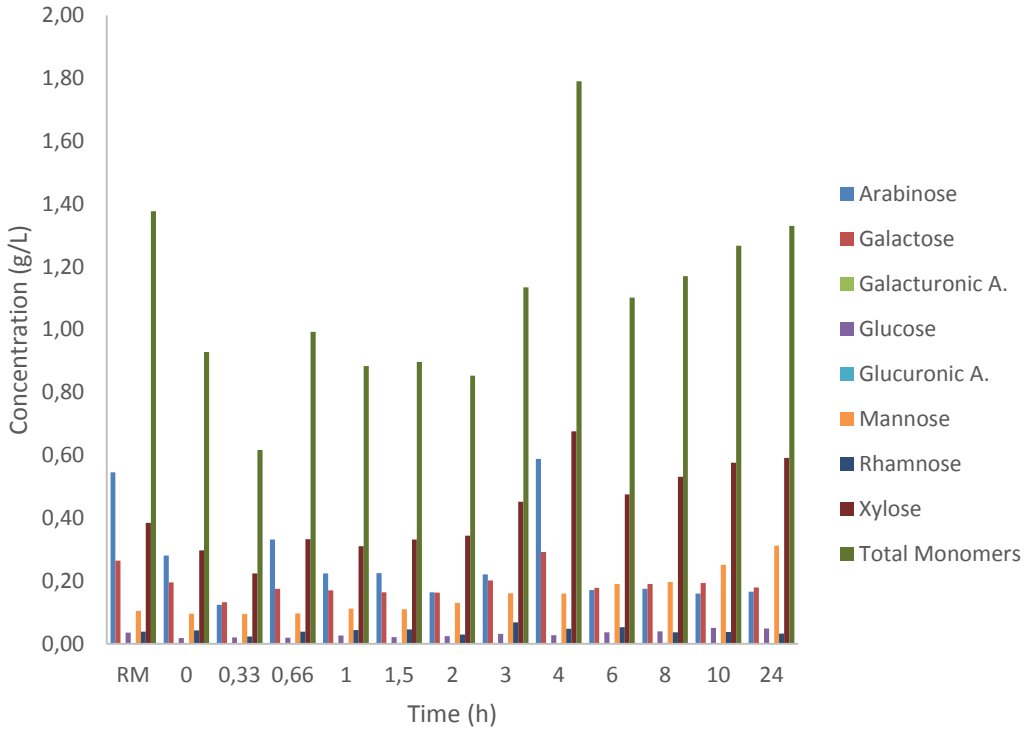


Figure 68-The monomer concentration as a function of time for Stone Pine extract hydrolysis catalyzed by Smopex-101 (T=85ºC, pH=0,5)

It can be observed that the concentration of monomers increased during the whole experiment, but the hydrolysis is very slow. The results from the oligomer analysis are shown in Figure 69 and Figure 70:

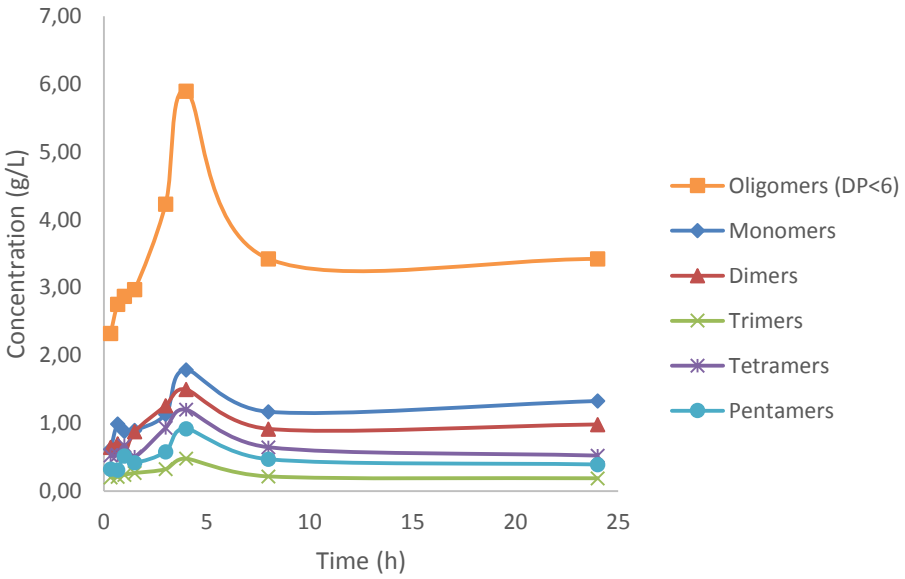


Figure 69-The oligomer (DP<6) concentration as a function of time for Stone Pine extract hydrolysis catalyzed by Smopex-101 (T=85ºC, pH=0,5)

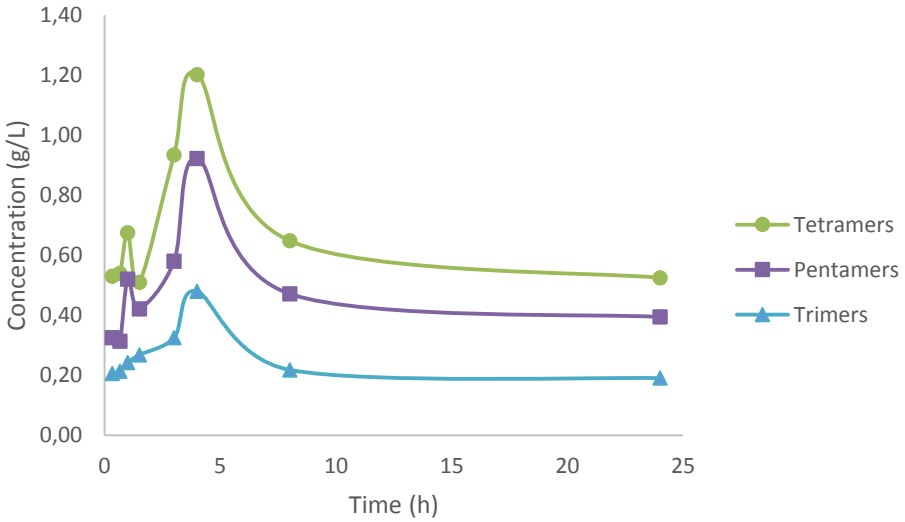


Figure 70-The tri, tetra and pentamers concentration as a function of time for Stone Pine extract hydrolysis catalyzed by Smopex-101 (T=85ºC, pH=0,5)

As shown in the figures, the hydrolysis is quite fast in the beginning reaching a maximum concentration after 5 hours of reaction. After that, degradation is observed.

In this case, the large oligomers (DP<6) were not degraded to monomers and dimers, their concentration increased during the first few hours and then they were slowly hydrolyzed.

Once the monomer and the oligomer analysis are done it is time to traduce these data into yield results so they can be comparable with previous results.

The yields towards oligomers (DP<6), monomers and dimers in the reactions at 75ºC and 85ºC are shown in Figure 71 and Figure 72:

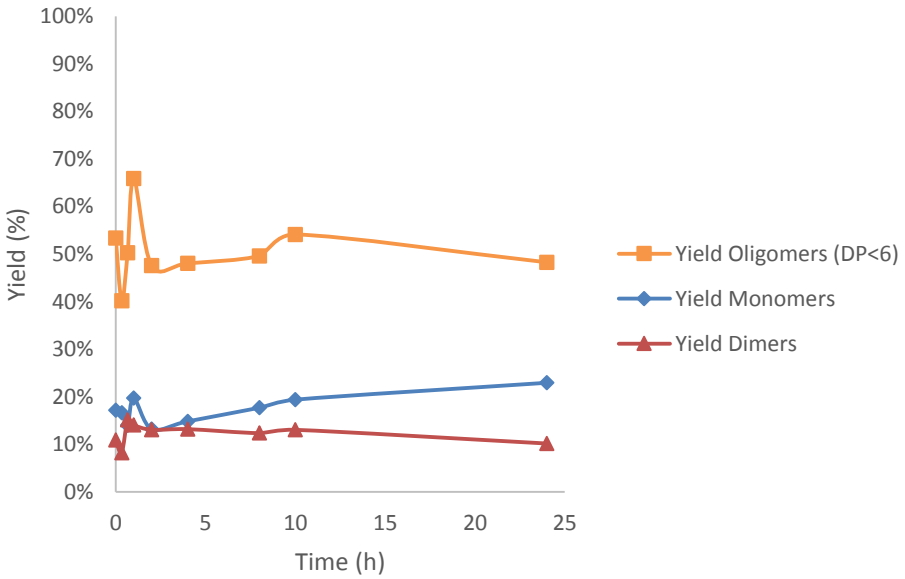


Figure 71-The yield of Stone Pine hemicelluloses to oligomers (DP<6), monomers and dimers as a function of time in a hydrolysis catalyzed by Smopex-101 (T=75ºC, pH=0,5)

The yields at 75ºC showed that hydrolysis barely occurred. The oligomer concentration and the monomer concentration increased slightly while the dimer concentration remained constant. No degradation was observed.

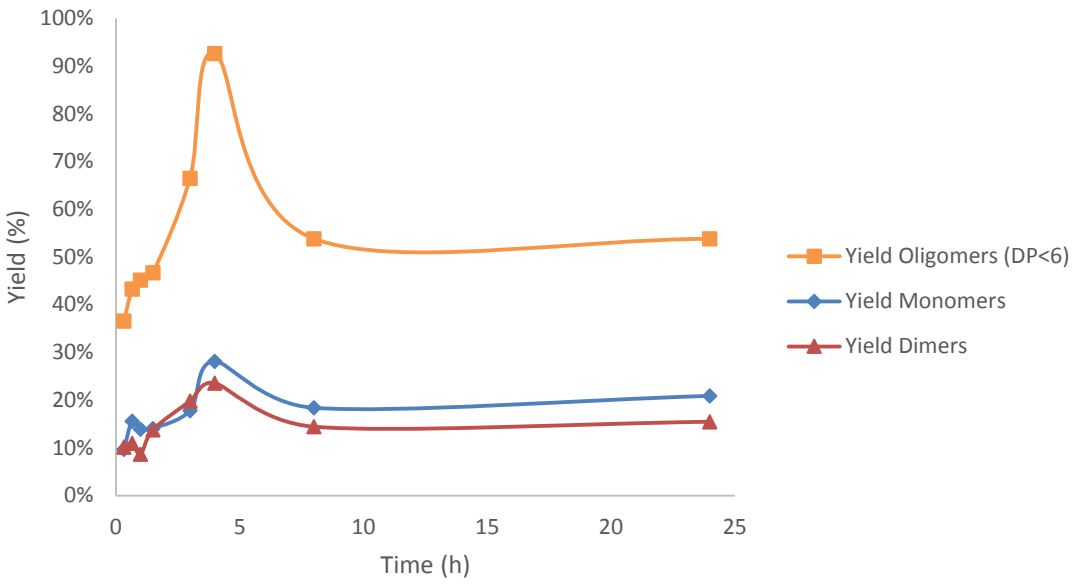


Figure 72-The yield of Stone Pine hemicelluloses to oligomers (DP<6), monomers and dimers as a function of time in a hydrolysis catalyzed by Smopex-101 (T=85ºC, pH=0,5)

As seen in Figure 72, during the first 5 hours of reaction the oligomer content increased as well as the monomer and the dimer concentrations. The highest yield obtained towards oligomers (DP<6) was about 95%. After the experimental point at 5 hours, degradation occurred and the observed yield dropped to about 55%.

It should be noted that the hydrolysis was not specific towards monomers and a mixture of oligomers (DP<6) was formed, at 85ºC. It can be observed again that the yield towards monomers is very close to the yields towards dimers.

The yields from the three experiments performed at pH 0,5 with Smopex-101 using Stone Pine extracts are plotted in Figure 73-Figure 75:

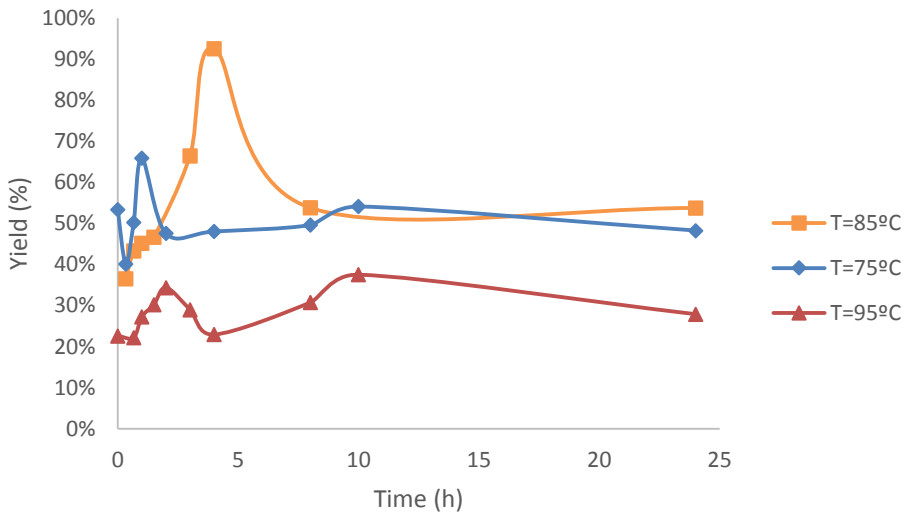


Figure 73-The influence of temperature on the yield of Stone Pine hemicelluloses to oligomers (DP<6) as a function of time in a hydrolysis catalyzed by Smopex-101 (pH=0,5)

As shown in Figure 73, the yields of the hydrolysis towards oligomers (DP<6) is very sensitive to temperature, especially as such low pH. The results indicate that the optimum window in the kinetics is very narrow. At 75ºC, the hydrolysis seemed very slow, at 85ºC a maximum of 95% yield was achieved followed by significant degradation and at 95ºC the yields were the lowest throughout the experiment indicating severe degradation of some sugars.

Compared to Holm Oak, the balance between hydrolysis and degradation seem much more delicate and care should be taken in the optimization.

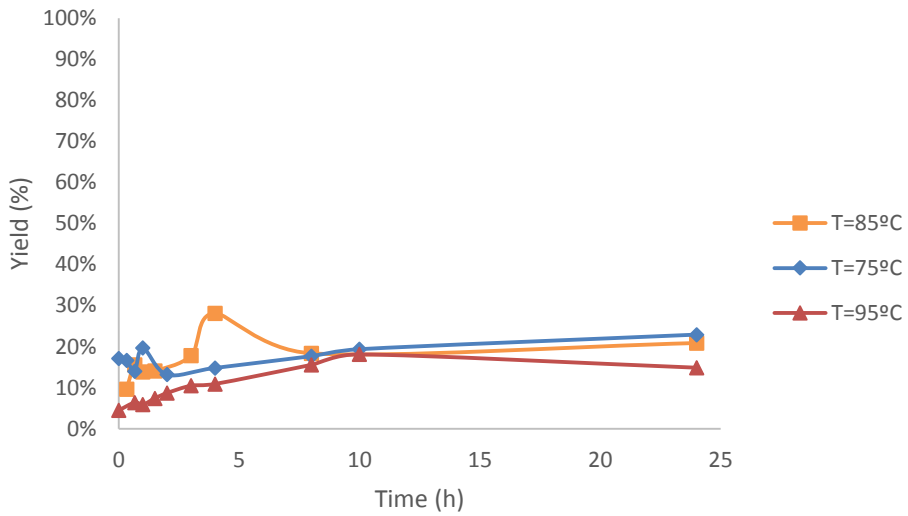


Figure 74-The influence of temperature on the yield of Stone Pine hemicelluloses to monomers as a function of time in a hydrolysis catalyzed by Smopex-101 (pH=0,5)

As seen in Figure 74, the concentration of monomers is rather low as a mixture of oligomers (DP<6) is produced in the hydrolysis.

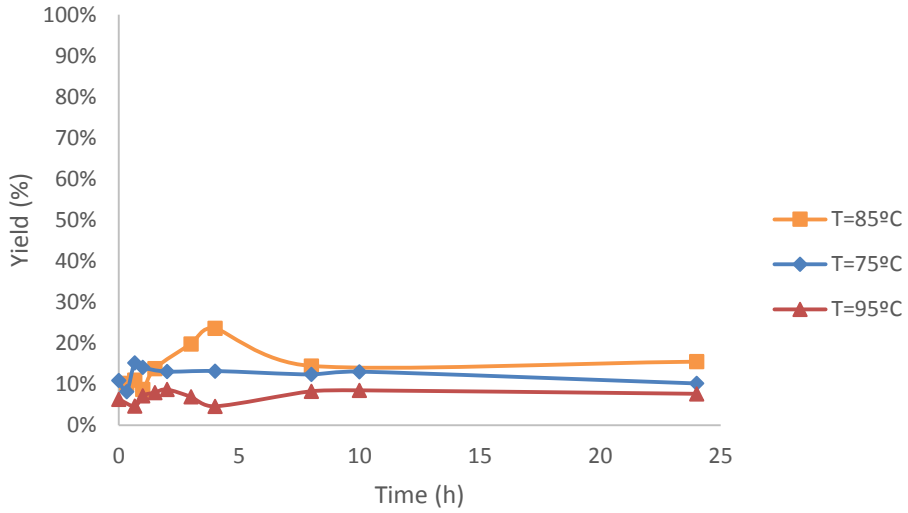


Figure 75-The influence of temperature on the yield of Holm Oak hemicelluloses to dimers as a function of time in a hydrolysis catalyzed by Smopex-101 (pH=0,5)

The low selectivity of the catalyst an also be observed in Figure 75, as the amount of dimers is rather low during the reactions being just slightly elevated at 85ºC. The hydrolysis produces a mixture of oligomers (DP<6).

3.6.3 The influence of pH

In order to study the dependence of the hydrolysis on the pH, the pH range was expanded to pH 1 and pH 1,5, at 95ºC.

Holm Oak Extract Hydrolysis (T=95ºC, Smopex-101)

The experiments were performed using a Holm Oak extract with a hemicellulose concentration of 2,02 g/L.

In order to study the formation of monomers and the degradation during the experiments at pH 1 and pH 1,5 monomer analysis was performed. The results of the analysis are shown in Figure 76 and Figure 77:

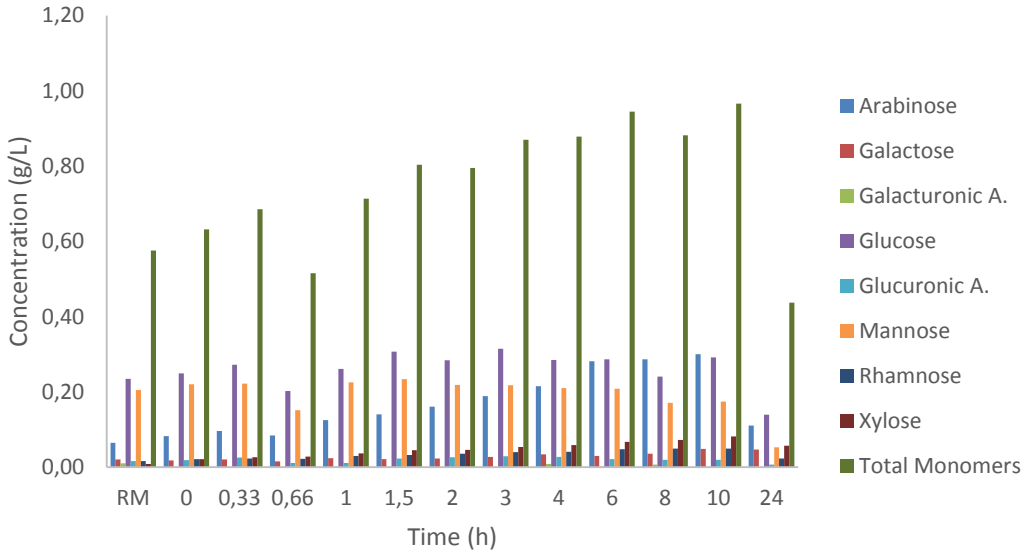


Figure 76-The monomer concentration as a function of time for Holm Oak extract hydrolysis catalyzed by Smopex-101 (T=95ºC, pH=1)

As seen in Figure 76, the concentration of the monomers increased trend steadily during the first 10 hours of reaction. This increase is constant but the concentrations remain rather low, always under 1 g/L. The results indicate that a maximum concentration might have been achieved between 10 hours and 24 hours as the concentration increased until 10 hours, but significant degradation was observed. It is possible to appreciate some degradation of the monomers at 24 hours. The results of the oligomer analysis are shown in Figure 77 and Figure 78:

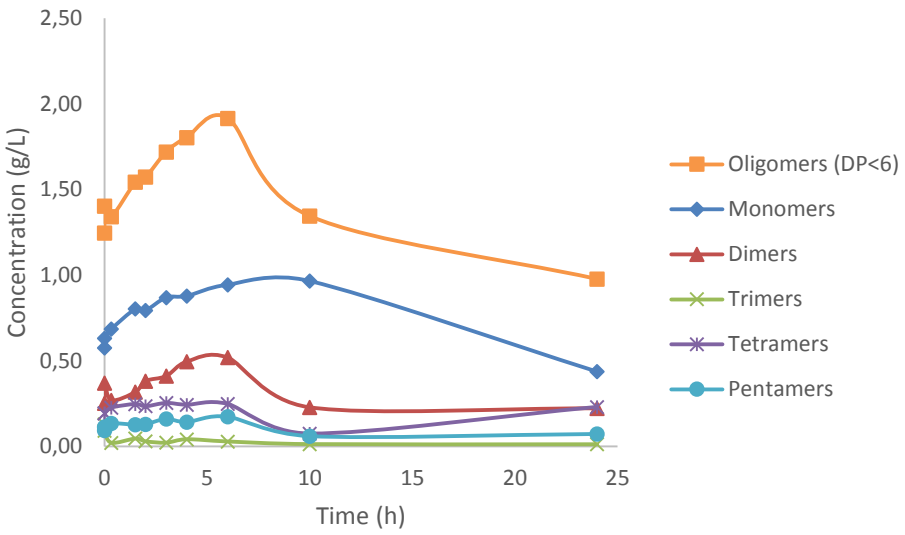


Figure 77-The oligomer (DP<6) concentration as a function of time for Holm Oak extract hydrolysis catalyzed by Smopex-101 (T=95ºC, pH=1)

It can be seen from the results that the kinetics of the hydrolysis at pH 1 is still rather fast. The monomer concentration reached a maximum after about 6 hours. Severe degradation of the sugars was observed after 10 hours of reaction. The dimers concentration also followed the same trend.

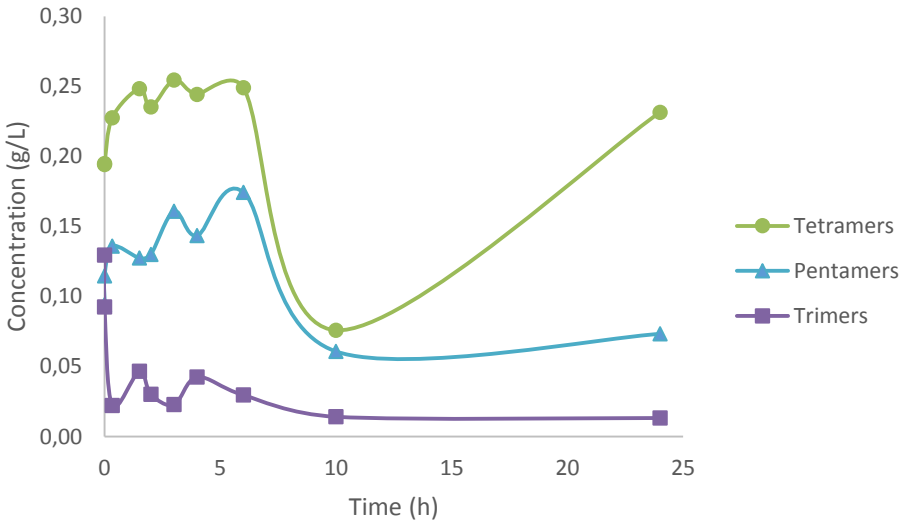


Figure 78-The tri, tetra and pentamers concentration as a function of time for Holm Oak extract hydrolysis catalyzed by Smopex-101 (T=95ºC, pH=1)

The concentration of tri, tetra and pentamers was very small in the raw material, and it decreased during the reaction. Their concentrations went through a maximum and decreased after that. The

observed increase in the final stages of reaction is most likely due to the formation of polymeric compounds from the sugar degradation products.

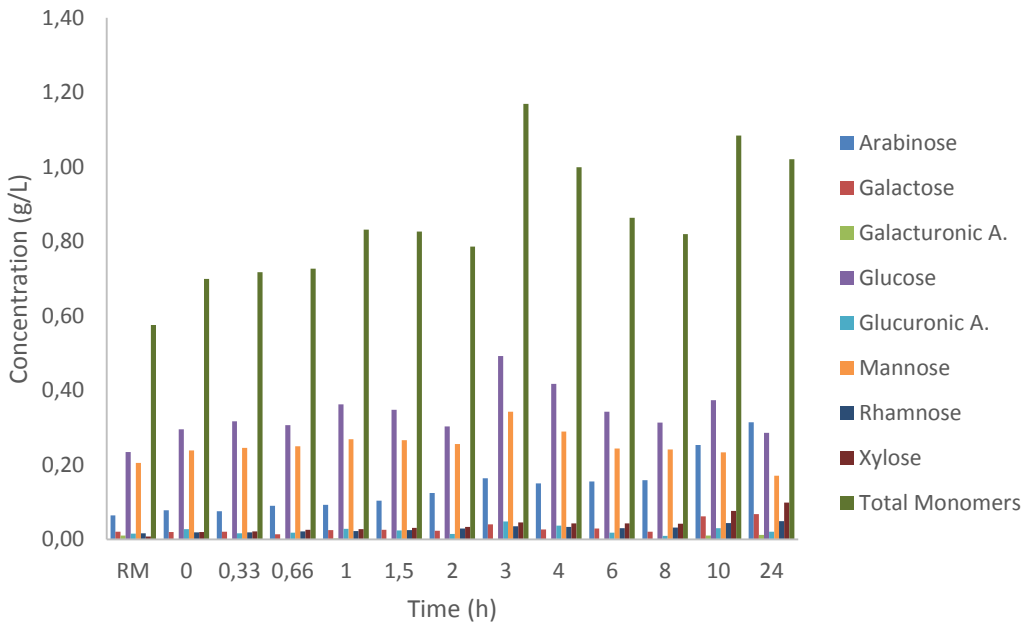


Figure 79-The monomer concentration as a function of time for Holm Oak extract hydrolysis catalyzed by Smopex-101 (T=95ºC, pH=1,5)

The increase in the monomer concentration at pH 1,5 is displayed in Figure 79. The concentrations are similar pH 1 but the trend is slightly different because there is no significant degradation.

The oligomer results are shown in Figure 80 and Figure 81:

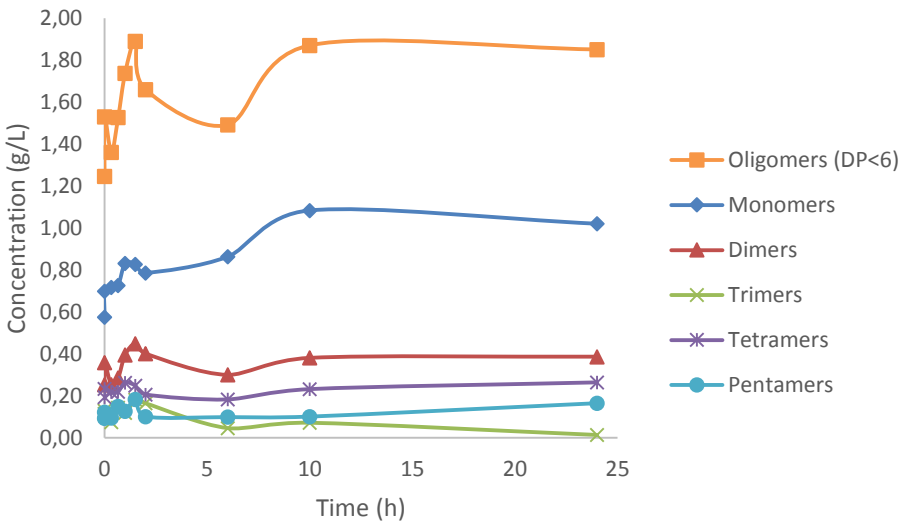


Figure 80-The oligomer (DP<6) concentration as a function of time for Holm Oak extract hydrolysis catalyzed by Smopex-101 (T=95ºC, pH=1,5)

Based on the results in Figure 80, it can be concluded that no degradation occurred during the experiment in pH 1,5 and that the concentration reached a maximum after 10 hours of reaction. The monomer concentration was high compared to the dimers and the rest of the oligomers (DP<6).

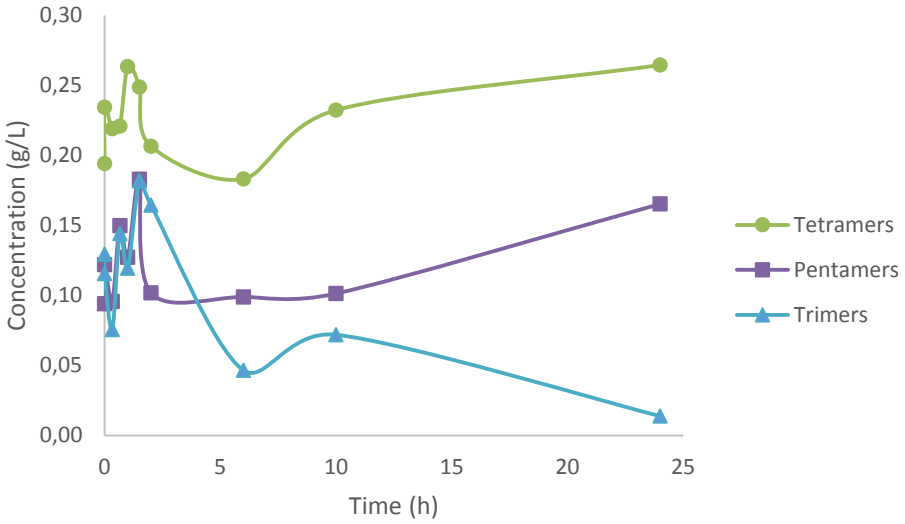


Figure 81-The tri, tetra and pentamers concentration as a function of time for Holm Oak extract hydrolysis catalyzed by Smopex-101 (T=95ºC, pH=1,5)

The oligomers (DP<6) (Figure 81) obtained values under 0,25 g/L. The trimers were still unusually low compared to the rest of the compounds. The observed increase in the final stages of reaction is most likely due to the formation of polymeric compounds from the sugar degradation products.

The yields for the reaction at pH 1 and pH 1,5 are depicted in Figure 82 and Figure 83:

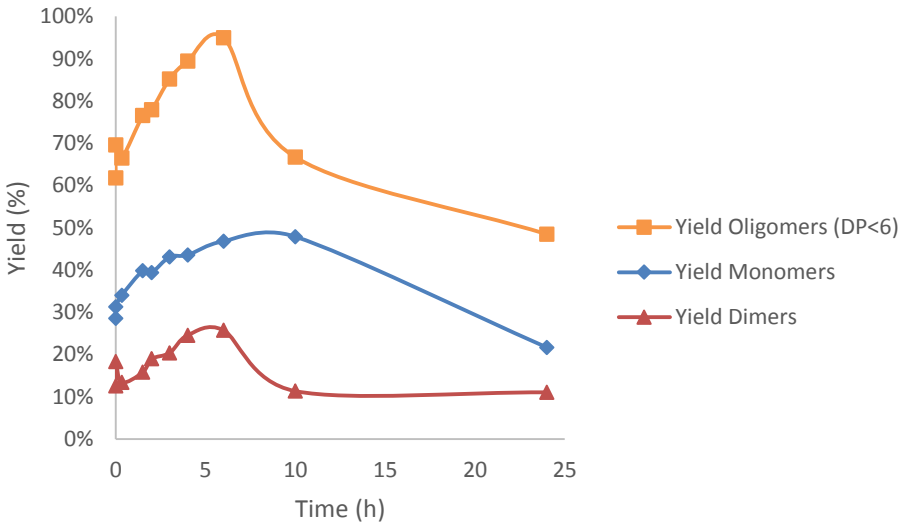


Figure 82-The yield of Holm Oak hemicelluloses to oligomers (DP<6), monomers and dimers as a function of time in a hydrolysis catalyzed by Smopex-101 (T=95ºC, pH=1)

High yields towards oligomers (DP<6), nearly 100%, were obtained at pH 1, however, the yields toward monomers did not achieve rates higher than 50%. The results indicate that the conditions were not harsh enough to result in the complete cleavage of the oligomers (DP<6) but on the other hand, the degradation of the sugars was avoided.

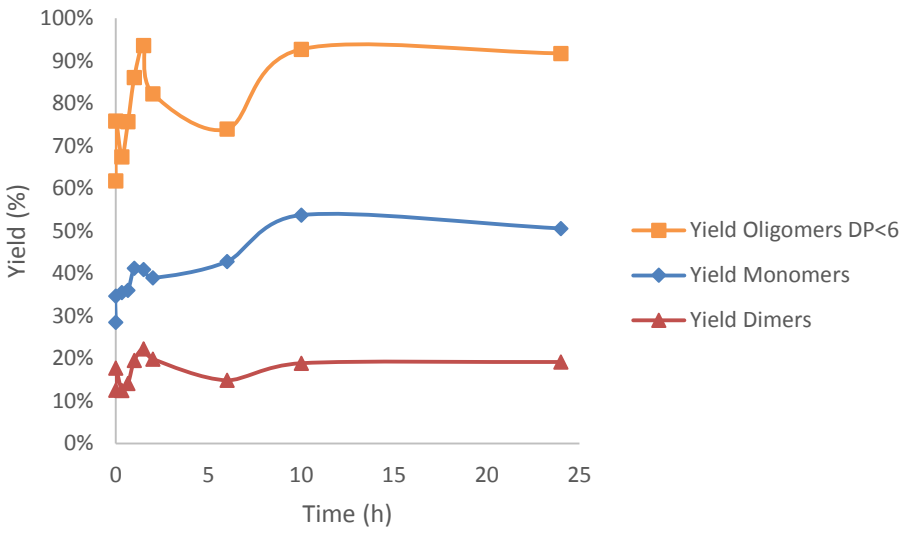


Figure 83-The yield of Holm Oak hemicelluloses to oligomers (DP<6), monomers and dimers as a function of time in a hydrolysis catalyzed by Smopex-101 (T=95ºC, pH=1,5)

At pH 1,5 the "stable" yield towards monomers was even higher, reaching stable values of over 90% for 14 hours. The monomer yield was similar to the experiment at pH 1, close to 50%. No significant degradation was observed.

After such interesting results regarding the pH of the hydrolysis of the holm oak, the monomer, dimer and oligomer results from the three experiments at different pH were plotted in the same figures in order to be able to better compare the results (Figure 84Figure 85Figure 86).

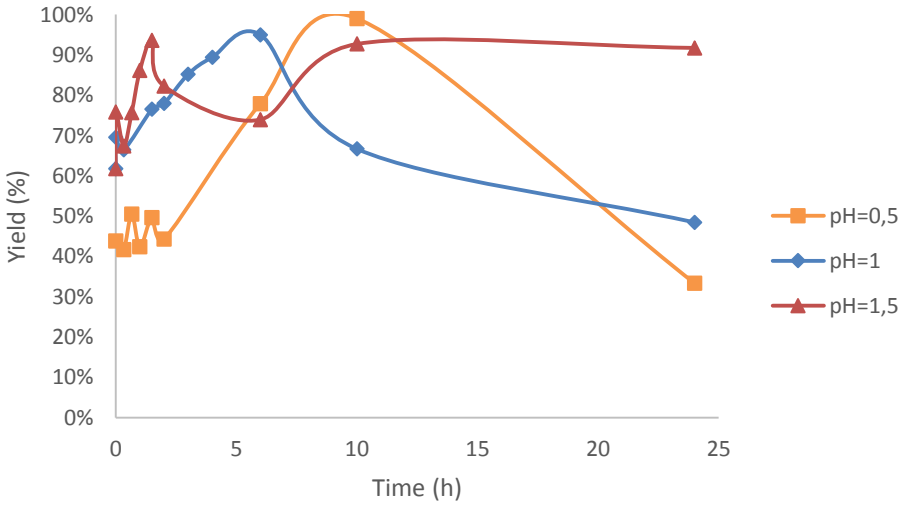


Figure 84-The influence of pH on the yield of Holm Oak hemicelluloses to oligomers (DP<6) as a function of time in a hydrolysis catalyzed by Smopex-101 (T=95ºC)

As shown in Figure 84, low pH conditions leads to degradation of the sugars. At high pH values, the reaction seems to be rather fast and no degradation was observed.

It should be noticed that the difference in conversion (not in yield) is significant, as the experiments at pH 1 and pH 1,5 go from an initial yield of 65% to a maximum yield of 90%, the reaction with pH 0,5 goes from 45% to almost 100%.

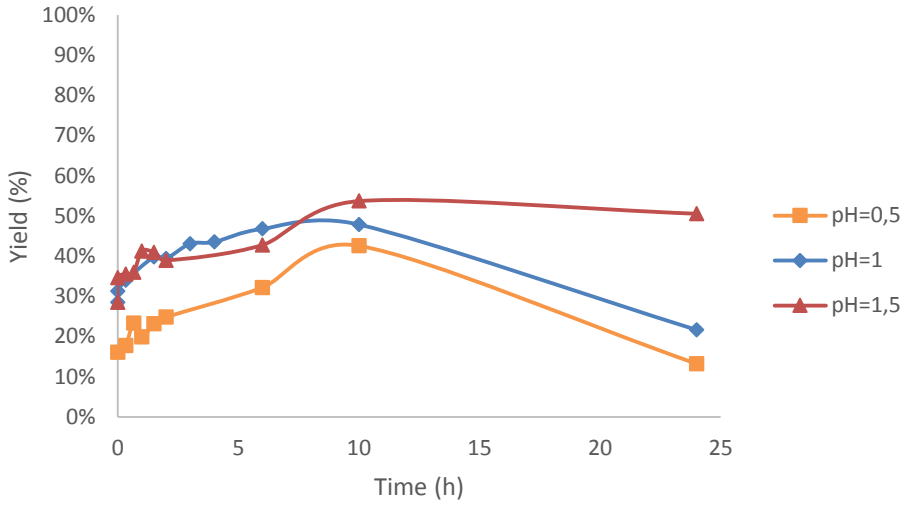


Figure 85-The influence of pH on the yield of Holm Oak hemicelluloses to monomers as a function of time in a hydrolysis catalyzed by Smopex-101 (T=95ºC)

The difference in production of monomers is significant (Figure 85). In each experiment the yields towards monomers reached a value close to 50% but in the reactions with lower pH, severe degradation of the monomers was observed, while at pH 1,5 no degradation occurred.

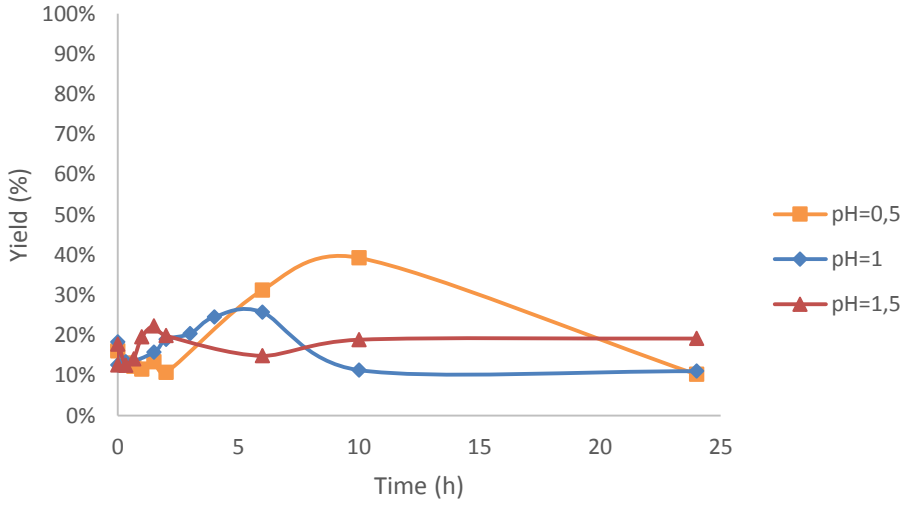


Figure 86-The influence of pH on the yield of Holm Oak hemicelluloses to dimers as a function of time in a hydrolysis catalyzed by Smopex-101 (T=95ºC)

As shown in Figure 86, low pH leads to higher dimer yields. It can be concluded that the dimers are stable at low pH values and that degradation only occurs after 10 hours of reaction. The yield in dimers is also very close to the yield towards monomers, as observed previously.

Stone Pine Extract Hydrolysis (T=95ºC, Smopex-101)

The experiments were performed using different concentrations, at pH 1 the hemicellulose concentration was 9,22 g/L and at pH 1,5 the hemicellulose concentration was 7,65 g/L. The extract was the same but it had to be diluted for the second experiment.

In order to study the production monomers and the degradation during the experiments at pH 1 and pH 1,5 monomer analysis was performed. The results of the analysis are shown in Figure 87 and Figure 88:

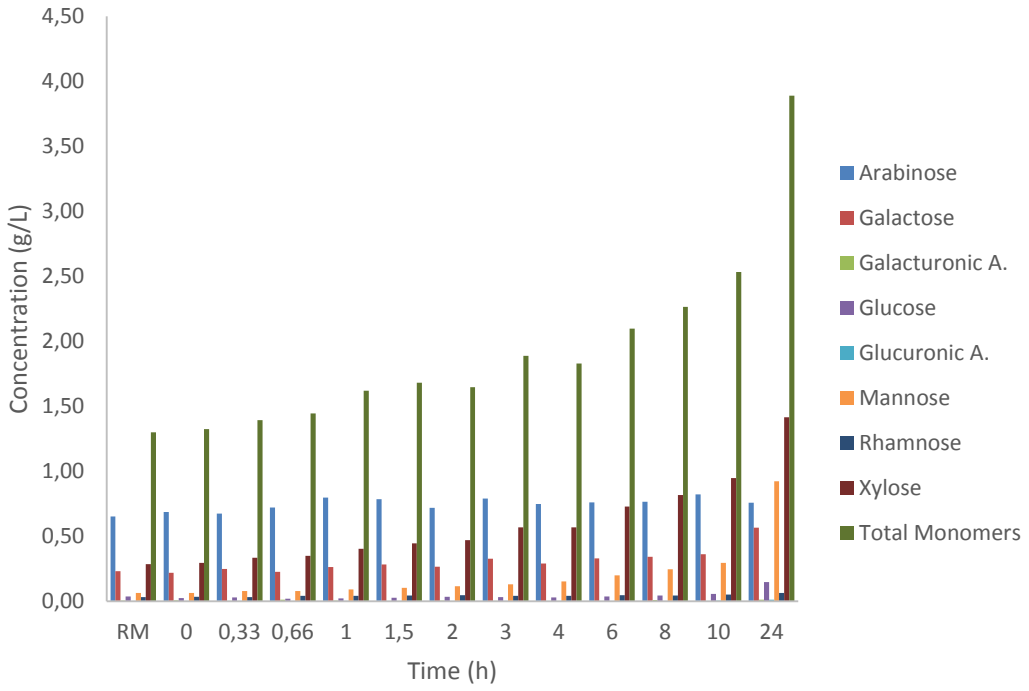


Figure 87- The monomer concentration as a function of time for Stone Pine extract hydrolysis catalyzed by Smopex-101 (T=95ºC, pH=1)

As shown in Figure 87, the monomer concentration at pH 1 increased during the whole reaction. The reaction does not seem to have reached the maximum at the end of the experiment. Consequently, no degradation was observed.

More information has been obtained from the oligomer analysis showed in Figure 88 and Figure 89:

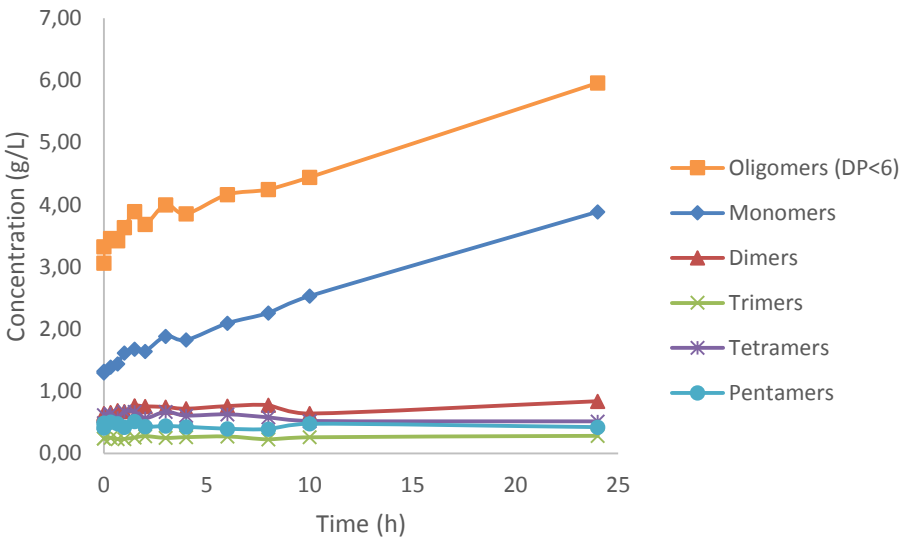


Figure 88-The oligomer (DP<6) concentration as a function of time for Stone Pine extract hydrolysis catalyzed by Smopex-101 (T=95ºC, pH=1)

From these results, it can be concluded that the kinetics of the hydrolysis at pH 1 are rather slow for the production of monomers, however, and that no degradation occurred.

The concentration of the oligomers (DP<6) remained rather constant during the reaction. This result would indicate either a consecutive reaction mechanism where the oligomers (DP<6) are intermediates or an end biting mechanism where the polymers are hydrolysed directly to monomers.

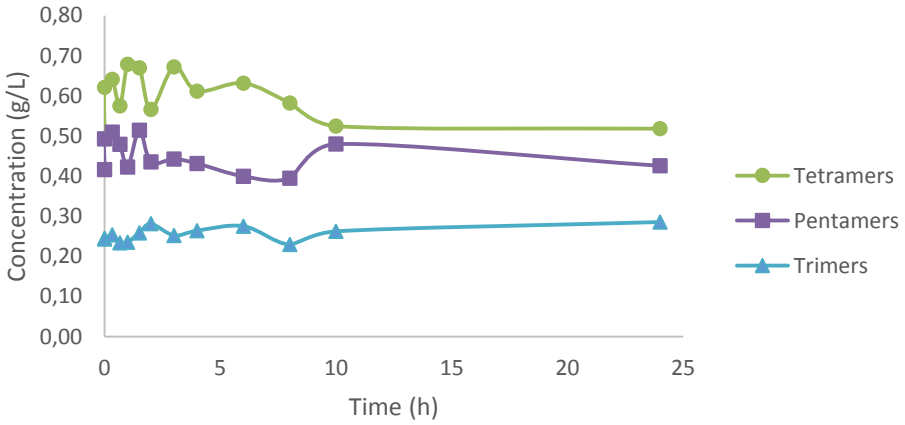


Figure 89-The tri, tetra and pentamers concentration as a function of time for Stone Pine extract hydrolysis catalyzed by Smopex-101 (T=95ºC, pH=1)

The trimers, tetramers and pentamers were slightly degraded in the first few hours of reaction but then the concentration remained constant. The trimer concentration was lower than for the tetra and pentamers.

For the experiment at pH 1,5 the monomer results are the following ones:

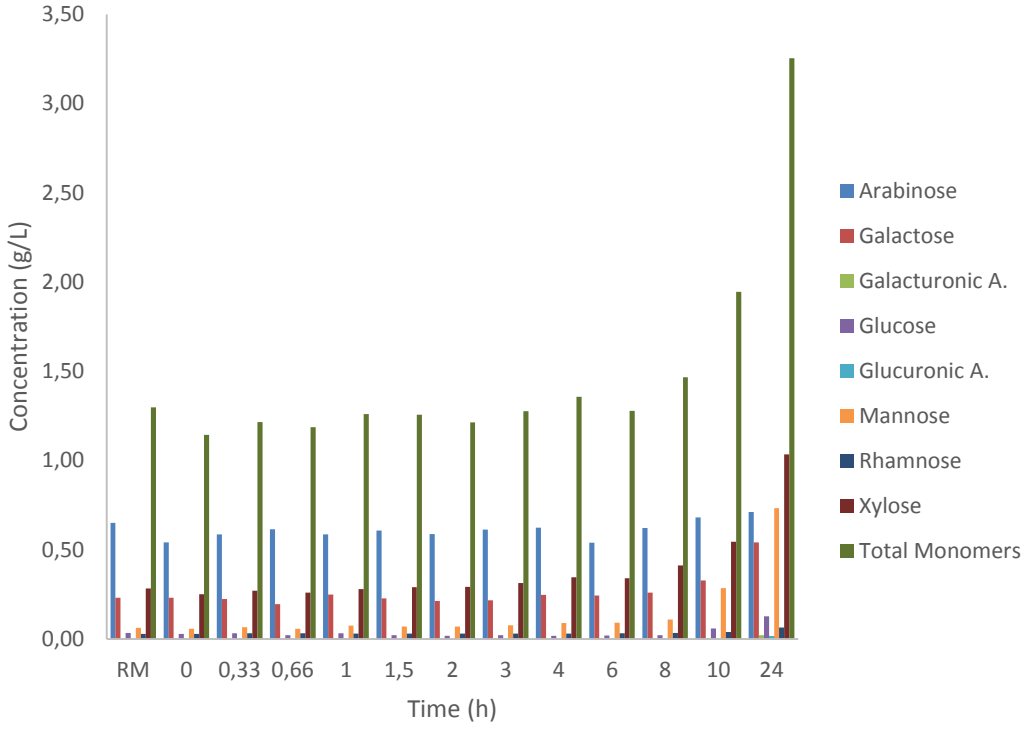


Figure 90- The monomer concentration as a function of time for Stone Pine extract hydrolysis catalyzed by Smopex-101 (T=95ºC, pH=1,5)

At pH 1,5, the trend is very similar to pH 1, the concentration increased during the whole 24 hours of reaction and no limiting maximum was reached, however, the concentrations achieved were lower than at pH 1.

The oligomer concentrations are depicted in Figure 91:

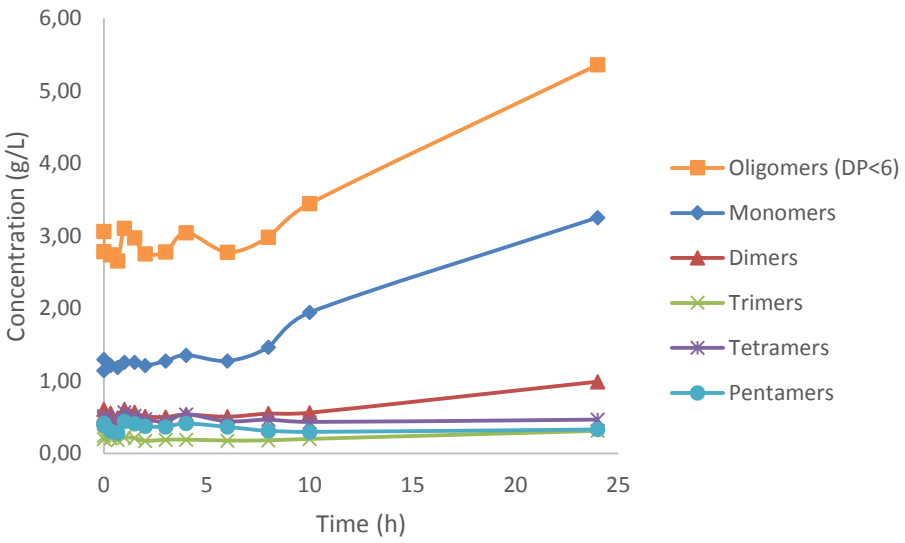


Figure 91-The oligomer (DP<6) concentration as a function of time for Stone Pine extract hydrolysis catalyzed by Smopex-101 (T=95ºC, pH=1,5)

It can be observed in Figure 91 that the monomer concentration increased even after 24 hours of reaction. The concentration of the oligomers (DP<6) was rather constant during the reaction.

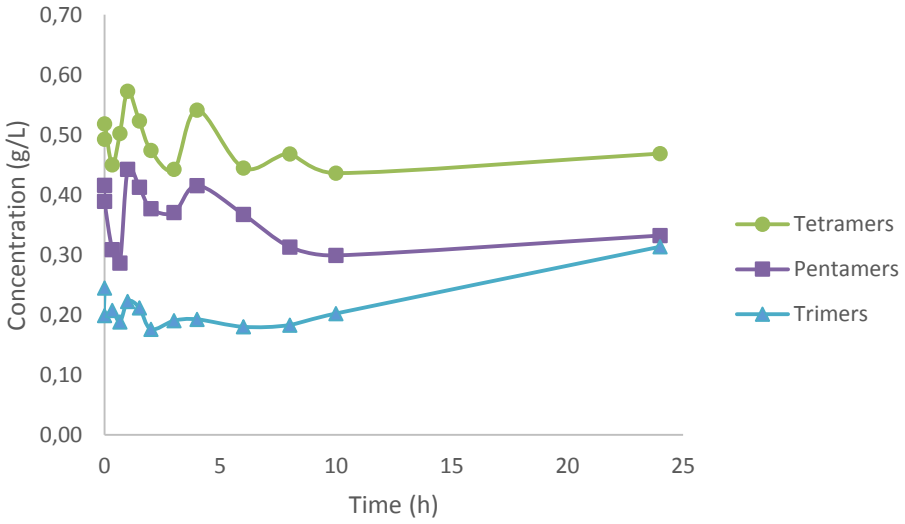


Figure 92-The tri, tetra and pentamers concentration as a function of time for Stone Pine extract hydrolysis catalyzed by Smopex-101 (T=95ºC, pH=1,5)

The trimers, tetramers and pentamers were not degraded, the concentrations remained constant during the reaction. Their concentrations are significantly high. The observed increase in the final stages of reaction is most likely due to the formation of polymeric compounds from the sugar degradation products. The yields for the reaction at pH 1 and pH 1,5 are displayed in Figure 93 and Figure 94:

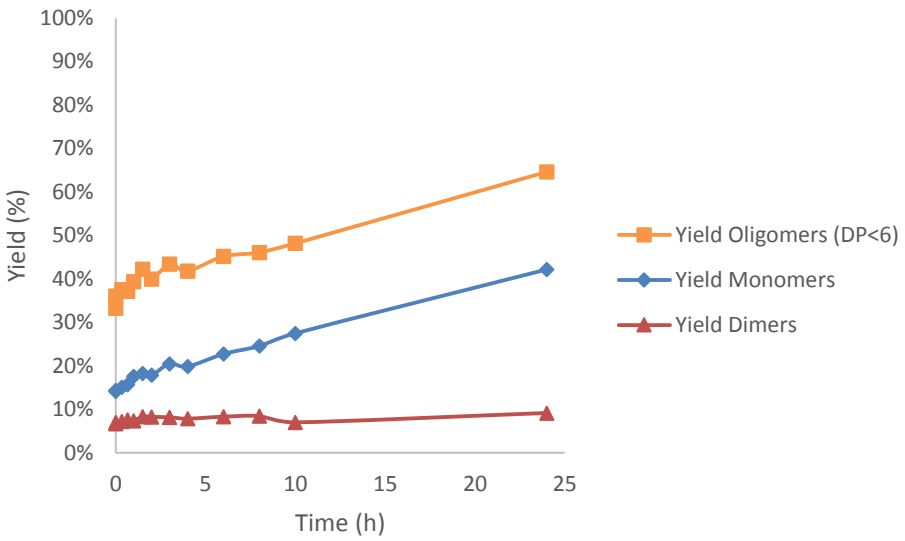


Figure 93-The yield of Stone Stone Pine hemicelluloses to oligomers (DP<6), monomers and dimers as a function of time in a hydrolysis catalyzed by Smopex-101 (T=95ºC, pH=1)

It can be observed in Figure 93 that the yield towards oligomers (DP<6) increased due to the formation of monomers, of nearly 40%. The concentration of the rest of sugars was not increased.

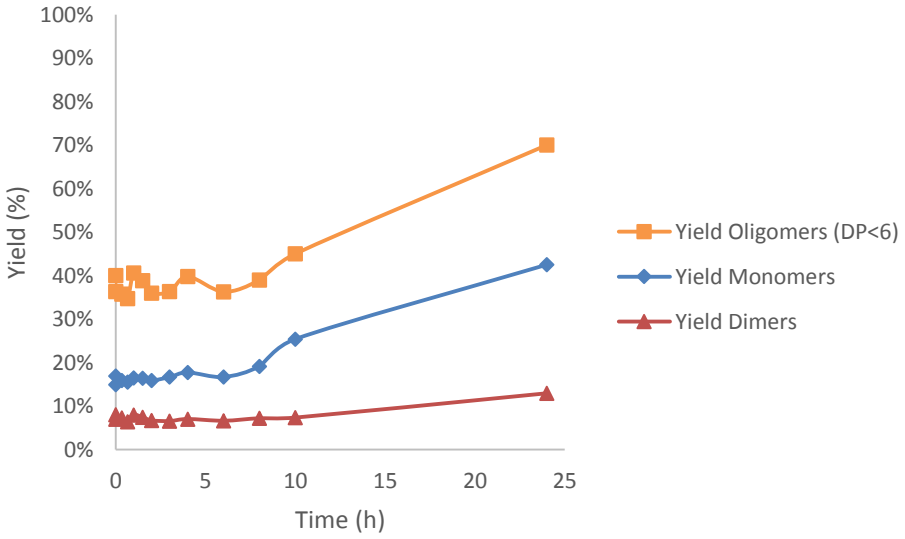


Figure 94-The yield of Stone Pine hemicelluloses to oligomers (DP<6), monomers and dimers as a function of time in a hydrolysis catalyzed by Smopex-101 (T=95ºC, pH=1,5)

The yields at pH 1,5 (Figure 94) were similar to the ones obtained at pH 0,5, the yield towards monomers increased steadily while concentration of the rest of the sugars remained stable. The results from the three experiments performed with Stone Pine at 95 ºC at different pH are presented in Figure 95Figure 97:

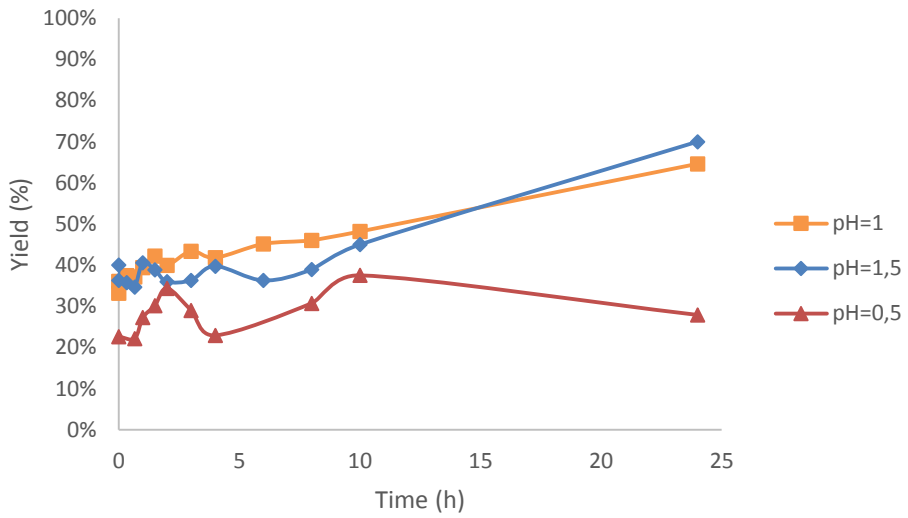


Figure 95-The influence of pH on the yield of Stone Pine hemicelluloses to oligomers (DP<6) as a function of time in a hydrolysis catalyzed by Smopex-101 (T=95ºC)

It is evident from the Figure 93Figure 95 that low pH (0,5) lead to severe degradation. The results obtained at pH 1 and pH 1,5 are rather similar. This indicates that over pH 1,5 could be enough for the hydrolysis without risking the degradation of the sugars.

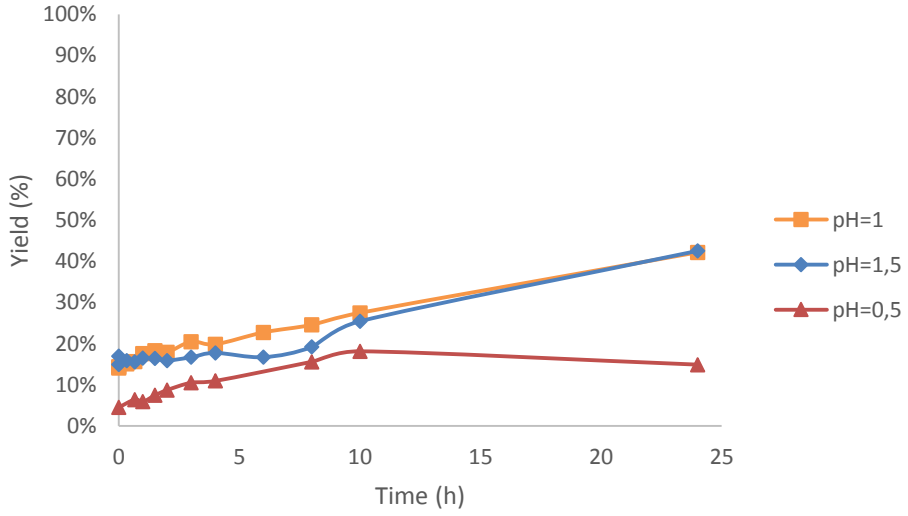


Figure 96-The influence of pH on the yield of Stone Pine hemicelluloses to monomers as a function of time in a hydrolysis catalyzed by Smopex-101 (T=95ºC)

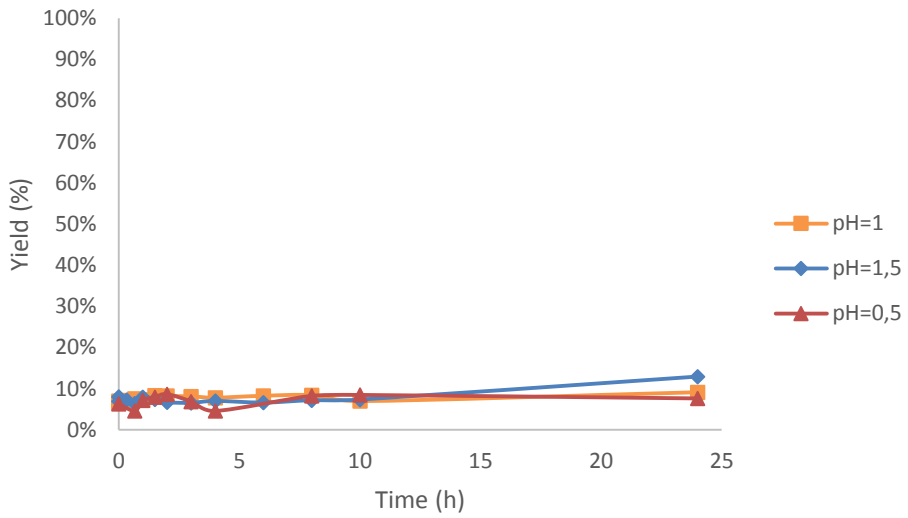


Figure 97-The influence of pH on the yield of Stone Pine hemicelluloses to dimers as a function of time in a hydrolysis catalyzed by Smopex-101 (T=95ºC)

As seen in Figure 97, the concentration of dimers stayed very constant during all of the experiments. This could indicate the presence of some very stable dimers in the solution.

4. CONCLUSIONS

Hemicelluloses extracted from Stone Pine and Holm Oak were hydrolysed in an isothermal batch reactor in the current study. Two different catalysts, HCl (homogeneous) and Smopex-101 (heterogeneous), were used in the experiments.

The different performance of the homogeneous and heterogeneous catalyst influenced strongly the degradation of the sugars and the kinetics of the hydrolysis. Also the product distribution obtained in the hydrolysis varied. The maximum yield towards oligomers (DP<6) was slightly higher with Smopex-101 (100% against 95%), however, the reaction was slower. Significant degradation was observed with both catalysts. The main difference in the hydrolysis was that with HCl, mainly monomers were formed while with Smopex-101 the concentration of monomers and dimers was almost equal. Both catalyst performed well in the hydrolysis of Holm Oak hemicelluloses but HCl showed higher selectivity toward monomers, while Smopex-101 produced also dimers. The yields towards oligomers (DP<6) was lower for Stone Pine extracts than for Holm Oak. The rapid kinetics obtained with HCl were counterweighed by the degradation, which is why as high yields were not obtained as with Smopex-101.

The temperature was concluded to be a crucial factor in the hydrolysis of hemicelluloses. In experiments performed at pH 0,5 (Smopex-101) with Holm Oak extracts, after 24 hours of reaction at 75ºC the reaction had barely taken place while at 85ºC the yield towards oligomers (DP<6) was around 80% and still increasing, with no observable degradation. At 95ºC the reaction reached a maximum close to 100% after 10 hours of experiment but severe degradation was observed after 24 hours. In each experiment, the concentrations of monomers and dimers was almost equal, indicating that the dimers are rather resistent against the hydrolysis with Smopex-101.

In the experiments performed with Stone Pine extracts (Smopex-101) at pH 0,5 the reaction proved to be even more temperature sensitive compared to Holm Oak. At 75ºC, no reaction took place, while at 85ºC a yield of nearly 95% was achieved. At 95ºC, the yields were very low because of severe degradation of the sugars. The balance between hydrolysis and degradation of the sugars proved to be much more delicate with Stone Pine compared to Holm Oak. The hydrolysis of Stone Pine also produced a mixture of monomers and dimers.

The pH played a crucial role in the hydrolysis of hemicelluloses. In the experiments performed at 95ºC with Holm Oak extracts, the degradation of the sugars proved to be strongly influenced by the pH. The yields towards oligomers (DP<6) was very high in each experiment, over 90%, but at low pH (0,5 and 1) severe degradation occurred, while at pH 1,5 the sugars were not significantly degraded. These results indicate that the hydrolysis of Holm Oak hemicelluloses can be optimized, the temperature being the main factor controlling the hydrolysis kinetics while the pH mainly influences the degradation.

In the experiments performed with Stone Pine extracts at 95ºC the degradation of the sugars proved to be strongly influenced by the pH. The yield towards oligomers (DP<6) was low and the kinetics rather slow at every pH. The hydrolysis of the Stone Pine extracts required a longer time than for the Holm Oak extracts. At pH 0,5, severe degradation occurred, but at pH 1 and 1,5 the degradation was mostly avoided. Moreover, the results showed no formation or degradation of dimers, which

indicates that very stable dimers were present in the solution. The kinetics of the acid hydrolysis of Stone Pine hemicelluloses with Smopex-101 proved to be rather slow and the reaction would require more than 24 hours to reach very high yields. The product of the hydrolysis was a mixture of different oligomers (DP<6), and, as described previously for the Holm Oak extracts, Smopex-101 can not be recommended for the production of highly concentrated monomer solutions.

The hydrolysis of the two different wood species revealed important differences. The experimental conditions for Holm Oak proved to be easier to optimize and the yields were higher than for Stone Pine extracts.

In several experiments, an increase in the trimer, tetramer and pentamer concentration was observed in the final stages of the reaction, while the sugar concentration decreased due to degradation of the sugars. The observed formation of tetra-pentamers was most likely due to some polymerization of the degradation products, possibly with the monomers, dimers and trimers present in the solution.

In some experiments, the concentration of the oligomers (DP<6) remained rather constant during the whole reaction. These results could indicate that the reaction mechanism of the hydrolysis of hemicelluloses proceeds via consecutive reactions in which the oligomers (DP<6) are intermediates as well as via an end biting mechanism where the polymers are hydrolysed directly to monomers.

The current study showed that the experimental work and the analysis of the sugars and oligomers is a challenging and time consuming task, however, by following precise laboratory practice, accurate results were obtained.

REFERENCES

[1] Tao Song, Andrey Pranovich, Bjarne Holmbom, "Hot-Water Extraction of ground Norway Spruce wood of different particle size," vol. 7, no. 3, pp. 4214-4225, 2012.

[2] Jens Krogell, Ekaterina Korotkova, Kari Eränen, Andrey Pranovich, Tapio Salmi, Dmitry Murzin, Stefan Willför, "Intensification of hemicellulose hot-water extraction from Norway Spruce wood," *Bioresource Technology,* no. 143, pp. 212-220, September 2013.

[3] Juben N. Chheda, George W. Huber, James A. Dumesic, "Liquid-Phase Catalytic Processing of Biomass-Derived Oxygenated Hydrocarbons to Fuels and Chemicals," *Angewandte Chemie,* no. 46, pp. 7164-7183, 2007.

[4] Tapio Salmi, "Groundbreaking Research in Biomass," *Public Service Review: Europe,* no. Issue 24.

[5] Jussi V. Rissanen, Henrik Grénman, Stefan Willför, Dmitry Yu. Murzin, Tapio Salmi, "Norway Spruce Hemicellulose for Chemicals Using Aqueous Extraction: Kinetics, Mass Transfer, and Modeling," *Ind. Eng. Chem. Res,* vol. 53, no. 14, pp. 6341-6350, 21 March 2014.

[6] Päivi Mäki-Arvela, Tapio Salmi, Bjarne Holmbom, Stefan Willför, Dmitry Yu. Murzin, "Synthesis of Sugars by Hydrolysis of Hemicelluloses-A review," *Chemical Reviews,* vol. 111, no. 9, pp. 5638-5666, 20 June 2011.

[7] M. J. González-Muñoz, Rosana Alvarez, Valentín Santos, J. C. Parajó, "Production of hemicellulosic sugars from Pinus pinaster wood by sequential steps of aqueous extraction and acid hydrolysis," *Wood Science and Technology,* vol. 46, pp. 271-285, January 2012.

[8] Henrik Grénman, Kari Eränen, Jens Krogell, Stefan Willför, Tapio Salmi, Dmitry Yu. Murzin, "Kinetics of Aqueous Extraction of Hemicelluloses from Norway Spruce in an Intensified Reactor system," *Ind. Eng. Chem. Res.,* vol. 50, no. 7, pp. 3818-3828, 3 March 2011.

[9] Rivas S., González-Muñoz MJ., Santos V., Parajó JC., "Acidic processing of hemicellulosic saccharides from Stone Pine wood: product distribution and kinetic modeling.," *Bioresource Technology,* vol. 9, pp. 162-192, June 2014.

[10] Jens Krogell, Kari Eränen, Kim Granholm, Andrey Pranovich, Stefan Willför, "High-temperature pH measuring during hot-water extraction of hemicelluloses from wood," *Industrial Crops and Products,* vol. 61, pp. 9-15, November 2014.

[11] Jussi V. Rissanen, Henrik Grénman, Chunlin Xu, Stefan Willför, Dmitry Yu Murzin, Tapio Salmi, "Obtaining Norway Spruce hemicelluloses of desired molar mass by using pressurized hot water extraction.," *ChemSusChem,* vol. 7, no. 10, pp. 2947-2953, October 2014.

[12] Nuwan Sella Kapu, Heather L. Trajano, "Review of hemicellulose hydrolysis in softwoods and bamboo," *Biofuels, Bioproducts & Biorefining,* vol. 8, pp. 857-870, 1 September 2014.

[13] Tapio Salmi, Dmitry Yu. Murzin, Päivi Mäki-Arvela, Bright Kusema, Bjarne Holmbom, Stefan Willför, Johan Wärnå, "Kinetic modeling of hemicellulose hydrolysis in the presence of homogeneous and heterogeneous catalysts," *Issue AIChE Journal,* vol. 60, no. 3, pp. 1066-1077, March 2014.

[14] Léa Vilcoq, Paula C. Castilho, Florblea Carvalheiro, Luís C. Duarte, "Hydrolysis of Oligosaccharides over solid acid catalysts: A review," *ChemSusChem,* vol. 7, no. 4, pp. 1010-1019, 2014.

[15] Piera Demma Carà, Mario Pagliaro, Ahmed Elmekawy, David. R. Brown, Peter Verschuren, N. Raveendran Shiju, Gadi Rothenberg, "Hemicelulose hydrolysis catalyzed by solid acids.," *Catalysis Science & Technology Issue 8, 2013,* vol. 3, no. 8, pp. 2057-2061, April 2013.

[16] Fengel,D. Wegener, G. Heizmann, A. Przyklenk, M., Cell. Chem. Technol., vol. 12, 1978, pp. 31-37.

[17] Fengel,D. Wegener, G, Wood Chemistry, Ultrastructure, Reactions, Walter de Gruyter, 1989, pp. 106-131.

[18] Eero Sjöström, Wood Chemistry: Fundamentals and Applications, London: Academic Press Inc., 1981.

[19] "RSC.org," [Online]. Available: http://www.rsc.org/Education/Teachers/Resources/cfb/carbohydrates.htm. [Accessed 14 04 2015].

[20] Roberts, J. C., The Chemistry of Paper, Cambridge: Royal Society of Chemistry, 1996.

[21] Bungay, Henry R., Energy, the biomass options, New York: John Wiley & Sons, 1981.

[22] Dieter Klemm, Brigitte Heublein, Hans-Peter Fink, Andreas Bohn., "Cellulose: Fascinating Biopolymer and Sustainable Raw Material," *Angewandte Chemie International Edition,* vol. 44, no. 22, pp. 3358-3393, May 2005.

[23] Fan, L.T., Gharpuray, M.M., Lee, Y, "Hydrolysis Hydrolysis," *Biotechnology Monographs,* p. 57, 1987.

[24] M. Lewin, J.S. Goldstein, Wood structure and composition, "Wood structure and composition," *International Fiber Science and Technology,* vol. 334, no. 8, p. 729, 1991.

[25] Aspinall, G.O, General introduction; in: The Polysaccharides, vol. 1, Academic Press, 1982, pp. 1-18.

[26] Wilkie, K. C. B., Adv. Carbohyd. Chem. Biochem., 1979, pp. 36-215.

[27] Casey, J. P., Pulp and Paper: Chemistry and Chemical Technology, New York: John Wiley & Sons., 1980.

[28] Westerlund E, Andersson R, Åman P., "Isolation and chemical characterization of water-soluble mixed-linked β-glucans and arabinoxylans in oat milling," *Carbohydr Polymers,* vol. 20, no. 2, pp. 115-123, 1993.

[29] Kiefer LL, York WS, Darvill AG, Albersheim P, "Xyloglucan isolated from suspension-cultured sycamore cell walls is O-acetylated," *Phytochemistry,* vol. 28, pp. 2105-2107, (1989).

[30] P. Hakkila, Utilisation of Residual Forest Biomass, Berlin: Springer-Verlag, 1989.

[31] Liyama, K., Lam, T. B. T., Stone, B. A., "Phenolic acid bridges between polysaccharides and lignin in wheat internodes," *Phytochemistry,* vol. 29, pp. 733-737, 1990.

[32] "www.wood-database.com," [Online]. Available: http://www.wood-database.com/wood-articles/what-is-wood/. [Accessed 15 April 2015].

[33] "paperonweb.com," [Online]. Available: http://www.paperonweb.com/wood.htm. [Accessed 16 April 2015].

[34] S. Willför, A. Sundberg, J. Hemming, B. Holmbom, "Polysaccharides in some industrially important softwood species," *Wood Science Technology,* vol. 39, pp. 245-258, 2005.

[35] Kagawa University, Milki, Japan, "google.com/site/raresugars," [Online]. Available: https://sites.google.com/site/raresugars/. [Accessed 24 April 2015].

[36] S. Dimitriu, Polysaccharides: Structural Diversity and Functional Versatility, 2 ed., New York: Marcel dekker, 2005, pp. 893-937.

[37] M. Auer, A.Sundberg, Wood and Paper Chemistry, Abo: Abo Akademi, 2012.

[38] Abatzoglou N, Chornet E, "Polysaccharides," no. 27, p. 1007–1045, 1998.

[39] Bright T. Kusema, Gerd Hilmann, Païvi Mäki-Arvela, Stefan Willför, Bjarne Holmbom, Tapio Salmi, Dmitry Yu. Murzin, "Selective Hydrolysis of Arabinogalactan into Arabinose," *Catal Lett,* no. 141, p. 408–412, 2011.

[40] B. Kusema, T.Tönnov, P. Mäki-Arvela, T. Salmi, S. Willför, B. Holmbom, D. Yu. Murzin., "Acid Hydrolysis of O-acetyl-galactoglucomannan," *Catalysis Science & Technology,* 2012.

[41] James H. Clark, "Catalysis for green chemistry," *Pure Appl. Chem,* vol. 73, no. 1, pp. 103-111, 2001.

[42] J. R. H. Ross., Heterogeneous catalysis. Fundamentals and applications, 1 ed., Oxford: Elsevier, 2012.

[43] J. Haber., "Manual on catalyst characterization," *Pure & Applied Chemistry,* vol. 63, no. 9, pp. 1227-1246, 1991.

[44] R. Kunin., Ion Exchange Resins, 2 ed., Philadelphia: John Wiley and Sons, 1958.

[45] Bruce C. Gates, "chms.ucdavis.edu," [Online]. Available: https://chms.ucdavis.edu/research/web/catalysis/PDF%20Pubs/Gates%20Pub%20-%20420.pdf. [Accessed 2015 April 20].

[46] Gale, Rajiv, Shahu and M. C. Payne J.D., "Acid-Base Catalysis in Zeolites from First Principles," *International Journal of Quantum Chemistry,* vol. 61, pp. 393-398, 1997.

[47] Sheffield Hallam University, "teaching.shu.ac.uk," 16 April 2015. [Online]. Available: http://teaching.shu.ac.uk/hwb/chemistry/tutorials/chrom/chrom1.htm.

[48] L. S. Ettre., "Nomenclature for Chromatography," *Pure & Applied Chemistry,* vol. 65, no. 4, pp. 819-872, 1993.

[49] Rebecca Carrier, Julie Bordonaro, "rpi.edu," 1994. [Online]. Available: http://www.rpi.edu/dept/chem-eng/Biotech-Environ/CHROMO/chromequip.html. [Accessed 16 April 2015].

[50] Jim Clark, "chemguide.co.uk," 2007. [Online]. Available: http://www.chemguide.co.uk/analysis/chromatography/gas.html. [Accessed 6 April 2015].

[51] E. Sjölstrom, R. Alén, Analytical Methods in Wood Chemistry, Pulping & Papermaking, Helsinki, Jyväskylä: Springer, 1998.

[52] J. Buchert, M. Siika-aho, M. Bailey, J. Puls, A. Valkeajärvi, J. Pere, L. Viikari, "Quantitative determination of wood-derived soluble oligosaccharides by HPLC," *Biotechnology Techniques,* vol. 7, no. 11, pp. 785-790, November 1993.

[53] Anna Sundberg, Kenneth Sundberg, Camilla Lillandt, Bjarne Holmbom, Abo Akademi University, "Determination of hemicelluloses and pectins in wood and pulp fibers by acid methanolysis and gas chromatography," *Nordic Pulp and Paper Research Journal no.4,* pp. 216-219, 1996.

[54] B. L. Browning, Methods of Wood Chemistry, New York: JohnWiley & Sons, 1967, pp. 589-590.

[55] Saeman, J., Wayne, M., Raymond, M. and Milett, M., Tappi, 1954, pp. 336-343.

[56] Saad Tayyab, Seema Qamar, Mozaffarul Islam, "Size exclusion chromatography and size exclusion HPLC of proteins," *Biochemical Education,* vol. 19, no. 3, p. 149–152, July 1991.

[57] Skoog, D. A.; Holler, F. J.; Crouch, S. R., Principles of Instrumental Analysis, Cole: Thomson Brooks, 2007.

[58] S. Aldrich, "sigmaaldrich.com," [Online]. Available: http://www.sigmaaldrich.com/analytical-chromatography/analytical-reagents/derivatization-reagents/silylation.html. [Accessed 16 April 2015].

[59] J. Gullichsen, H. Paulapuro, Forest Products Chemistry, Helsinki: Fapet Oy, 2000.

[60] Boer, Desty, D. H, Vapour phase Chromatography, London, UK: Butterworths Sci. Pub, 1957.

APPENDIX

I Analysis description

A. Monomer Analysis Procedure

The necessary steps of the Monomer analysis are divided in three working days:

Day 1: Sample preparation

-Transfer the liquid sample to a test tube in order to have between 0,8 mg and 1,2 mg of sugars. The amount of liquid is determined for the concentration of the sample. The goal is to have enough amount of sugars, so the peaks in the chromatograms will be big enough.

-Freeze the samples.

-Open the screw cup enough to let the vapor out and allow the sample to dry in the freeze-dryer during 24 h.

Day 2: Transformation of the samples-Silylation

Preparation of the calibration samples

-Add in three test tubes

- 1 mL of calibration solution of pure monosaccharides (0,1 mg/mL of each polysaccharide; arabinose, xylose, rhamnose, glucose, mannose, galactose, glucuronic acid and galacturonic acid, all solved in MeOH).
- 1 mL of sucrose (0,1 mg/mL in MeOH).

Internal standard solution

- Add 1 mL of xylitol (0,1 mg/mL in MeOH) to the samples as well as the calibration samples).

-Cap and shake the flasks in the vortex and immerse them one by one in an ultrasonic bath.

-Evaporate to dryness under nitrogen gas in purpose built sand placed on a hot plate at 50ºC. (Approx. 45 min)

-The samples are further dried in a vacuum desiccator until they are nearly dry. (Approx. 15 min)

Silylation process

-Add 150 µL of Pyridine, 150 µL of HDMS and 70 µL of TMCS.

-Cap and shake the flasks in a vortex and immerse them one by one in an ultrasonic bath. Allow time for samples to dissolve.

-Let the samples stay for a while and shake them again. This helps to break the ammonium chloride flocks by placing the sample flasks in an ultrasonic bath. After that, the content of the flasks should look like a fairly homogeneous sludge. (To ensure if enough reagents are

added, I can be checked by opening the screw caps and look for smoke. If there is no smoke, more silylation reagents can be added).

-Let the flask stay overnight at room temperature.

Day 3: GC Analysis of the samples

-Prepare the GC vials by labeling them.

-The silylated samples are transferred to GC auto sampler vials using clean Pasteur pipettes. Try to avoid transferring any precipitate to prevent clogging the injection needle of the gas chromatograph.

-Analyze by GC.

For the monomer analysis, GC-5 is used. The characteristics of this GC are: GC-FI detector, which is a PerkinElmer AutoSystemXL. The used column is 25 m x 0,2 mm and coated with cross-linked methyl polysiloxane (HP-1). The heating process of the column oven is as follows: starting at 100 ºC raised at 4 ºC/min to 170 ºC, then 12 ºC/min to 300 ºC and hold this final temperature for 7 minutes. The used carrier gas was H2 with a flow rate of 45 mL/min. The split injector has a temperature of 250 ºC and the ratio is 1:15. A sample volume of 1 µL is injected in the GC.

B. Oligomer Analysis Procedure

The necessary steps of the Oligomers analysis are divided in three working days:

Day 1: Sample preparation

-Transfer the liquid sample to a test tube in order to have between 0,8 mg and 1,2 mg of sugars. The amount of liquid is determined for the concentration of the sample. The goal is to have enough amount of sugars, so the peaks in the chromatograms will be big enough.

-Freeze the samples.

-Open the screw cup enough to let the vapor out and allow the sample to dry in the freeze-dryer during 24 h.

Day 2: Transformation of the samples-Silylation

Preparation of the calibration samples

-Add in three test tubes

- 1 mL of xylitol (0,1 mg/mL in MeOH).
- 1 mL of sucrose (0,1 mg/mL in MeOH).

Internal standard solution

Different standards are added depending on the type of wood used in the experiments:

- Hardwood: Add 2 mL of cholesterol (0,02 mg/mL in MTBE) to the samples as well as the calibration samples.
- Softwood: Add 2 mL of betulinol (0,02 mg/mL in MTBE) to the samples as well as the calibration samples.

-Cap and shake the flasks in the vortex and immerse them one by one in an ultrasonic bath.

-Evaporate to dryness under nitrogen gas in purpose built sand placed on a hot plate at 50ºC. (Approx. 45 min)

-The samples are further dried in a vacuum desiccator until they are nearly dry. (Approx. 15 min)

Silylation process

-Add 150 µL of Pyridine, 150 µL of HDMS and 70 µL of TMCS.

-Cap and shake the flasks in a vortex and immerse them one by one in an ultrasonic bath. Allow time for samples to dissolve.

-Let the samples stay for a while and shake them again. This helps to break the ammonium chloride flocks by placing the sample flasks in an ultrasonic bath. After that, the content of the flasks should look like a fairly homogeneous sludge. (To ensure if enough reagents are

added, I can be checked by opening the screw caps and look for smoke. If there is no smoke, more silylation reagents can be added).

-Let the flask stay overnight at room temperature.

Preparation of a blank

-In the oligomer analysis it is important to use a blank in order to eliminate the undesired noise from the chromatograms. This blank is prepared by adding in an empty test tubes the same proportions of silylation reagents as in the samples.

Day 3: GC Analysis of the samples

-Prepare the GC vials by labeling them.

-The silylated samples are transferred to GC auto sampler vials using clean Pasteur pipettes. Try to avoid transferring any precipitate to prevent clogging the injection needle of the gas chromatograph.

-Analyze by GC.

For the monomer analysis, GC-1 is used. The characteristics of this GC are: GC-FI detector, which is a PerkinElmer Clarus 500. The used column is 6,2 m x 0,53 mm and coated with cross-linked methyl polysiloxane (HP-1). The heating process of the column oven is as follows: starting at 100 ºC raised at 12 ºC/min to 340 ºC, then hold this final temperature for 5 minutes. The used carrier gas was H2 with a flow rate of 7 mL/min. The column injector had a temperature of 80 ºC and sample volume of 0,5 µL was injected in the GC.

C. Total Sugar Analysis (Methanolysis) Procedure-Liquid Phase

The necessary steps of the Methanolysis analysis are divided in three working days:

Day 1: Sample preparation

-Transfer the liquid sample to a pressure resistant pear-shaped flask with a screw cap in order to have between 0,8 mg and 1,2 mg of sugars, close tightly. The amount of liquid is determined for the concentration of the sample. The goal is to have enough amount of sugars, so the peaks in the chromatograms will be big enough.

-The pear-shaped flasks must have no defects. The Teflon inner side of the cap should be new.

-Freeze the samples.

-Open the screw cup enough to let the vapor out and allow the sample to dry in the freeze-dryer during 24 h.

Day 2: Transformation of the samples-Silylation

-Take from the freezer the methanolysis reagent and allow reaching room temperature before open it to prevent air-moisture from condensing in this solution.

Preparation of the calibration samples

-Add 1 mL of a carbohydrate calibration solution (0,1 mg/mL in MeOH) in three empty pear-shaped flasks.

-Evaporate to dryness under nitrogen gas in purpose built sand placed on a hot plate at 50ºC. (Approx. 45 min).

-The samples are further dried in a vacuum desiccator until they are nearly dry. (Approx. 15 min)

Methanolysis

-Add about 2 mL of methanolysis reagent (water free 2 M HCl in MeOH) to the freeze-dried sample and the evaporated calibration mixture.

-The samples are shaken in the vortex and briefly treated in the ultrasonic bath for less than 1 minute.

-Put the samples in the oven at 100 ºC for 3 hours.

-Take the samples every 30 minute and shake them carefully. This step must be done under safety conditions (mask and gloves) because due to the internal pressure and heat inside the bottles they could explode.

-After 3 hours, take out the samples from the oven and allow them to reach room temperature to equalize the pressure.

-Open carefully the bottles because they will have some pressure remaining in the inside.

-Add 170 µL of pyridine to neutralize the excess of HCl.

-The samples are shaken in the vortex.

Internal standard solution

-Add 1 mL of sorbitol (0,1 mg/mL in MeOH) to the samples as well as the calibration samples).

-Add 1 mL of resorcinol (0,1 mg/mL in MeOH) to the samples as well as the calibration samples).

-Evaporate to dryness under nitrogen gas in purpose built sand placed on a hot plate at 50ºC. (Approx. 45 min)

-The samples are further dried in a vacuum desiccator until they are nearly dry. (Approx. 15 min)

Silylation process

-Add 150 µL of Pyridine, 150 µL of HDMS and 70 µL of TMCS.

-Cap and shake the flasks in a vortex and immerse them one by one in an ultrasonic bath. Allow time for samples to dissolve.

-Let the samples stay for a while and shake them again. This helps to break the ammonium chloride flocks by placing the sample flasks in an ultrasonic bath. After that, the content of the flasks should look like a fairly homogeneous sludge. (To ensure if enough reagents are added, I can be checked by opening the screw caps and look for smoke. If there is no smoke, more silylation reagents can be added).

-Let the flask stay overnight at room temperature.

Day 3: GC Analysis of the samples

-Prepare the GC vials by labeling them.

-The silylated samples are transferred to GC auto sampler vials using clean Pasteur pipettes. Try to avoid transferring any precipitate to prevent clogging the injection needle of the gas chromatograph.

-Analyze by GC.

For the methanolysis analysis, GC-5 is used. The characteristics of this GC are: GC-FI detector, which is a PerkinElmer AutoSystemXL. The used column is 25 m x 0,2 mm and coated with cross-linked methyl polysiloxane (HP-1). The heating process of the column oven is as follows: starting at 100 ºC raised at 2 ºC/min to 170 ºC, then 12 ºC/min to 300 ºC and hold this final temperature for 7 minutes.

The used carrier gas was H2 with a flow rate of 45 mL/min. The split injector has a temperature of 250 ºC and for the detectors 310 ºC. The ratio is 1:15. A sample volume of 1 µL is injected in the GC.

D. Total Sugar Analysis (Methanolysis) Procedure-Solid Phase

The necessary steps of the Methanolysis analysis of the solid samples are divided in four working days:

Day 0 : Preparation of the wood material

-Freeze-dry the solid material.

-Grind the dry wood material fine enough, almost powder like using a specific grinder machine.

Day 1: Sample preparation

-Weight approximately 10 mg (from 8 mg to 12 mg is acceptable) wood powder to the pear-shaped flasks.

-The pear-shaped flasks must have no defects. The Teflon inner side of the cap should be new.

-Freeze the samples.

-Open the screw cup enough to let the vapor out and allow the sample to dry in the freeze-dryer during 24 h.

Day 2: Transformation of the samples-Silylation

-Take from the freezer the methanolysis reagent and allow reaching room temperature before open it to prevent air-moisture from condensing in this solution.

Preparation of the calibration samples

-Add 1 mL of a carbohydrate calibration solution (0,1 mg/mL in MeOH) in three empty pear-shaped flasks.

-Evaporate to dryness under nitrogen gas in purpose built sand placed on a hot plate at 50ºC. (Approx. 45 min).

-The samples are further dried in a vacuum desiccator until they are nearly dry. (Approx. 15 min)

Methanolysis

-Add about 2 mL of methanolysis reagent (water free 2 M HCl in MeOH) to the freeze-dried sample and the evaporated calibration mixture.

-The samples are shaken in the vortex and briefly treated in the ultrasonic bath for less than 1 minute.

-Put the samples in the oven at 100 ºC for 5 hours.

-Take the samples every 30 minute and shake them carefully. This step must be done under safety conditions (mask and gloves) because due to the internal pressure and heat inside the bottles they could explode.

-After 35hours, take out the samples from the oven and allow them to reach room temperature to equalize the pressure.

-Open carefully the bottles because they will have some pressure remaining in the inside.

-Add 150 µL of pyridine to neutralize the excess of HCl.

-The samples are shaken in the vortex.

Internal standard solution

-Add 1 mL of sorbitol (0,1 mg/mL in MeOH) and 1 mL resorcinol to the calibration samples.

-Add 1 mL of sorbitol (0,1 mg/mL in MeOH) and 4 mL resorcinol to the rest of the samples.

-Mix and shake well.

-Transfer 1 mL of liquid phase to new pear-shaped bottles and evaporate to dryness under nitrogen gas in purpose built sand placed on a hot plate at 50ºC. (Approx. 45 min)

-The samples are further dried in a vacuum desiccator until they are nearly dry. (Approx. 15 min)

Silylation process

-Add 170 µL of Pyridine, 170 µL of HDMS and 85 µL of TMCS.

-Cap and shake the flasks in a vortex and immerse them one by one in an ultrasonic bath. Allow time for samples to dissolve.

-Let the samples stay for a while and shake them again. This helps to break the ammonium chloride flocks by placing the sample flasks in an ultrasonic bath. After that, the content of the flasks should look like a fairly homogeneous sludge. (To ensure if enough reagents are added, I can be checked by opening the screw caps and look for smoke. If there is no smoke, more silylation reagents can be added).

-Let the flask stay overnight at room temperature.

Day 3: GC Analysis of the samples

-Prepare the GC vials by labeling them.

-The silylated samples are transferred to GC auto sampler vials using clean Pasteur pipettes. Try to avoid transferring any precipitate to prevent clogging the injection needle of the gas chromatograph.

-Analyze by GC.

For the methanolysis analysis, GC-5 is used. The characteristics of this GC are: GC-FI detector, which is a PerkinElmer AutoSystemXL. The used column is 25 m x 0,2 mm and coated with cross-linked methyl polysiloxane (HP-1). The heating process of the column oven is as follows: starting at 100 ºC raised at 2 ºC/min to 170 ºC, then 12 ºC/min to 300 ºC and hold this final temperature for 7 minutes. The used carrier gas was H2 with a flow rate of 45 mL/min. The split injector has a temperature of 250 ºC and for the detectors 310 ºC. The ratio is 1:15. A sample volume of 1 µL is injected in the GC.

II Chromatograms

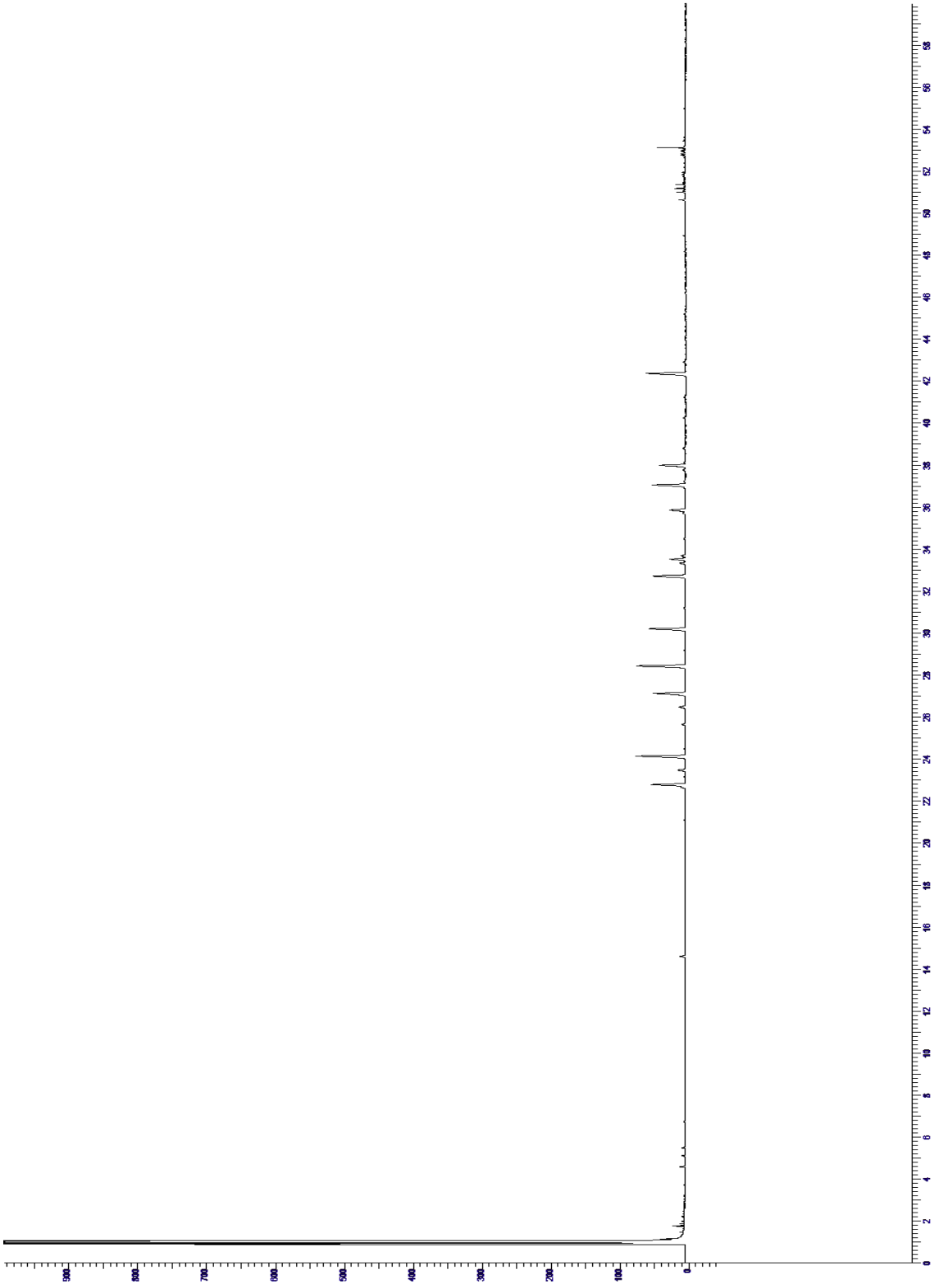


Figure 98-Monomer content chromatogram for holm oak

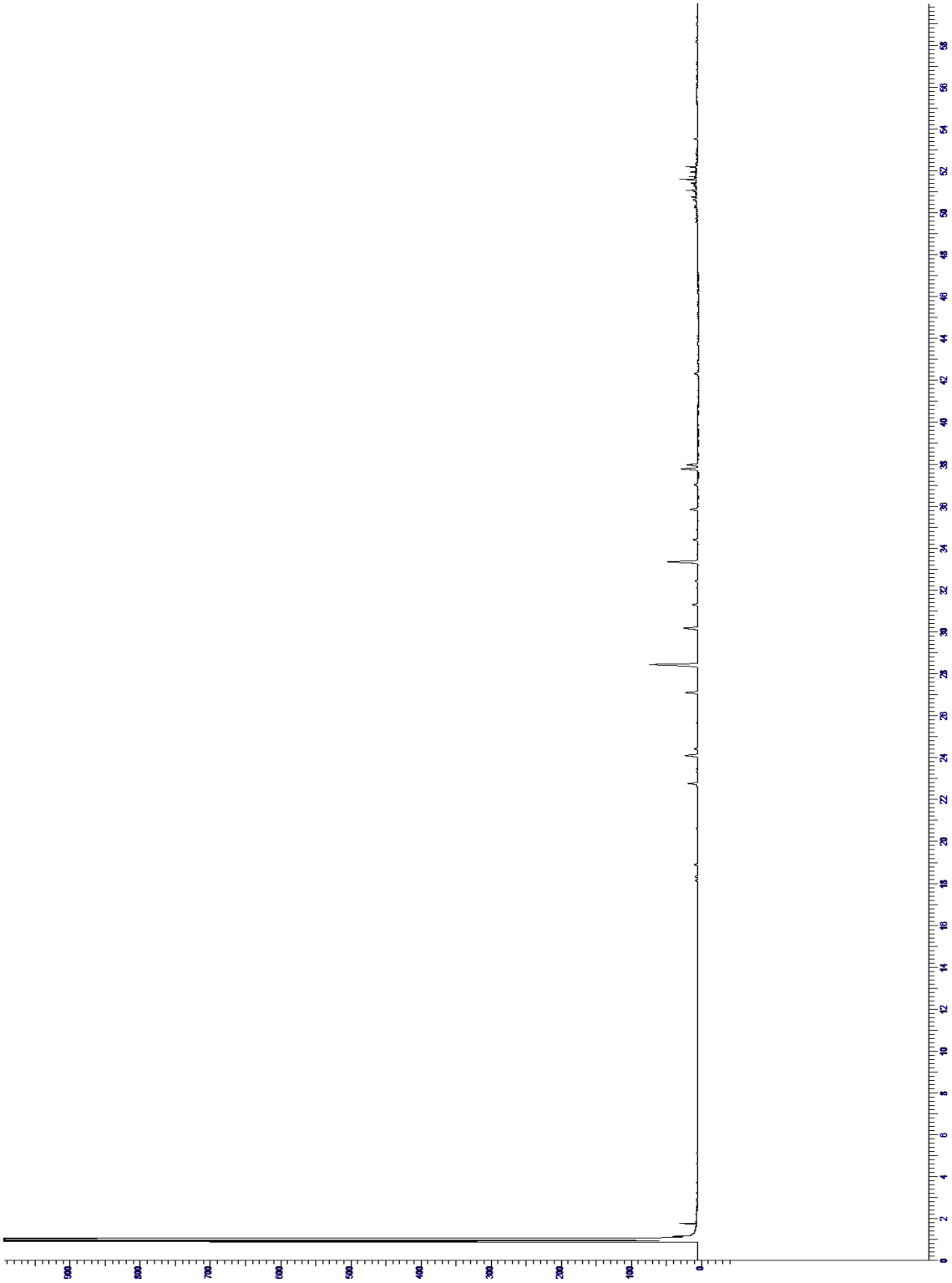


Figure 99- Monomer content chromatogram for Stone Pine

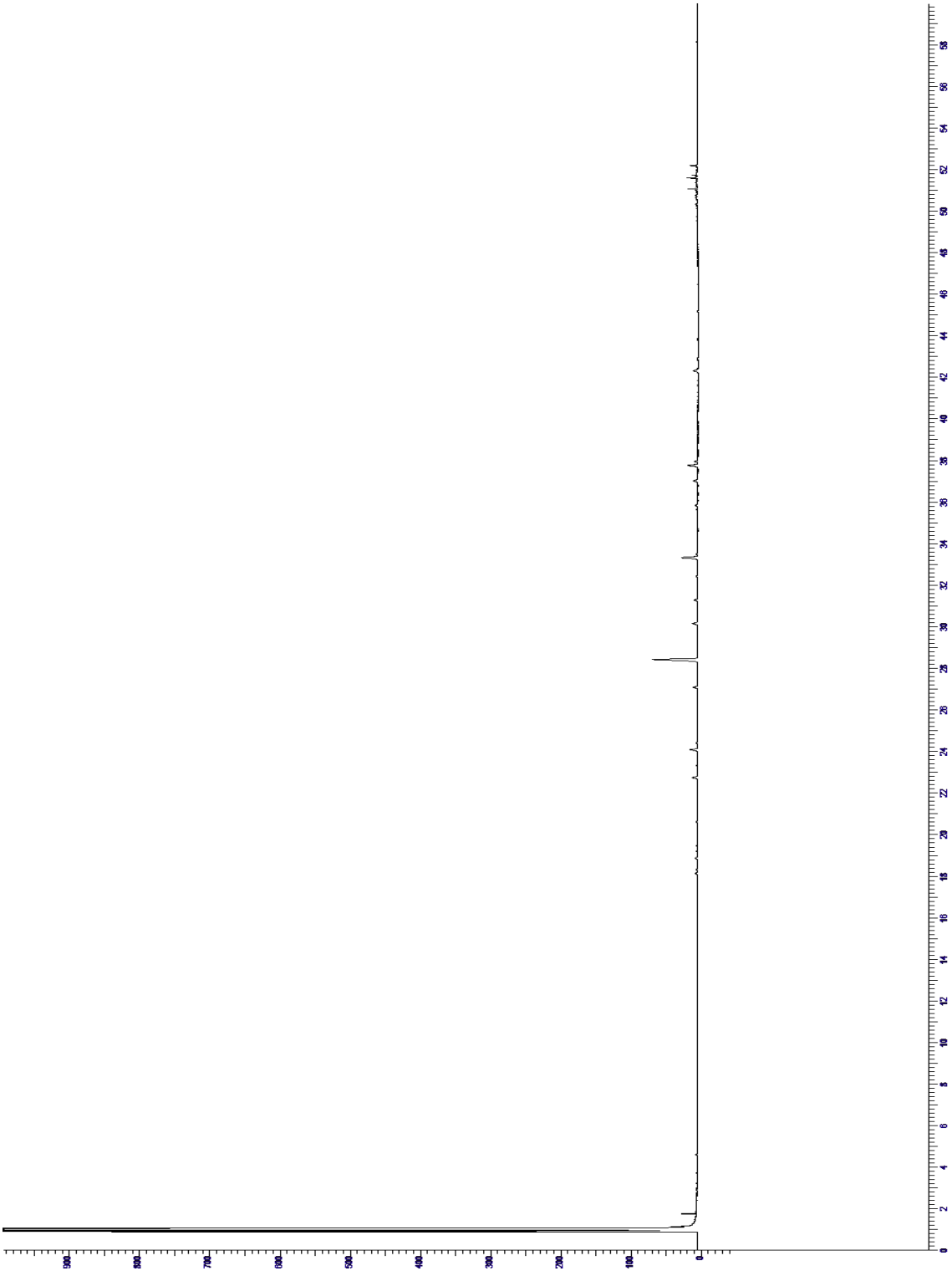


Figure 100-Monomer content chromatogram for Norway Spruce

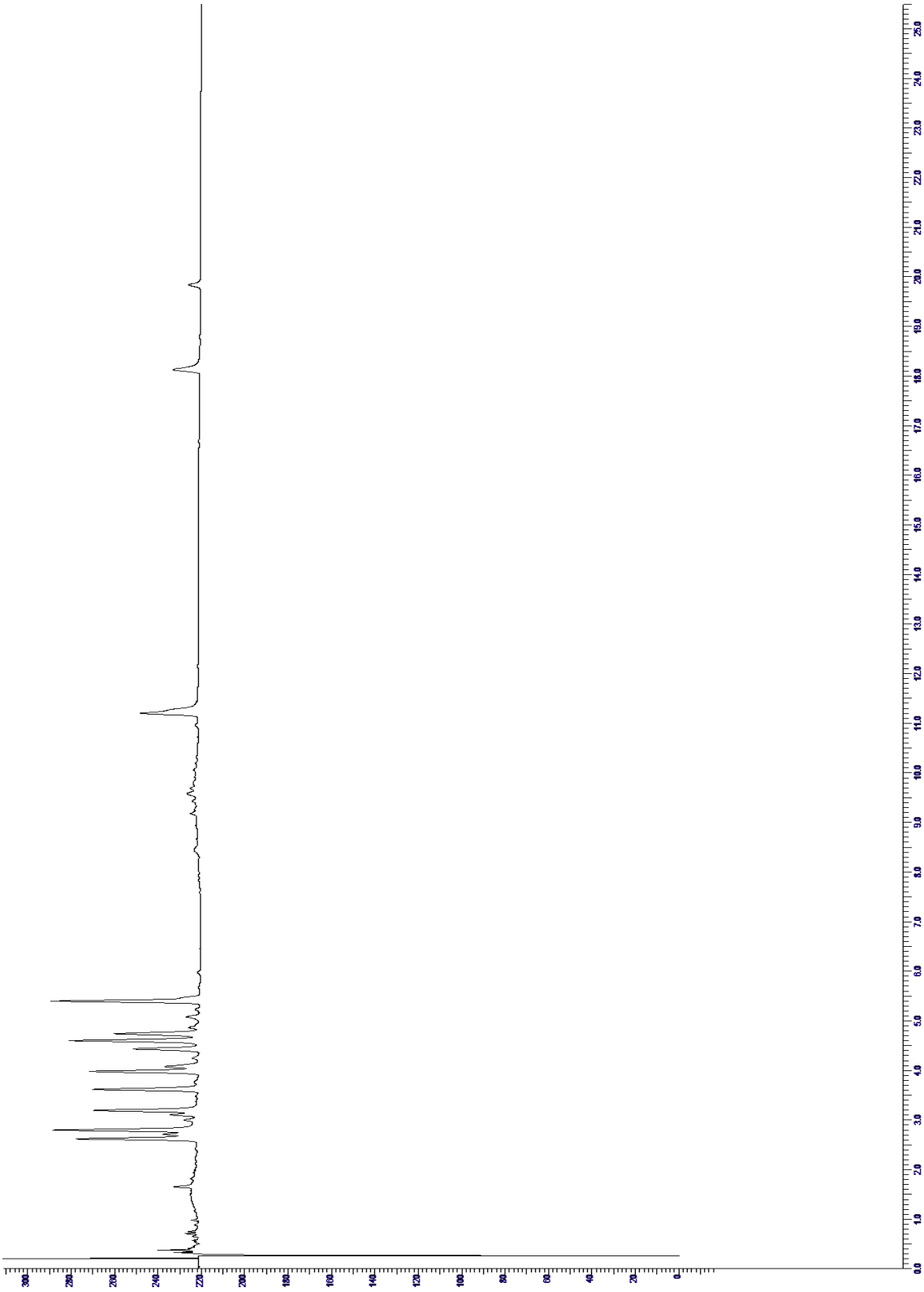


Figure 101- Oligomer content chromatogram for holm oak

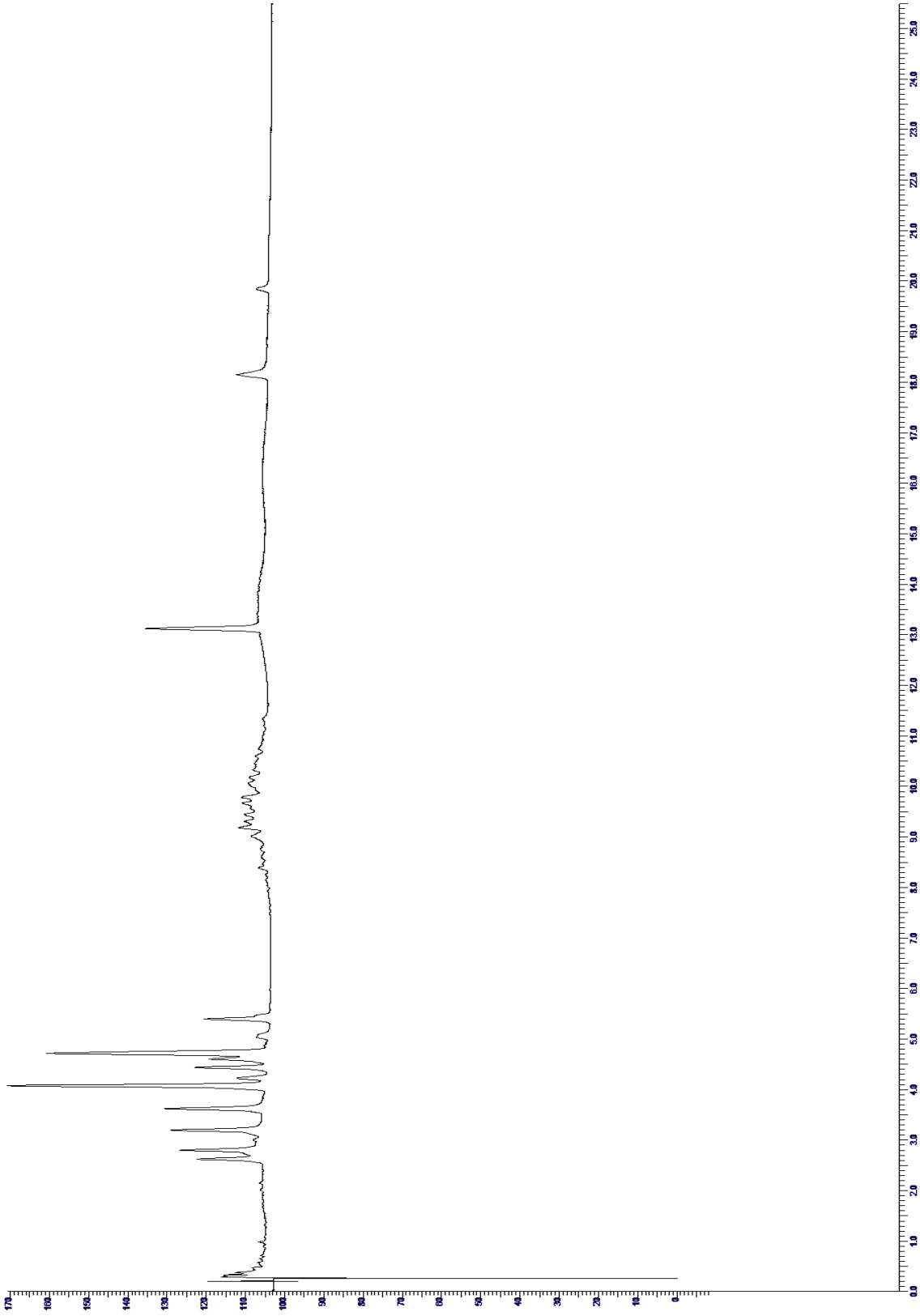


Figure 102- Oligomer content chromatogram for Stone Pine

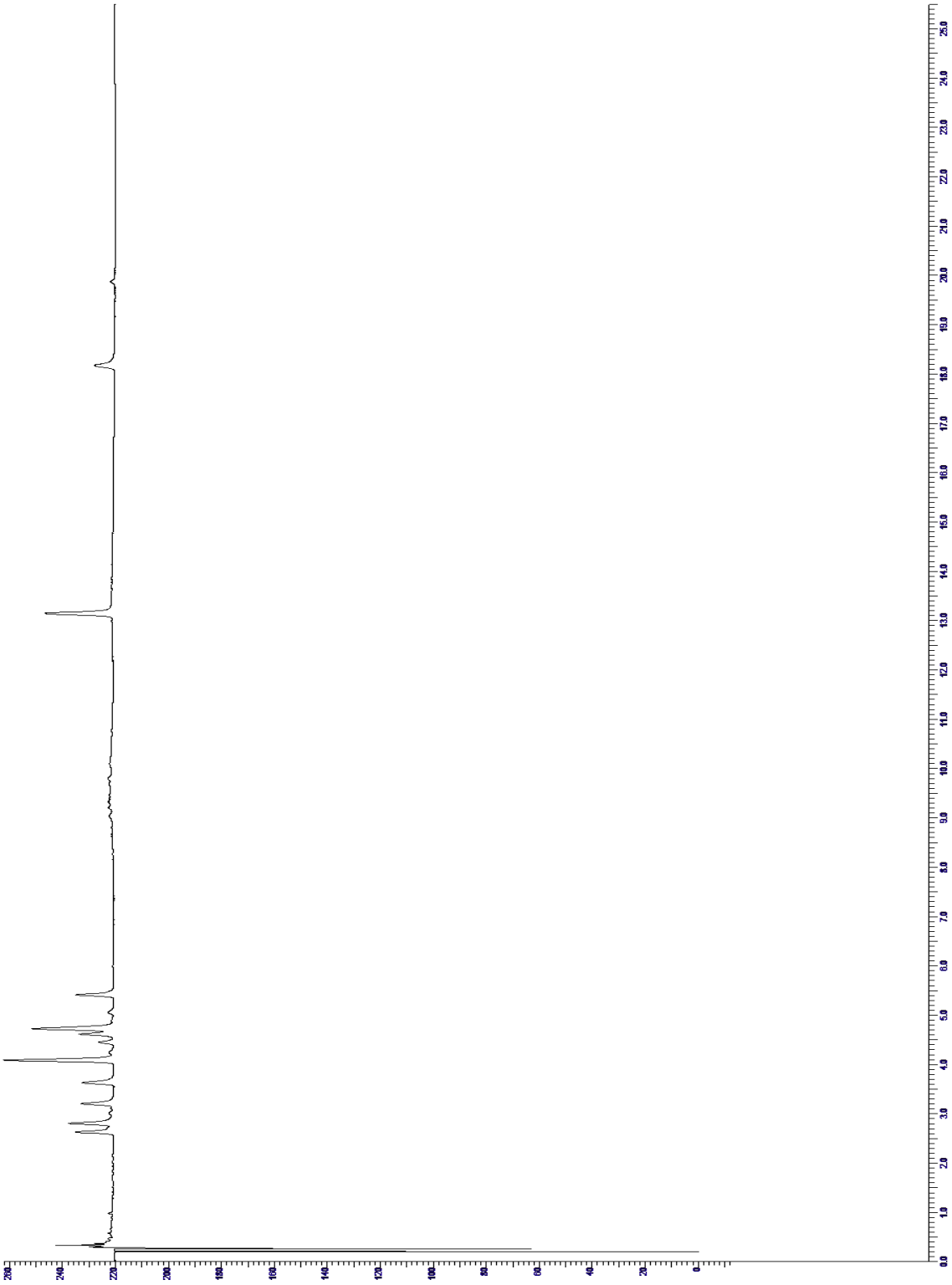


Figure 103-Oligomer content chromatogram for Norway Spruce

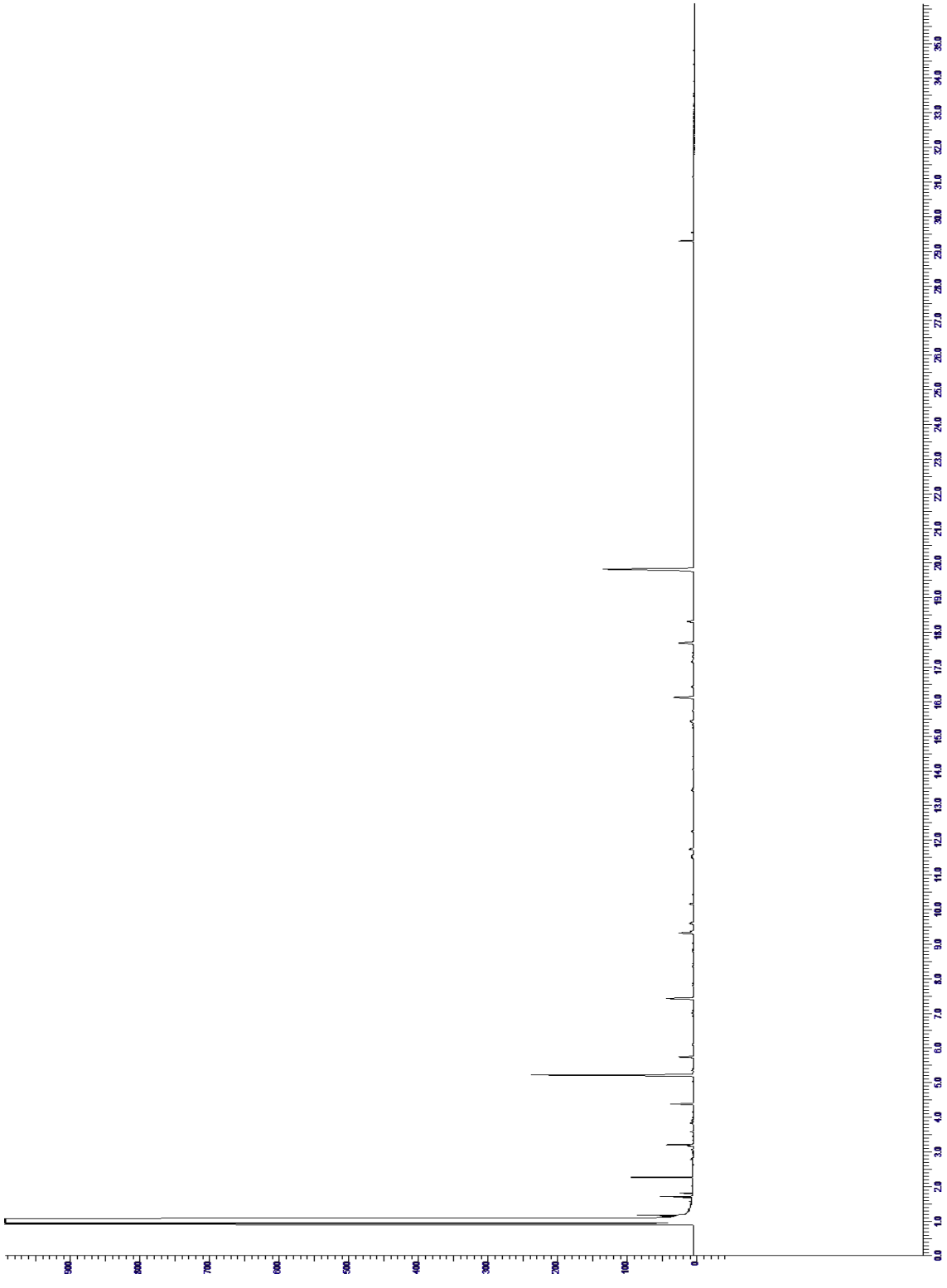


Figure 104-Total sugar content chromatogram for holm oak

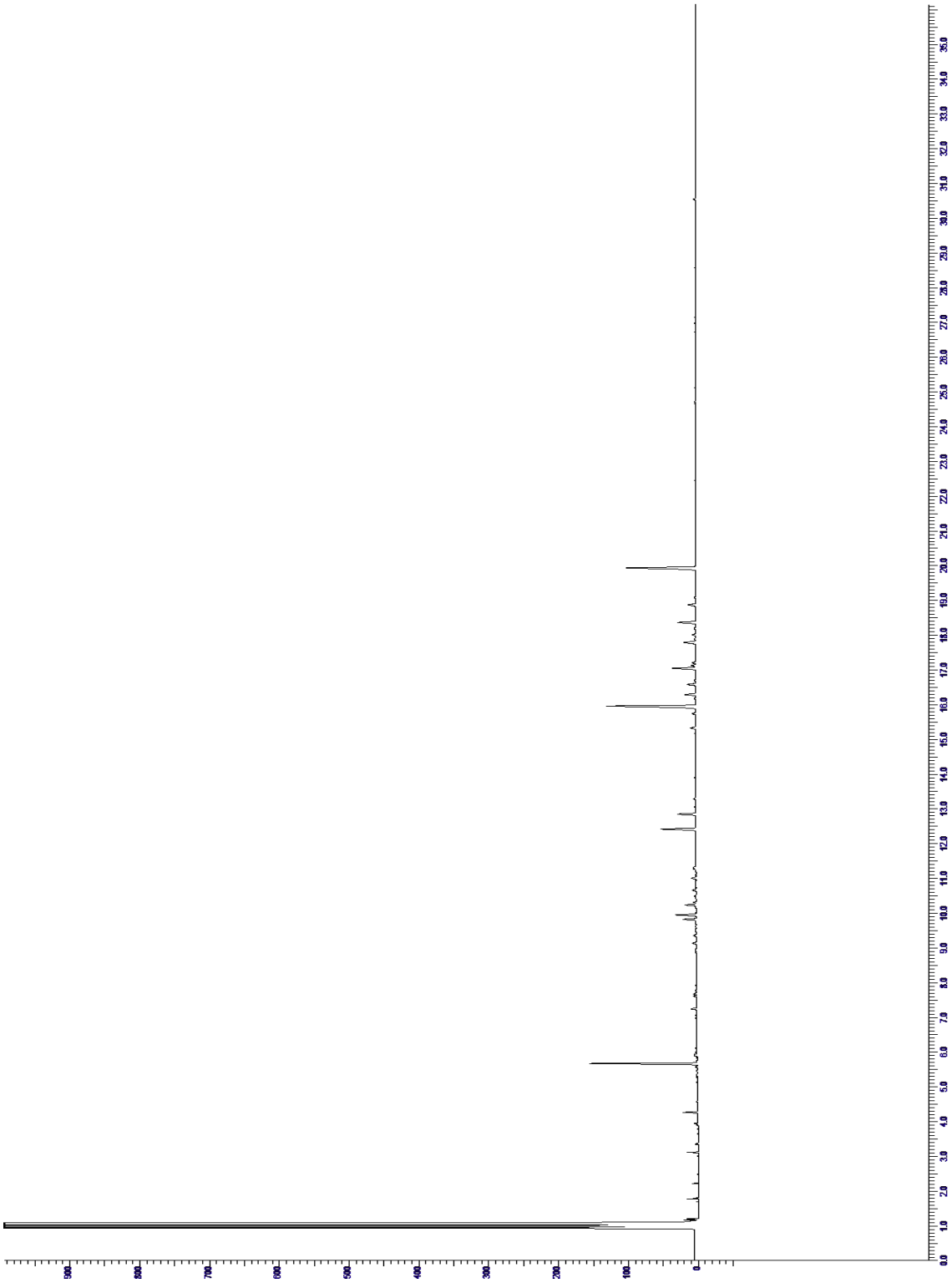


Figure 105-Total sugar content chromatogram for Stone Pine

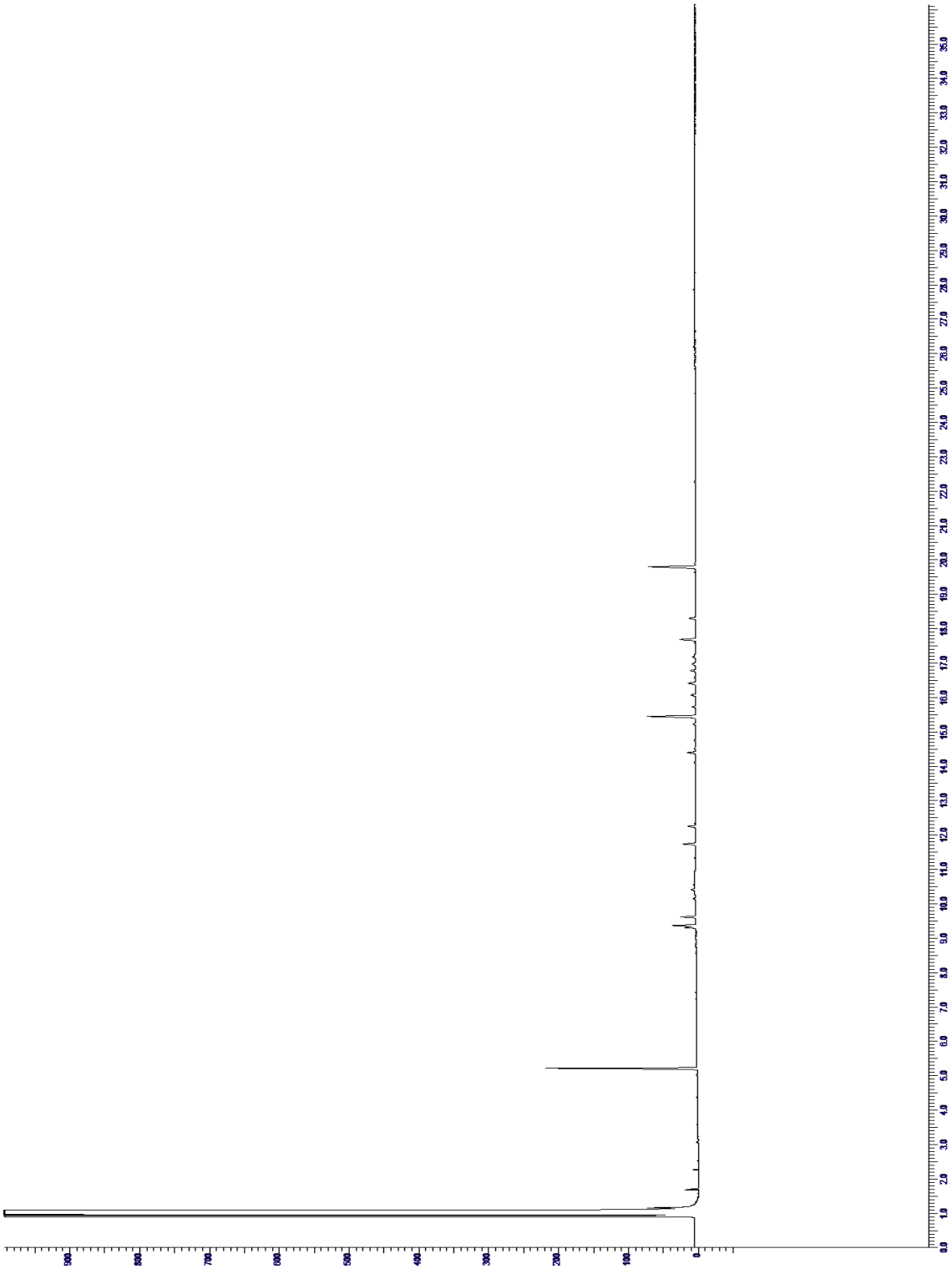


Figure 106-Total sugar content chromatogram for Norway Spruce

III Experimental results

Experiment 1: Catalyst-HCl, Wood-Holm Oak, T=95ºC, pH=0,5

Extract Nº1 has been used, this extract has been extracted from 1,25-2 mm Holm Oak chips at 130ºC during 220 minutes. It presents a pH of 4,33, a solid concentration of 6,75 g/L, a hemicellulose concentration of 4,06 g/L and a molecular mass of 10650 g/mol (DP≈59).

100 mL of this extract were used for the experiment. 8,58 mL of HCl 4 M were added as an acid catalyst in order to reach a value of pH 0,5. 12 samples of 6 mL were extracted from the mixture and they were all neutralized with 18,96 mL of a NaOH 0,1 N solution. The results from the monomer and oligomer analysis are presented in Table 15 and in Table 16:

Table 15-Monomer Concentrations (g/L) Experiment 1, HCl, Holm Oak, T=95ºC, pH=0,5

Time (h)	ARA	GAL	GALA	GLC	GLCA	MAN	RHA	XYL	TOTAL
0	0,16	0,04	0,00	0,30	0,03	0,29	0,03	0,03	0,87
0,33	0,64	0,10	0,02	0,34	0,04	0,23	0,06	0,17	1,60
0,66	0,74	0,16	0,03	0,41	0,04	0,25	0,08	0,36	2,07
1	0,65	0,19	0,02	0,44	0,04	0,23	0,09	0,45	2,11
1,5	0,81	0,28	0,04	0,53	0,04	0,27	0,10	0,57	2,65
2	0,72	0,31	0,05	0,56	0,05	0,25	0,11	0,56	2,60
3	0,68	0,35	0,07	0,60	0,05	0,23	0,11	0,54	2,62
4	0,65	0,35	0,06	0,58	0,05	0,20	0,12	0,53	2,54
6	0,53	0,30	0,08	0,52	0,05	0,15	0,11	0,44	2,19
8	0,73	0,39	0,11	0,67	0,05	0,12	0,14	0,57	2,77
10	0,60	0,35	0,15	0,58	0,05	0,13	0,13	0,49	2,49
24	0,31	0,21	0,16	0,41	0,06	0,06	0,12	0,28	1,61

Table 16-Oligomer Concentrations (g/L) Experiment 1, HCl, Holm Oak, T=95ºC, pH=0,5

Time (h)	Monomers	Dimers	Trimers	Tetramers	Pentamers	Oligomers (DP<6)
0	0,87	0,09	0,12	0,01	0,00	1,11
0,33	1,60	0,26	0,22	0,16	0,02	2,27
0,66	2,07	0,32	0,19	0,04	0,02	2,64
1	2,11	0,43	0,09	0,03	0,01	2,67
1,5	2,65	0,32	0,12	0,03	0,00	3,12
2	2,60	0,38	0,05	0,01	0,00	3,05
3	2,62	0,49	0,03	0,01	0,00	3,15
4	2,54	0,45	0,01	0,01	0,00	3,01
6	2,19	0,72	0,06	0,02	0,00	3,00
8	2,77	0,59	0,04	0,02	0,00	3,41
10	2,49	0,63	0,09	0,03	0,00	3,24
24	1,61	0,39	0,03	0,01	0,00	2,04

Experiment 2: Catalyst-HCl, Wood-Stone Pine, T=95ºC, pH=0,5

Extract Nº2 has been used, this extract has been extracted from 1,25-2 mm Stone Pine chips at 130ºC during 220 minutes. It presents a pH of 3,77, a solid concentration of 5,76 g/L, a hemicellulose concentration of 5,70 g/L and a molecular mass of 5702 g/mol (DP≈32).

100 mL of this extract were used for the experiment. 8,58 mL of HCl 4 M were added as an acid catalyst in order to reach a value of pH 0,5. 12 samples of 6 mL were extracted from the mixture and they were all neutralized with 18,95 mL of a NaOH 0,1 N solution. The results from the monomer and oligomer analysis are presented in Table 17 and in Table 18:

Table 17-Monomer Concentrations (g/L) Experiment 2, HCl, Stone Pine, T=95ºC, pH=0,5

Time (h)	ARA	GAL	GALA	GLC	GLCA	MAN	RHA	XYL	TOTAL
RM	0,48	0,10	0,00	0,02	0,00	0,01	0,01	0,05	0,68
0	0,67	0,12	0,01	0,02	0,00	0,01	0,01	0,07	0,91
0,33	0,36	0,14	0,01	0,02	0,00	0,08	0,03	0,20	0,84
0,66	0,33	0,17	0,01	0,03	0,00	0,17	0,05	0,26	1,03
1	0,24	0,18	0,01	0,04	0,00	0,27	0,07	0,24	1,05
1,5	0,22	0,21	0,01	0,06	0,00	0,36	0,05	0,23	1,15
2	0,24	0,24	0,02	0,07	0,00	0,49	0,03	0,27	1,36
3	0,49	0,69	0,04	0,14	0,01	0,85	0,03	0,48	2,73
4	0,20	0,28	0,03	0,11	0,01	0,53	0,08	0,21	1,47
6	0,20	0,30	0,03	0,15	0,00	0,59	0,07	0,23	1,58
8	0,25	0,38	0,07	0,20	0,01	0,77	0,08	0,30	2,05
10	0,24	0,34	0,06	0,17	0,00	0,68	0,06	0,28	1,83
24	0,22	0,33	0,14	0,18	0,01	0,69	0,05	0,27	1,90

Table 18-Oligomer Concentrations (g/L) Experiment 2, HCl, Stone Pine, T=95ºC, pH=0,5

Time (h)	Monomers	Dimers	Trimers	Tetramers	Pentamers	Oligomers (DP<6)
0	0,91	0,16	0,09	0,12	0,02	1,30
0,33	0,84	0,46	0,28	0,24	0,13	1,94
0,66	1,03	0,45	0,26	0,20	0,13	2,07
1	1,05	0,80	0,45	0,35	0,06	2,71
1,5	1,15	0,46	0,22	0,12	0,04	1,98
2	1,36	0,52	0,17	0,03	0,00	2,08
3	2,73	0,77	0,13	0,03	0,00	3,67
4	1,47	0,81	0,05	0,07	0,01	2,40
6	1,58	0,61	0,10	0,01	0,00	2,31
8	2,05	0,96	0,28	0,07	0,01	3,37
10	1,83	0,67	0,02	0,01	0,00	2,54
24	1,90	0,35	0,05	0,05	0,01	2,36

Experiment 3: Catalyst-HCl, Wood-Norway Spruce, T=95ºC, pH=0,5

Extract Nº3 has been used, this extract has been extracted from 1,25-2 mm Norway Spruce chips at 130ºC during 220 minutes. It presents a pH of 4,5, a solid concentration of 4,81 g/L, a hemicellulose concentration of 2,81 g/L and a molecular mass of 5900 g/mol (DP≈33).

100 mL of this extract were used for the experiment. 8,58 mL of HCl 4 M were added as an acid catalyst in order to reach a value of pH 0,5. 12 samples of 6 mL were extracted from the mixture and they were all neutralized with 18,95 mL of a NaOH 0,1 N solution. The results from the monomer and oligomer analysis are presented in Table 19 and in Table 20:

Table 19-Monomer Concentrations (g/L) Experiment 3, HCl, Picea Abies, T=95ºC, pH=0,5

Time (h)	ARA	GAL	GALA	GLC	GLCA	MAN	RHA	XYL	TOTAL
0	0,29	0,03	0,00	0,06	0,00	0,10	0,00	0,03	0,52
0,33	0,14	0,04	0,00	0,05	0,00	0,08	0,01	0,07	0,41
0,66	0,16	0,04	0,00	0,06	0,00	0,11	0,02	0,09	0,49
1	0,18	0,05	0,00	0,07	0,00	0,18	0,01	0,12	0,61
1,5	0,20	0,07	0,00	0,08	0,00	0,29	0,01	0,15	0,81
2	0,13	0,06	0,01	0,07	0,00	0,25	0,01	0,09	0,62
3	0,14	0,07	0,01	0,09	0,00	0,32	0,01	0,11	0,76
4	0,14	0,07	0,01	0,11	0,00	0,35	0,01	0,11	0,79
6	0,16	0,08	0,01	0,14	0,00	0,41	0,01	0,13	0,95
8	0,13	0,07	0,00	0,12	0,00	0,34	0,01	0,11	0,79
10	0,11	0,06	0,01	0,10	0,01	0,28	0,01	0,09	0,66
24	0,11	0,07	0,01	0,11	0,00	0,30	0,02	0,09	0,72

Table 20-Oligomer Concentrations (g/L) Experiment 3, HCl, Picea Abies, T=95ºC, pH=0,5

Time (h)	Monomers	Dimers	Trimers	Tetramers	Pentamers	Oligomers (DP<6)
0	0,52	0,06	0,04	0,02	0,01	0,65
0,33	0,41	0,17	0,09	0,05	0,04	0,75
0,66	0,49	0,16	0,12	0,05	0,04	0,86
1	0,61	0,13	0,10	0,03	0,01	0,87
1,5	0,81	0,24	0,09	0,03	0,01	1,17
2	0,62	0,19	0,05	0,01	0,00	0,87
3	0,76	0,32	0,08	0,01	0,00	1,17
4	0,79	0,31	0,03	0,01	0,00	1,15
6	0,95	0,20	0,02	0,00	0,00	1,17
8	0,79	0,27	0,03	0,01	0,00	1,09
10	0,66	0,21	0,01	0,01	0,00	0,88
24	0,72	0,07	0,03	0,00	0,01	0,83

Experiment 4: Catalyst-Smopex-101, Wood-Holm Oak, T=95ºC, pH=1

Extract Nº4 has been used, this extract has been extracted from 1,25-2 mm Holm Oak chips at 130ºC during 140 minutes. It presents a pH of 4,61, a solid concentration of 6,23 g/L, a hemicellulose concentration of 2,02 g/L and a molecular mass of 13750 g/mol (DP≈76).

100 mL of this extract were used for the experiment. 6,5084 g of Smopex-101 were added as an acid catalyst in order to reach a value of pH 0,5 (Calculated value was 6,5088 g). 12 samples of 6 mL were extracted from the mixture. The results from the monomer and oligomer analysis are presented in Table 21 and in Table 22:

Table 21-Monomer Concentrations (g/L) Experiment 4, Smopex-101, Holm Oak, T=95ºC, pH=1

Time (h)	ARA	GAL	GALA	GLC	GLCA	MAN	RHA	XYL	TOTAL
RM	0,06	0,02	0,01	0,23	0,02	0,21	0,02	0,01	0,58
0	0,08	0,02	0,00	0,25	0,02	0,22	0,02	0,02	0,63
0,33	0,10	0,02	0,00	0,27	0,03	0,22	0,02	0,03	0,69
0,66	0,08	0,02	0,00	0,20	0,01	0,15	0,02	0,03	0,51
1	0,13	0,02	0,00	0,26	0,01	0,23	0,03	0,04	0,71
1,5	0,14	0,02	0,00	0,31	0,02	0,23	0,03	0,04	0,80
2	0,16	0,02	0,00	0,28	0,03	0,22	0,04	0,05	0,79
3	0,19	0,03	0,00	0,31	0,03	0,22	0,04	0,05	0,87
4	0,21	0,03	0,01	0,28	0,03	0,21	0,04	0,06	0,88
6	0,28	0,03	0,00	0,29	0,02	0,21	0,05	0,07	0,94
8	0,29	0,04	0,01	0,24	0,02	0,17	0,05	0,07	0,88
10	0,30	0,05	0,00	0,29	0,02	0,17	0,05	0,08	0,97
24	0,11	0,05	0,00	0,14	0,01	0,05	0,02	0,06	0,44

Table 22-Oligomer Concentrations (g/L) Experiment 4, Smopex-101, Holm Oak, T=95ºC, pH=1

Time (h)	Monomers	Dimers	Trimers	Tetramers	Pentamers	Oligomers (DP<6)
0	0,58	0,25	0,13	0,19	0,09	1,25
0	0,63	0,37	0,09	0,19	0,11	1,40
0,33	0,69	0,27	0,02	0,23	0,14	1,34
0,66	0,51	0,23	0,04	0,20	0,09	1,07
1	0,71	0,26	0,03	0,20	0,12	1,32
1,5	0,80	0,32	0,05	0,25	0,13	1,54
2	0,79	0,38	0,03	0,24	0,13	1,57
3	0,87	0,41	0,02	0,25	0,16	1,72
4	0,88	0,50	0,04	0,24	0,14	1,80
6	0,94	0,52	0,03	0,25	0,17	1,92
8	0,88	0,34	0,03	0,23	0,09	1,57
10	0,97	0,23	0,01	0,08	0,06	1,35
24	0,44	0,22	0,01	0,23	0,07	0,98

Experiment 5: Catalyst-Smopex-101, Wood-Holm Oak, T=95ºC, pH=1,5

Extract Nº4 has been used, this extract has been extracted from 1,25-2 mm Holm Oak chips at 130ºC during 140 minutes. It presents a pH of 4,61, a solid concentration of 6,23 g/L, a hemicellulose concentration of 2,02 g/L and a molecular mass of 13750 g/mol (DP≈76).

100 mL of this extract were used for the experiment. 2,0570 g of Smopex-101 were added as an acid catalyst in order to reach a value of pH 0,5 (Calculated value was 2,0571 g). 12 samples of 6 mL were extracted from the mixture. The results from the monomer and oligomer analysis are presented in Table 23 and Table 24:

Table 23-Monomer Concentrations (g/L) Experiment 5, Smopex-101, Holm Oak, T=95ºC, pH=1,5

Time (h)	ARA	GAL	GALA	GLC	GLCA	MAN	RHA	XYL	TOTAL
RM	0,06	0,02	0,01	0,23	0,02	0,21	0,02	0,01	0,58
0	0,08	0,02	0,00	0,30	0,03	0,24	0,02	0,02	0,70
0,33	0,08	0,02	0,00	0,32	0,02	0,25	0,02	0,02	0,72
0,66	0,09	0,01	0,00	0,31	0,02	0,25	0,02	0,03	0,73
1	0,09	0,02	0,00	0,36	0,03	0,27	0,02	0,03	0,83
1,5	0,10	0,03	0,00	0,35	0,02	0,27	0,03	0,03	0,83
2	0,13	0,02	0,00	0,30	0,02	0,26	0,03	0,03	0,79
3	0,16	0,04	0,00	0,49	0,05	0,34	0,04	0,05	1,17
4	0,15	0,03	0,00	0,42	0,04	0,29	0,03	0,04	1,00
6	0,16	0,03	0,00	0,34	0,02	0,24	0,03	0,04	0,86
8	0,16	0,02	0,00	0,31	0,01	0,24	0,03	0,04	0,82
10	0,25	0,06	0,01	0,37	0,03	0,23	0,04	0,08	1,08
24	0,31	0,07	0,01	0,29	0,02	0,17	0,05	0,10	1,02

Table 24-Oligomer Concentrations (g/L) Experiment 5, Smopex-101, Holm Oak, T=95ºC, pH=1,5

Time (h)	Monomers	Dimers	Trimers	Tetramers	Pentamers	Oligomers (DP<6)
0	0,58	0,25	0,13	0,19	0,09	1,25
0	0,70	0,36	0,12	0,23	0,12	1,53
0,33	0,72	0,25	0,08	0,22	0,10	1,36
0,66	0,73	0,29	0,14	0,22	0,15	1,53
1	0,83	0,40	0,12	0,26	0,13	1,74
1,5	0,83	0,45	0,18	0,25	0,18	1,89
2	0,79	0,40	0,16	0,21	0,10	1,66
3	1,17	0,21	0,04	0,19	0,07	1,68
4	1,00	0,37	0,07	0,25	0,15	1,84
6	0,86	0,30	0,05	0,18	0,10	1,49
8	0,82	0,34	0,06	0,21	0,12	1,54
10	1,08	0,38	0,07	0,23	0,10	1,87
24	1,02	0,39	0,01	0,26	0,17	1,85

Experiment 6: Catalyst-Smopex-101, Wood-Holm Oak, T=95ºC, pH=0,5

Extract Nº1 has been used, this extract has been extracted from 1,25-2 mm Holm Oak chips at 130ºC during 220 minutes. It presents a pH of 4,33, a solid concentration of 6,75 g/L, a hemicellulose concentration of 4,06 g/L and a molecular mass of 10650 g/mol (DP≈59).

100 mL of this extract were used for the experiment. 20,5790g of Smopex-101 were added as an acid catalyst in order to reach a value of pH 0,5 (Calculated value was 20,5847 g). 12 samples of 6 mL were extracted from the mixture. The results from the monomer and oligomer analysis are presented in Table 25 and in Table 26:

Table 25-Monomer Concentrations (g/L) Experiment 6, Smopex-101, Holm Oak, T=95ºC, pH=0,5

Time (h)	ARA	GAL	GALA	GLC	GLCA	MAN	RHA	XYL	TOTAL
0	0,14	0,03	0,00	0,25	0,03	0,15	0,03	0,03	0,65
0,33	0,17	0,04	0,00	0,28	0,02	0,15	0,04	0,04	0,72
0,66	0,28	0,04	0,00	0,32	0,03	0,18	0,05	0,05	0,95
1	0,23	0,03	0,00	0,29	0,02	0,14	0,05	0,05	0,81
1,5	0,27	0,04	0,00	0,33	0,03	0,17	0,05	0,06	0,94
2	0,32	0,04	0,00	0,31	0,02	0,18	0,06	0,08	1,01
3	0,34	0,06	0,01	0,33	0,03	0,18	0,06	0,10	1,09
4	0,63	0,07	0,01	0,36	0,03	0,24	0,08	0,15	1,58
6	0,39	0,08	0,03	0,36	0,04	0,14	0,08	0,18	1,31
8	0,38	0,09	0,03	0,36	0,04	0,15	0,08	0,21	1,33
10	0,56	0,13	0,00	0,39	0,03	0,18	0,11	0,33	1,73
24	0,15	0,06	0,00	0,12	0,01	0,04	0,03	0,13	0,54

Table 26-Oligomer Concentrations (g/L) Experiment 6, Smopex-101, Holm Oak, T=95ºC, pH=0,5

Time (h)	Monomers	Dimers	Trimers	Tetramers	Pentamers	Oligomers (DP<6)
0	0,65	0,65	0,07	0,27	0,13	1,78
0,33	0,72	0,53	0,01	0,27	0,15	1,69
0,66	0,95	0,50	0,16	0,29	0,15	2,05
1	0,81	0,47	0,01	0,30	0,14	1,72
1,5	0,94	0,54	0,01	0,36	0,17	2,02
2	1,01	0,44	0,01	0,22	0,13	1,80
3	1,09	0,72	0,02	0,25	0,13	2,22
4	1,58	0,49	0,14	0,31	0,16	2,66
6	1,31	1,27	0,02	0,36	0,20	3,16
8	1,33	0,65	0,02	0,30	0,16	2,45
10	1,73	1,59	0,04	0,42	0,24	4,02
24	0,54	0,42	0,00	0,29	0,11	1,35

Experiment 7: Catalyst-Smopex-101, Wood-Holm Oak, T=85ºC, pH=0,5

Extract Nº5 has been used, this extract has been extracted from 1,25-2 mm Holm Oak chips at 140ºC during 160 minutes. It presents a pH of 3,88, a solid concentration of 10,89 g/L, a hemicellulose concentration of 8,43 g/L and a molecular mass of 5370 g/mol (DP≈30).

100 mL of this extract were used for the experiment. 20,4798 g of Smopex-101 were added as an acid catalyst in order to reach a value of pH 0,5 (Calculated value was 20,5792 g). 12 samples of 6 mL were extracted from the mixture. The results from the monomer and oligomer analysis are presented in Table 27 and in Table 28:

Table 27-Monomer Concentrations (g/L) Experiment 7, Smopex-101, Holm Oak, T=85ºC, pH=0,5

Time (h)	ARA	GAL	GALA	GLC	GLCA	MAN	RHA	XYL	TOTAL
RM	0,20	0,05	0,03	0,29	0,02	0,20	0,04	0,07	0,91
0	0,20	0,04	0,00	0,29	0,02	0,14	0,05	0,08	0,81
0,33	0,18	0,03	0,00	0,24	0,02	0,12	0,04	0,07	0,70
0,66	0,19	0,03	0,00	0,19	0,01	0,11	0,04	0,08	0,65
1	0,23	0,04	0,00	0,28	0,02	0,13	0,05	0,10	0,86
1,5	0,22	0,03	0,00	0,23	0,01	0,12	0,05	0,11	0,76
2	0,25	0,04	0,00	0,25	0,01	0,11	0,05	0,12	0,84
3	0,27	0,04	0,00	0,25	0,02	0,12	0,06	0,16	0,91
4	0,32	0,06	0,01	0,32	0,02	0,13	0,09	0,30	1,25
6	0,38	0,07	0,00	0,36	0,02	0,15	0,09	0,41	1,48
8	0,36	0,07	0,00	0,29	0,02	0,14	0,09	0,45	1,41
10	0,38	0,07	0,00	0,31	0,01	0,14	0,09	0,56	1,57
24	0,49	0,14	0,01	0,41	0,02	0,17	0,15	1,89	3,29

Table 28-Oligomer Concentrations (g/L) Experiment 7, Smopex-101, Holm Oak, T=85ºC, pH=0,5

Time (h)	Monomers	Dimers	Trimers	Tetramers	Pentamers	Oligomers (DP<6)
0	0,91	0,60	0,16	0,48	0,28	2,43
0	0,81	0,94	0,06	0,67	0,40	2,88
0,33	0,70	0,74	0,01	0,46	0,29	2,20
0,66	0,65	0,61	0,05	0,37	0,25	1,93
1	0,86	0,63	0,01	0,40	0,24	2,14
1,5	0,76	0,93	0,04	0,60	0,38	2,71
2	0,84	0,56	0,01	0,47	0,27	2,15
3	0,91	0,97	0,01	0,48	0,31	2,68
4	1,25	1,05	0,02	0,58	0,34	3,23
6	1,48	0,68	0,00	0,47	0,17	2,79
8	1,41	0,83	0,00	0,46	0,27	2,97
10	1,57	0,88	0,00	0,54	0,13	3,12
24	3,29	1,80	0,23	0,70	0,39	6,40

Experiment 8: Catalyst-Smopex-101, Wood-Holm Oak, T=75ºC, pH=0,5

Extract Nº5 has been used, this extract has been extracted from 1,25-2 mm Holm Oak chips at 140ºC during 160 minutes. It presents a pH of 3,88, a solid concentration of 10,89 g/L, a hemicellulose concentration of 8,43 g/L and a molecular mass of 5370 g/mol (DP≈30).

100 mL of this extract were used for the experiment. 20,5661 g of Smopex-101 were added as an acid catalyst in order to reach a value of pH 0,5 (Calculated value was 20,5792 g). 12 samples of 6 mL were extracted from the mixture. The results from the monomer and oligomer analysis are presented in Table 29 and in Table 30:

Table 29-Monomer Concentrations (g/L) Experiment 8, Smopex-101, Holm Oak, T=75ºC, pH=0,5

Time (h)	ARA	GAL	GALA	GLC	GLCA	MAN	RHA	XYL	TOTAL
RM	0,20	0,05	0,03	0,29	0,02	0,20	0,04	0,07	0,91
0	0,16	0,04	0,00	0,25	0,01	0,16	0,04	0,06	0,70
0,33	0,14	0,03	0,00	0,23	0,02	0,09	0,04	0,06	0,61
0,66	0,11	0,03	0,01	0,25	0,04	0,08	0,03	0,05	0,60
1	0,14	0,03	0,00	0,25	0,01	0,09	0,04	0,06	0,62
1,5	0,15	0,04	0,00	0,24	0,02	0,10	0,04	0,06	0,65
2	0,15	0,04	0,00	0,26	0,03	0,10	0,03	0,07	0,68
3	0,19	0,04	0,01	0,25	0,02	0,10	0,04	0,10	0,75
4	0,14	0,03	0,00	0,25	0,02	0,06	0,04	0,09	0,64
6	0,26	0,04	0,00	0,26	0,02	0,12	0,05	0,14	0,90
8	0,16	0,04	0,00	0,34	0,02	0,09	0,05	0,14	0,85
10	0,25	0,05	0,00	0,23	0,01	0,10	0,05	0,17	0,86
24	0,16	0,03	0,00	0,19	0,01	0,06	0,04	0,17	0,64

Table 30-Oligomer Concentrations (g/L) Experiment 8, Smopex-101, Holm Oak, T=75ºC, pH=0,5

Time (h)	Monomers	Dimers	Trimers	Tetramers	Pentamers	Oligomers (DP<6)
0	0,91	0,60	0,16	0,48	0,28	2,43
0	0,70	0,48	0,08	0,32	0,25	1,84
0,33	0,61	0,30	0,05	0,21	0,17	1,32
0,66	0,60	0,43	0,06	0,30	0,24	1,64
1	0,62	0,29	0,04	0,20	0,16	1,31
1,5	0,65	0,38	0,06	0,27	0,22	1,57
2	0,68	0,45	0,08	0,32	0,26	1,78
3	0,75	0,43	0,06	0,33	0,26	1,83
4	0,64	0,39	0,07	0,31	0,24	1,64
6	0,90	0,46	0,07	0,29	0,20	1,92
8	0,85	0,47	0,04	0,30	0,24	1,90
10	0,86	0,60	0,05	0,41	0,29	2,22
24	0,64	0,45	0,06	0,35	0,25	1,74

Experiment 9: Catalyst-Smopex-101, Wood-Stone Pine, T=95ºC, pH=1

Extract Nº6 has been used, this extract has been extracted from 1,25-2 mm Stone Pine chips at 150ºC during 120 minutes. It presents a pH of 3,62, a solid concentration of 10,46 g/L, a hemicellulose concentration of 9,22 g/L and a molecular mass of 3649 g/mol (DP≈20).

100 mL of this extract were used for the experiment. 6,4940 g of Smopex-101 were added as an acid catalyst in order to reach a value of pH 0,5 (Calculated value was 6,4948 g). 12 samples of 6 mL were extracted from the mixture. The results from the monomer and oligomer analysis are presented in Table 31 and in Table 32:

Table 31-Monomer Concentrations (g/L) Experiment 9, Smopex-101, Stone Pine, T=95ºC, pH=1

Time (h)	ARA	GAL	GALA	GLC	GLCA	MAN	RHA	XYL	TOTAL
RM	0,65	0,23	0,00	0,03	0,00	0,06	0,03	0,28	1,30
0	0,68	0,22	0,01	0,02	0,00	0,06	0,03	0,29	1,32
0,33	0,67	0,25	0,00	0,03	0,00	0,08	0,03	0,33	1,39
0,66	0,72	0,23	0,01	0,02	0,00	0,08	0,04	0,35	1,44
1	0,80	0,26	0,00	0,02	0,00	0,09	0,04	0,40	1,62
1,5	0,78	0,28	0,00	0,03	0,00	0,10	0,04	0,44	1,68
2	0,72	0,27	0,00	0,03	0,00	0,11	0,05	0,47	1,65
3	0,79	0,33	0,00	0,03	0,00	0,13	0,04	0,57	1,89
4	0,75	0,29	0,00	0,03	0,00	0,15	0,04	0,57	1,83
6	0,76	0,33	0,00	0,04	0,00	0,20	0,05	0,73	2,10
8	0,77	0,34	0,01	0,04	0,00	0,24	0,04	0,82	2,26
10	0,82	0,36	0,00	0,05	0,00	0,29	0,05	0,95	2,53
24	0,76	0,57	0,01	0,15	0,01	0,92	0,06	1,41	3,89

Table 32-Oligomer Concentrations (g/L) Experiment 9, Smopex-101, Stone Pine, T=95ºC, pH=1

Time (h)	Monomers	Dimers	Trimers	Tetramers	Pentamers	Oligomers (DP<6)
0	1,30	0,61	0,25	0,49	0,42	3,06
0	1,32	0,64	0,24	0,62	0,49	3,33
0,33	1,39	0,66	0,25	0,64	0,51	3,46
0,66	1,44	0,69	0,23	0,58	0,48	3,43
1	1,62	0,68	0,24	0,68	0,42	3,63
1,5	1,68	0,77	0,26	0,67	0,51	3,89
2	1,65	0,76	0,28	0,57	0,44	3,69
3	1,89	0,75	0,25	0,67	0,44	4,00
4	1,83	0,72	0,26	0,61	0,43	3,86
6	2,10	0,76	0,28	0,63	0,40	4,16
8	2,26	0,78	0,23	0,58	0,39	4,25
10	2,53	0,64	0,26	0,52	0,48	4,44
24	3,89	0,84	0,29	0,52	0,43	5,96

Experiment 10: Catalyst-Smopex-101, Wood-Stone Pine, T=95ºC, pH=1,5

Extract Nº6 has been used, this extract has been extracted from 1,25-2 mm Stone Pine chips at 150ºC during 120 minutes. It had to be diluted in a proportion 83 mL of extract and 17 mL of diluted water. The new mixture presents a pH of 3,62, a solid concentration of 8,68 g/L, a hemicellulose concentration of 7,65 g/L and a molecular mass of 3649 g/mol (DP≈20).

100 mL of this modified extract were used for the experiment. 2,0430g of Smopex-101 were added as an acid catalyst in order to reach a value of pH 0,5 (Calculated value was 2,0432 g). 12 samples of 6 mL were extracted from the mixture. The results from the monomer and oligomer analysis are presented in Table 33 and in Table 34:

Table 33-Monomer Concentrations (g/L) Experiment 10, Smopex-101, Stone Pine, T=95ºC, pH=1,5

Time (h)	ARA	GAL	GALA	GLC	GLCA	MAN	RHA	XYL	TOTAL
RM	0,65	0,23	0,00	0,03	0,00	0,06	0,03	0,28	1,30
0	0,54	0,23	0,00	0,03	0,00	0,06	0,03	0,25	1,14
0,33	0,59	0,23	0,00	0,03	0,00	0,07	0,03	0,27	1,22
0,66	0,62	0,20	0,00	0,02	0,00	0,06	0,03	0,26	1,19
1	0,59	0,25	0,00	0,03	0,00	0,08	0,03	0,28	1,26
1,5	0,61	0,23	0,00	0,02	0,00	0,07	0,03	0,29	1,26
2	0,59	0,21	0,00	0,02	0,00	0,07	0,03	0,29	1,21
3	0,61	0,22	0,00	0,02	0,00	0,08	0,03	0,31	1,28
4	0,62	0,25	0,00	0,02	0,00	0,09	0,03	0,35	1,36
6	0,54	0,24	0,00	0,02	0,00	0,09	0,03	0,34	1,28
8	0,62	0,26	0,00	0,02	0,00	0,11	0,03	0,41	1,47
10	0,68	0,33	0,01	0,06	0,00	0,29	0,04	0,55	1,95
24	0,71	0,54	0,02	0,13	0,01	0,73	0,06	1,03	3,25

Table 34-Oligomer Concentrations (g/L) Experiment 10, Smopex-101, Stone Pine, T=95ºC, pH=1,5

Time (h)	Monomers	Dimers	Trimers	Tetramers	Pentamers	Oligomers (DP<6)
0	1,30	0,61	0,25	0,49	0,42	3,06
0	1,14	0,53	0,20	0,52	0,39	2,78
0,33	1,22	0,56	0,21	0,45	0,31	2,74
0,66	1,19	0,49	0,19	0,50	0,29	2,66
1	1,26	0,61	0,22	0,57	0,44	3,11
1,5	1,26	0,57	0,21	0,52	0,41	2,97
2	1,21	0,51	0,18	0,47	0,38	2,76
3	1,28	0,50	0,19	0,44	0,37	2,78
4	1,36	0,54	0,19	0,54	0,42	3,04
6	1,28	0,51	0,18	0,44	0,37	2,78
8	1,47	0,55	0,18	0,47	0,31	2,98
10	1,95	0,56	0,20	0,44	0,30	3,45
24	3,25	0,99	0,31	0,47	0,33	5,36

Experiment 11: Catalyst-Smopex-101, Wood-Stone Pine, T=95ºC, pH=0,5

Extract Nº2 has been used, this extract has been extracted from 1,25-2 mm Stone Pine chips at 130ºC during 220 minutes. . It had to be diluted in a proportion 90 mL of extract and 10 mL of diluted water. The new mixture presents a pH of 3,77, a solid concentration of 5,19 g/L, a hemicellulose concentration of 5,13 g/L and a molecular mass of 5702 g/mol (DP≈32).

100 mL of this modified extract were used for the experiment. 20,5765 g of Smopex-101 were added as an acid catalyst in order to reach a value of pH 0,5 (Calculated value was 20,5767 g). 12 samples of 6 mL were extracted from the mixture. The results from the monomer and oligomer analysis are presented in Table 35 and in Table:

Table 35-Monomer Concentrations (g/L) Experiment 11, Smopex-101, Stone Pine, T=95ºC, pH=0,5

Time (h)	ARA	GAL	GALA	GLC	GLCA	MAN	RHA	XYL	TOTAL
RM	0,48	0,10	0,00	0,02	0,00	0,01	0,01	0,05	0,68
0	0,08	0,10	0,00	0,01	0,00	0,01	0,00	0,02	0,23
0,33	0,10	0,10	0,00	0,01	0,00	0,01	0,01	0,03	0,26
0,66	0,15	0,09	0,00	0,01	0,00	0,02	0,01	0,05	0,33
1	0,10	0,11	0,00	0,02	0,00	0,02	0,01	0,04	0,30
1,5	0,15	0,12	0,00	0,02	0,00	0,03	0,00	0,06	0,38
2	0,21	0,10	0,00	0,01	0,00	0,02	0,01	0,09	0,45
3	0,23	0,11	0,00	0,02	0,00	0,04	0,02	0,12	0,54
4	0,20	0,12	0,00	0,02	0,00	0,05	0,03	0,14	0,56
6	0,10	0,13	0,00	0,03	0,00	0,07	0,05	0,08	0,46
8	0,19	0,16	0,00	0,05	0,00	0,15	0,02	0,23	0,80
10	0,18	0,19	0,00	0,06	0,00	0,21	0,04	0,25	0,93
24	0,10	0,14	0,00	0,06	0,00	0,26	0,04	0,16	0,76

Table 36-Oligomer Concentrations (g/L) Experiment 11, Smopex-101, Stone Pine, T=95ºC, pH=0,5

Time (h)	Monomers	Dimers	Trimers	Tetramers	Pentamers	Oligomers <6
0	0,23	0,32	0,01	0,35	0,25	1,16
0,66	0,33	0,24	0,02	0,33	0,23	1,14
1	0,30	0,37	0,03	0,41	0,30	1,40
1,5	0,38	0,41	0,02	0,44	0,31	1,55
2	0,45	0,44	0,03	0,49	0,36	1,77
3	0,54	0,35	0,02	0,33	0,24	1,49
4	0,56	0,24	0,03	0,21	0,15	1,18
8	0,80	0,42	0,04	0,19	0,13	1,58
10	0,93	0,44	0,07	0,29	0,20	1,93
24	0,76	0,39	0,03	0,15	0,10	1,43

Experiment 12: Catalyst-Smopex-101, Wood-Stone Pine, T=85ºC, pH=0,5

Extract Nº7 has been used, this extract has been extracted from 1,25-2 mm Stone Pine chips at 160ºC during 80 minutes. It had to be diluted in a proportion 90 mL of extract and 10 mL of diluted water. The new mixture presents a pH of 3,62, a solid concentration of 11,90 g/L, a hemicellulose concentration of 6,36 g/L and a molecular mass of 2254 g/mol (DP≈13).

100 mL of this modified extract were used for the experiment. 20,5713 g of Smopex-101 were added as an acid catalyst in order to reach a value of pH=0,5 (Calculated value was 20,5721 g). 12 samples of 6 mL were extracted from the mixture. The results from the monomer and oligomer analysis are presented in Table 37 and in Table 38:

Table 37-Monomer Concentrations (g/L) Experiment 12, Smopex-101, Stone Pine, T=85ºC, pH 0,5

Time (h)	ARA	GAL	GALA	GLC	GLCA	MAN	RHA	XYL	TOTAL
RM	0,55	0,26	0,00	0,04	0,00	0,10	0,04	0,39	1,38
0	0,28	0,19	0,00	0,02	0,00	0,10	0,04	0,30	0,93
0,33	0,12	0,13	0,00	0,02	0,00	0,09	0,02	0,22	0,62
0,66	0,33	0,18	0,00	0,02	0,00	0,10	0,04	0,33	0,99
1	0,22	0,17	0,00	0,03	0,00	0,11	0,04	0,31	0,88
1,5	0,23	0,16	0,00	0,02	0,00	0,11	0,05	0,33	0,90
2	0,16	0,16	0,00	0,02	0,00	0,13	0,03	0,34	0,85
3	0,22	0,20	0,00	0,03	0,00	0,16	0,07	0,45	1,13
4	0,59	0,29	0,00	0,03	0,00	0,16	0,05	0,68	1,79
6	0,17	0,18	0,00	0,04	0,00	0,19	0,05	0,48	1,10
8	0,17	0,19	0,00	0,04	0,00	0,20	0,04	0,53	1,17
10	0,16	0,19	0,00	0,05	0,00	0,25	0,04	0,58	1,27
24	0,17	0,18	0,00	0,05	0,00	0,31	0,03	0,59	1,33

Table 38-Oligomer Concentrations (g/L) Experiment 12, Smopex-101, Stone Pine, T=85ºC, pH=0,5

Time (h)	Monomers	Dimers	Trimers	Tetramers	Pentamers	Oligomers (DP<6)
0	1,38	0,73	0,31	0,63	0,42	3,47
0	0,93	0,87	0,19	0,67	0,50	3,16
0,33	0,62	0,65	0,21	0,53	0,33	2,33
0,66	0,99	0,70	0,21	0,54	0,31	2,76
1	0,88	0,55	0,24	0,67	0,52	2,87
1,5	0,90	0,88	0,27	0,51	0,42	2,97
2	0,85	0,73	0,19	0,53	0,46	2,76
3	1,13	1,26	0,32	0,93	0,58	4,23
4	1,79	1,50	0,48	1,20	0,92	5,89
6	1,10	0,76	0,19	0,62	0,46	3,12
8	1,17	0,92	0,22	0,65	0,47	3,43
10	1,27	0,21	0,12	0,21	0,20	2,00
24	1,33	0,98	0,19	0,52	0,39	3,42

Experiment 13: Catalyst-Smopex-101, Wood-Stone Pine, T=75ºC, pH=0,5

Extract Nº7 has been used, this extract has been extracted from 1,25-2 mm Stone Pine chips at 160ºC during 80 minutes. It had to be diluted in a proportion 90 mL of extract and 10 mL of diluted water. The new mixture a pH of 3,62, a solid concentration of 11,90 g/L, a hemicellulose concentration of 6,36 g/L and a molecular mass of 2254 g/mol (DP≈13).

100 mL of this modified extract were used for the experiment. 20,5722 g of Smopex-101 were added as an acid catalyst in order to reach a value of pH 0,5 (Calculated value was 20,5721 g). 12 samples of 6 mL were extracted from the mixture. The results from the monomer and oligomer analysis are presented in Table 39 and in Table 40:

Table 39-Monomer Concentrations (g/L) Experiment 13, Smopex-101, Stone Pine, T=75ºC, pH=0,5

Time (h)	ARA	GAL	GALA	GLC	GLCA	MAN	RHA	XYL	TOTAL
RM	0,58	0,25	0,00	0,03	0,00	0,10	0,04	0,40	1,40
0	0,38	0,22	0,00	0,02	0,00	0,09	0,04	0,35	1,09
0,33	0,38	0,19	0,00	0,02	0,00	0,09	0,03	0,35	1,06
0,66	0,22	0,19	0,00	0,02	0,00	0,11	0,05	0,30	0,89
1	0,44	0,25	0,00	0,02	0,00	0,11	0,04	0,39	1,25
1,5	0,13	0,17	0,00	0,03	0,00	0,11	0,06	0,22	0,71
2	0,21	0,17	0,00	0,02	0,00	0,10	0,04	0,29	0,84
3	0,12	0,14	0,00	0,02	0,00	0,09	0,06	0,18	0,61
4	0,20	0,21	0,00	0,03	0,00	0,12	0,05	0,34	0,94
6	0,17	0,16	0,00	0,02	0,00	0,11	0,04	0,29	0,79
8	0,32	0,20	0,00	0,02	0,00	0,12	0,04	0,42	1,13
10	0,35	0,20	0,00	0,02	0,00	0,14	0,04	0,48	1,23
24	0,30	0,23	0,00	0,04	0,00	0,25	0,04	0,61	1,46

Table 40-Oligomer Concentrations (g/L) Experiment 13, Smopex-101, Stone Pine, T=75ºC, pH=0,5

Time (h)	Monomers	Dimers	Trimers	Tetramers	Pentamers	Oligomers (DP<6)
0	1,40	0,58	0,28	0,57	0,37	3,21
0	1,09	0,69	0,37	0,78	0,47	3,40
0,33	1,06	0,52	0,21	0,56	0,21	2,56
0,66	0,89	0,96	0,23	0,80	0,32	3,20
1	1,25	0,89	0,41	1,08	0,55	4,19
1,5	0,71	0,51	0,22	0,50	0,18	2,11
2	0,84	0,83	0,27	0,68	0,41	3,03
3	0,61	0,78	0,19	0,53	0,20	2,30
4	0,94	0,84	0,31	0,58	0,38	3,06
6	0,79	0,20	0,05	0,19	0,11	1,34
8	1,13	0,79	0,24	0,72	0,29	3,16
10	1,23	0,83	0,33	0,75	0,30	3,45
24	1,46	0,65	0,17	0,58	0,22	3,07