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HOT WATER EXTRACTION AND MEMBRANE FILTRATION PROCESSES IN FRACTIONATION AND RECOVERY OF VALUE-ADDED COMPOUNDS FROM WOOD AND PLANT RESIDUES

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ABSTRACT

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The production of green chemicals and sustainable energy from renewable resources is gaining global interest. Special attention is paid to the refining of biomass residues, e.g. forest and agricultural wastes, into high value bio-based products. However, although biomass residues contain many valuable extractable compounds, their complex nature makes their full exploitation challenging. To overcome such difficulties, the development of efficient extraction, fractionation, concentration, and purification processes for the recovery of good quality natural-based biochemicals and biopolymers is essential. The aim of this study is to develop sustainable separation processes that could be applied on a large scale for the recovery of high value-added compounds from various kinds of biomass residues.

In the first part of this work, an ultrafiltration (UF)-based separation process was performed for the recovery and fractionation of galactoglucomannans (GGMs) from spruce autohydrolysates. The evaluation of different membrane materials showed that the regenerated cellulose (RC) membranes (10 and 30 kDa) could offer rather high permeability of autohydrolys with a quite low fouling tendency. The purity of the GGMs in the hemicellulose fractions after UF was from 60 to 80%, and the concentration of hemicelluloses reached even 400 g/L. This was achieved by using hydrophilic membranes and a high shear rate filter. The average molar masses of the different concentrates were from 5 to 18 kDa. These specifications provide great potential for the concentrated fractions to be utilized as raw material for manufacturing e.g. sustainable packaging films and hydrogels.

In order to purify the rich fractions of the GGMs further, diafiltration (DF) and oxidation were applied. Diafiltration of concentrated fractions containing high molar mass GGMs leads to the increase of their average molar mass by removing small molar mass compounds. The results also proved that only partial removal of lignin could be achieved by DF. Oxidation of the autohydrolysates improved the purity of the GGMs slightly, although the total amount of phenolic compounds (lignin) was not decreased notably. Mainly lignans and wood extractives were degraded by the oxidation. Oxidation increased the filterability of the autohydrolysates significantly, mainly due to the decrease of the viscosity of the oxidized autohydrolysates. This

indicates that some changes in high molar mass compounds occurred in the oxidation process, which had a clear effect on the viscosity and filterability of the autohydrolysate.

In the second part of this work, nonedible carob residues were processed in a hybrid separation process consisting of aqueous extraction and membrane-based separation techniques. This process aimed at extracting phenolic compounds and sugars from carob kibbles, and then fractionating and concentrating these value-added compounds from the aqueous extracts. One-step extraction recovered only about 20% of the phenolic compounds, but the extract contained a significant amount of sugars (110 g/L). The membrane-based separation of phenolic compounds and sugars from this extract was inadequate. Therefore, two-step aqueous extraction at different temperatures (30 and 100 °C) was developed. It gave a superior yield of phenolic compounds, i.e. about 70%. It also upgraded the quality of the extracts obtained from the carob residues by improving the separation of the sugars from the phenolic compounds in the extraction stages. By the membrane processes, two distinct natural streams from carob kibbles could be produced. The first stream is enriched in antioxidant content, namely catechin and its derivatives, for the nutraceuticals market. While the second stream is enriched in sugars for the food industry. In addition, the proposed process, including the two-step extraction process combined with nanofiltration (NF) and reverse osmosis (RO) fulfils the zero-discharge principle.

Hybrid processes based on combining membrane filtration with aqueous extraction could effectively be applied as a sustainable recovery and separation approach in biorefinery. This approach utilizes the environmental benefits of water as a green solvent to upgrade the exploitation of biomass residues. The proposed hybrid process is able to scale up and extend to other biomass residues, which makes it a promising alternative when biorefinery processes are developed and implemented.

Keywords: biomass residues, hot water extraction, membrane-based separation processes, hemicelluloses, phenolic compounds

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Almanasrah Mohammad

January 2017
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List of Publications

This thesis is based on the following journal papers. The rights from the publisher have been granted to include the papers in a dissertation.

I. Al Manasrah, M., Kallioinen, M., Ilvesniemi, H., Mänttäri, M., ***Recovery of galactoglucomannan from wood hydrolysate using regenerated cellulose ultrafiltration membranes***, Bioresource Technology, 114 (2012) 375-381.

II. Mänttäri, M., Al Manasrah, M., Strand, E., Laasonen, H., Preis, S., Puro, L., Xu, C., Kisonen, V., Korpinen, R., Kallioinen M., ***Improvement of ultrafiltration performance by oxidation treatment in the recovery of galactoglucomannan from wood autohydrolyzate,*** Separation and Purification Technology 149 (2015), 428–436.

III. Almanasrah, M., Roseiro, L. B., Bogel-Lukasik, R., Carvalheiro, F., Brazinha, C., Crespo, J., Kallioinen, M., Mänttäri, M., Duarte, L., ***Selective recovery of phenolic compounds and carbohydrates from carob kibbles using water-based extraction***, Industrial Crops and Products 70 (2015) 443-450.

IV. Almanasrah, M., Brazinha, C., Kallioinen, M., Duarte, L., Roseiro, L. B., Bogel-Lukasik, R., Carvalheiro, F., Mänttäri, M., Crespo, J., ***Nanofiltration and reverse osmosis as a platform for production of natural botanic extracts: The case study of carob by-products,*** Separation and Purification Technology 149 (2015), 389-397.

Author's contribution

The author was responsible for the preparation of papers I–IV. In papers I, III and IV, most of the experimental planning and measurements were performed by the author. In these papers, the manuscripts were mainly written by the author with some contribution from the co-writers. In paper II, the author planned some of the experiments and did part of the experimental work. In all the papers (I–IV), the author interpreted the results together with the co-writers. The technicians of the Laboratory of Membrane Technology and Technical Polymer Chemistry and reseach projects partners contributed to the analysis work.

Abbreviations and symbols

Abbreviations

ABTS	2, 2’-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)
ASE	Accelerated solvent extractors
BHA	Butylated hydroxyanisole
CAE	Carob aqueous extract
CF	Concentration factor
CR	Cross-rotational
CTMP	Chemithermomechanical pulp
CZE	Capillary zone electrophoresis
Da	Dalton
DF	Diafiltration
Di-NF	Dia-nanofiltration
DPPH	2, 2-diphenyl-1-picrylhydrazyl
FR (PWF)	Flux reduction (pure water flux)
Fru	Fructose
GAE	Gallic acid equivalent
Gal.A	Gallic acid
GGM	Galactoglucomannan
Glc	Glucose
HMF	Hydroxymethylfurfural
HPLC	High performance liquid chromatography
H-PS	Hydrophilized polysulphone
LSR	Liquid to solid ratio
MALLS	Multi-angle laser light scattering
MF	Microfiltration

Mw	Molar mass (average)
MTBE	Methyl *tert*-butyl ether
NERL	National Renewable Energy Laboratory
NF	Nanofiltration
OMWW	Olive mill wastewater
PAC	Polyaluminium chloride
PCD	Pulsed corona discharge
PDI	Polydispersity index
PES	Polyethersulphone
PHWE	Pressurized hot water extraction
PS	Polysulphone
PVDF	Polyvinylidene difluoride
PWP	Pure water permeability
R	Retentate
RI	Refractive index
RC	Regenerated cellulose
RO	Reverse osmosis
SEC	Size exclusion chromatography
SWaH	Spruce wood autohydrolysate
Suc	Sucrose
TOC	Total organic carbon
TMP	Thermomechanical pulp
TDS	Total dissolved solids
TOC	Total organic carbon
TP	Total phenolic compounds
UF	Ultrafiltration
UV	Ultraviolet

VR	Volume reduction
VRF	Volume reduction factor

Symbols

A_m	Membrane surface area, m^2
C_c	Concentration of solutes in the concentrate, g/L
C_f	Concentration of solutes in the feed, g/L
C_c	Concentration of solutes in the permeate, g/L
D	Number of dia-volumes, -
J_m	Mass permeate flux, kg/ (m^2h)
m_p	Mass flow rate, kg/h
M	Molarity, mol/L
Δp	Pressure difference across the membrane, bar
P	Permeability, kg/ (m^2h bar)
P_{corr}	Permeability corrected for the osmotic pressure effect, kg/ (m^2h bar)
$PWFa$	Pure water flux after filtration, kg/ (m^2h)
$PWFb$	Pure water flux before filtration, kg/ (m^2h)
PWP	Pure water permeability, kg/ (m^2h bar)
R_o	Observed retention, %
R	Gas constant, L bar /mole °K
V_{fb}	Volume of feed solution in the beginning of filtration, L
V_p	Volume of permeate, L
T	Temperature, °K
Δ	Recovery rate, -
$\Delta\pi$	Osmotic pressure difference across the membrane, bar

1 Introduction

Biomass is a renewable resource representing sustainable feedstock supply for a wide range of biomaterials. It is a versatile resource with potential applications to replace non-renewable fossil resources in the production of biofuel, biopolymers and fine chemicals (Werpy et al. 2004; Ruane et al. 2010). Even though biomass materials such as lignocellulose materials are highly abundant, their exploitation in a sustainable manner is still limited. Biomass-based products will have great market potential in the near future. For example, the US national vision is to use biomass as the source for 25% of the chemicals produced by the year 2030 (Perlack et al. 2005). To increase the contribution of biomass in the production of chemicals, their efficient refining is a necessity. Biomass refining, known as biorefining, is considered a long-term sustainable environmentally friendly alternative to petroleum refining (Octave and Thomas, 2009; Ruane et al. 2010). According to the National Renewable Energy Laboratory (NREL, September 2009), biorefinery is defined as "a facility that integrates biomass conversion processes and equipment to produce multiple fuels, power and chemicals from biomass".

Lignocellulosic residues, e.g. forestry and agricultural wastes are low-value feedstock with promising features for developing numerous value-added products. Even though some products, mainly biofuel, are made in lignocellulosic feedstock biorefineries, several opportunities remain for exploiting the full potential of this feedstock in producing many other bio-based materials. This type of biorefineries have the possibility to improve the competence of the forest industry by e.g. integration with conventional pulp and paper mills (Kenealy et al. 2007; Hu et al. 2008). Such biorefinery should be operated without radical changes in the existing processes or undesirable impacts on the quality of the main products, like pulp. Its operation aims at enhancing the overall profitability and productivity of any forest industry.

The biopolymers of lignocellulosic materials are primarily polysaccharides (cellulose and hemicellulose) and lignin. Among polysaccharides, cellulose has been found to have various commercial applications in bioethanol production and good potential in the manufacture of nano-fibril films (Henriksson et al. 2008; Menon and Rao, 2012). On the other hand, hemicelluloses have been recognized as potential raw material for making of renewable barrier, coating and packaging films (Hartman et al. 2006 a; Hansen and Plackett, 2008) and hydrogels (Söderqvist-Lindblad et al. 2001). They also can be utilized as food emulsion stabilizers (Mikkonen et al. 2016)

or even paper additives (Willför et al. 2008). As claimed by Persson et al. (2007), the production of concentrated hemicelluloses fraction suitable for packaging films from the process water of wood pulping could present a lower-cost raw material supply than ethylene vinyl alcohol, which is today used as an oxygen barrier in commercial packaging materials. The attention towards such applications is growing so that developing novel processes to promote commercial applications for hemicelluloses is still open for further research and investigation.

In biorefinery processes, recovery and fractionation of lignocellulosic materials can be achieved using various extraction methods. In the extraction, a process stream, i.e. an extract or autohydrolysate containing valuable compounds, e.g. hemicelluloses, together with other co-extracted compounds, is formed. Membrane filtration processes could perform well in recovering, fractionating and purifying hemicelluloses and other compounds from these extracts (Liu et al. 2012). Unlike traditional concentration separation methods, e.g. evaporation, membrane filtration processes could provide rather efficient fractionation, selective separation capabilities and high quality of the final product. Moreover, no chemical additions are necessarily needed in the membrane filtration process, and the energy consumption could be lower than in evaporation, as no phase transition needs to be applied. The main challenge in the utilization of membrane processes, especially in the case of complex mixtures, such as biomass autohydrolysates, is membrane fouling. Because of fouling, the filtration capacity of the membrane is reduced, which means increase in the operating cost of the filtration process.

Phenolic compounds are another group of valuable renewable materials that could be recovered from biomass. A lot of attention has been recently paid to biomass wastes like plant and agricultural residues as sources of phenolic compounds with antioxidant activity. These compounds have a high ability to offer benefits for human health as dietary antioxidants (Scalbert et al. 2005). Agro-industrial effluents, mainly winery and grape processing effluents (Giacobbo et al. 2013 a, b) and olive mill wastewater (Paraskeva et al. 2007 a, b), are considered the most common sources of valuable phenolic compounds. They are also recognized in the wood residues especially in the knotwood of trees (Kähkönen et al. 1999; Pietarinen et al. 2006).

The recovery of bioactive phenolic compounds is usually made by solvent extraction. In addition, aqueous extraction has been employed in a small scale and for analysis purposes. To separate and concentrate phenolic compounds from these effluents and extracts, membrane-based separation processes have been studied to some extent (Conde et al. 2013).

In particular carob kibbles are recognized as promising nonedible biomass residues, not only due to their high content of easily fermentable sugars, but also their phenolic compounds content (Petit and Pinilla, 1995; Avallone et al. 1997). Extraction of phenolic compounds from carob residues has been carried out with alcoholic solvents or water (Kumazawa et al. 2002; Papagiannopoulos et al. 2004). Even though extraction with alcohols, especially methanol, has demonstrated good performance at a laboratory scale (Owen et al. 2003), their conversion to a larger scale has several economic and environmental impacts. Drawbacks in the common extraction methods in terms of toxic solvents, low yield, scaling up, and further purification challenges limit the sustainable exploitation of carob kibbles. Therefore, developing novel processes to overcome these drawbacks and perform well at a large scale is required. Aqueous extraction and membrane filtration could have a substantial contribution in this development. Extraction with water is a sustainable and green process that could be developed for the recovery of phenolic compounds and sugars from carob kibbles in a large scale. After the extraction, membrane processes could play an important role in the separation and fractionation of the extracted compounds. The operation of membrane filtration under mild temperature conditions is beneficial for preserving the biological activity of the separated phenolic compounds (Conidi et al. 2011).

In ideal biorefinery, multiple products should be produced, and all types of renewable material residues should be able to be processed. To realize this concept, efficient separation processes are needed.

1.1 Aims and scope of the study

The present study focuses on developing a separation approach for the recovery of valuable compounds from biomass residues. In this approach, aqueous extraction and membrane separation processes are mainly employed due to their environmentally friendly, sustainable and cost-effective features. In the first part of this study, membrane-based separation processes are investigated to recover, concentrate and fractionate hemicelluloses from spruce wood autohydrolysates. In the second part, recovery and fractionation of phenolic compounds and sugars from carob kibbles through aqueous extraction and membrane separation processes are developed. The scope of this study is shown in **Fig. 1.1**.

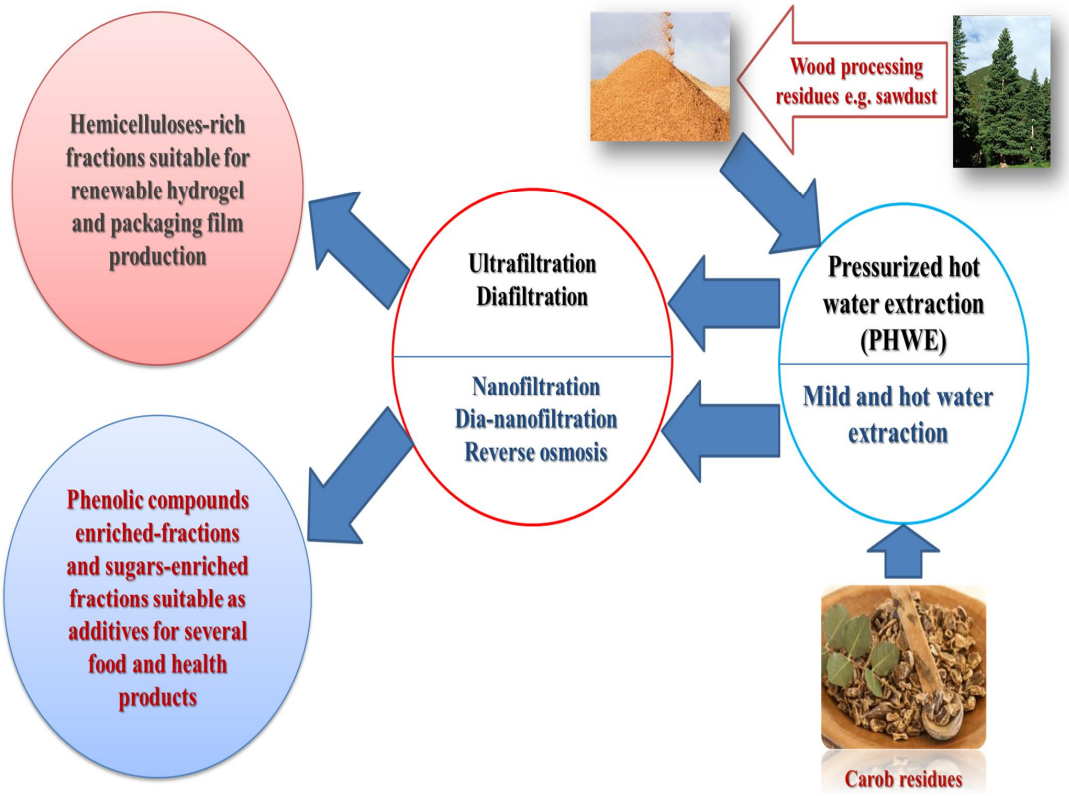


Figure 1.1 Scope of the study, presenting raw materials, separation processes and potential products.

The aims of the research focus on the following aspects:

- Increasing the understanding of the possibilities of enhancing an ultrafiltration-based fractionation and concentration of galactoglucomannans (GGMs) from spruce autohydrolysates using diafiltration and oxidation. The aim of applying diafiltration and oxidation is to improve the filterability of the autohydrolysates during fractionation and to increase the purity of the produced GGMs. It was assumed that GGMs will be concentrated and partially purified from unwanted impurities like lignin and extractives with UF meanwhile the pre- and/or posttreatment will modify the content of the concentrate fractions by removing these impurities.

- Developing a separation approach to recover, fractionate and concentrate phenolic compounds and sugars from carob residues. In this approach, aqueous extraction procedures to produce carob extracts with a certain quality are developed. Moreover, the applicability of nanofiltration and reverse osmosis in improving the quality of these extracts is assessed. It was assumed that the sugars and valuable phenolic compounds could be extracted at different operating conditions. Furthermore, the recovery of these compounds into two distinct extracts could be achieved with nanofiltration and reverse osmosis.

1.2 Outline of the study

In this research, the separation of high value-added compounds, in particular GGMs and phenolic compounds from biomass residues through aqueous extraction and membrane filtration processes are investigated. In order to develop this hybrid separation approach, advance understanding of each separation technique applied is of major importance. Therefore, the literature review of this thesis begins in **Chapter 2** with a summary of the extraction methods of hemicelluloses and the background of hot water extraction with focus on the principles and applications of pressurized hot water extraction. **Chapter 2** also includes the methods to extract phenolic compounds from wood and agro-food residues. **Chapter 3** covers the utilization of membrane processes in the recovery of hemicelluloses, mainly GGMs, from spruce autohydrolysates, as well as the possible challenges of such separation. The recovery of phenolic compounds from biomass-based liquors with a membrane is presented in the second section of **Chapter 3**. **Chapter 4** contains a description of the raw materials used in this study. The procedures for performing aqueous extraction and

membrane filtration experiments and the analysis methods for the characterization of various process streams are also described in **Chapter 4**.

The experimental part of this study focuses on presenting the performance of extraction and membrane separation processes. The main results and findings that serve the objectives of this work are presented in **Chapter 5**. This chapter is divided into two sections. In the first section, the results of the ultrafiltration scheme used to produce concentrated GGMs fractions from spruce autohydrolysates are presented and discussed **(Paper I)**. Moreover, evaluation of the performance of the membrane-based hybrid process in the enhancement of GGMs recovery is presented **(Papers I and II)**. The second section of this chapter contains discussion of the main findings that were reached when phenolic compounds and sugars were recovered from carob kibbles **(Papers III and IV)**. In the end of the thesis, concluding remarks and the core findings of the research valuable for future research are discussed in **Chapter 6**.

2 Extraction of hemicelluloses and phenolic compounds from biomass

In this chapter, the isolation of hemicelluloses and phenolic compounds from biomass with extraction using different solvents is discussed. The focus is on the extraction of these compounds with water-based extraction processes and the effect of different parameters on the extraction result are reviewed in detail.

2.1 Extraction of hemicelluloses from lignocellulosic biomass

The choice of the appropriate extraction method for any biomass depends on the feedstock and the target compounds as well as the economic prospect and environmental impacts of the process. As each technique has its benefits and drawbacks, an effective extraction process is characterized by several criteria. These criteria include the quality of the produced streams and the generation of by-products, as well as energy consumption and the cost efficiency. The main challenge in the extraction of hemicelluloses from biomass is being able to recover them as polymers with a feasible yield.

Table 2.1 presents various extraction methods used in the isolation of hemicelluloses from lignocellulosic biomass. The choice of the extraction method has a significant effect on the form in which hemicelluloses can be separated (as hemicelluloses or as monosaccharides and/or oligosaccharides). For instance, it is easier to maintain hemicelluloses and not to degrade them to monosaccharides when the extraction is done with water, compared to extraction with strong acids. Compared to other methods, water is one of the green reagents that make lignocellulosic fractionation a nontoxic, sustainable and probably cost-efficient process (Yu et al. 2008; Peng et al. 2012).

Table 2.1 The most common methods utilized for the extraction of hemicelluloses from lignocellulosic biomass (based on Kumar et al. 2009; Menon and Rao, 2012; Bundhoo et al. 2013).

Biological pretreatment	Chemical pretreatment	Physico-chemical pretreatment
• Enzymatic hydrolysis	• Acid hydrolysis • Alkaline hydrolysis • Organosolv hydrolysis • Ozonolysis • Hydrolysis with ionic liquids	• Hydrolysis with hot water • Steam explosion • CO_2 explosion • Wet oxidation • Ammonia fibre explosion

2.2 Extraction of hemicelluloses with hot water

The fractionation of lignocellulosic biomass into its components using hot liquid water extraction (> 100 °C) has been studied widely (Garrote et al. 1999; Ando et al. 2000; Allén et al. 2001). This type of extraction is known as a pressurized hot water extraction (PHWE). In the literature also other terms, such as hydrothermal treatment, auto-hydrolysis with liquid water, sub-critical water extraction, and hot compressed water extraction have been used to describe this extraction method.

PHWE has been considered an environmentally friendly separation technique of different compounds from samples taken for environmental monitoring purposes as well as for the extraction of bioactive compounds from of natural matrices (Hartonen, 1999; Garrote et al. 1999; Teo et al. 2010). The major advantages of this method include easy handling and disposal of water, and the fact that the use of water as the extraction solvent does not increase the chemical load caused by the extraction process. In many cases, the availability of water is also good. The costs of water use are also lower compared to other extraction solvents. Hence, PHWE is recognized as a green extraction method for different target compounds present in several kinds of biomass (Hartonen et al. 2007; Lo et al. 2007; Kilpeläinen et al. 2014).

Table 2.2 presents examples on studies where the extraction of hemicelluloses from different raw materials with PHWE has been in focus. The results of these extraction experiments are difficult to compare due to the fact that the feedstocks and extraction conditions (temperature, time, pH) and procedures applied in the experiments have been different. PHWE is not selective for the extraction and dissolution of hemicelluloses. Typically, other compounds, such as lignin, monosaccharides and extractives are also dissolved. The recovery and purification of hemicelluloses from these co-extraction compounds in the autohydrolysates often needs other separation processes, such as ultrafiltration (Persson et al. 2010). As **Table 2.2** shows, extraction at higher than 200 °C leads to significant degradation of hemicelluloses to monosaccharides independent of the raw materials. For instance, Mok and Antal (1992) extracted almost 100% of the hemicelluloses, 90% of them as degradation products i.e. monosaccharides, when biomass materials (eucalyptus and populus woods) were exposed to water extraction at a temperature of 200-230 °C **(Table 2.2)**. Moreover, the solubilisation of lignin was about 50% of its content in the wood. In general, it is very difficult to avoid the dissolution of lignin completely with PHWE, although exact values of lignin in the extraction liquors (autohydrolysates) are not usually reported.

Von Schoultz (2014) recently suggests new approach to enhance the extraction of hemicelluloses from biomass. In this approach, the circulation and impregnation of extract under reduced pressure were applied during hot water extraction of Scots pine chips at 150 °C. This allows to purify the extract from unwanted impurities mainly lignin. It also helps to minimize the oxidation and degradation of the extract. The produced extract had high concentration and purity (96% carbohydrates with only 0.5% lipophilic compounds) compared to the extract obtained without impregnation and circulation (55% hemicelluloses, 35% lignin and 5% lipophilic extractives).

Table 2.2 Examples of studies on hot water extraction to isolate hemicelluloses from several lignocellulosic biomass materials.

Biomass	Operating conditions	Observations	Reference
Cellulosic matter mainly biomass straw	Continuous water flow through a static biomass. 200-275 ºC	Most of sugars-hemicelluloses dissolved at 200 °C; yield enhanced by increasing the hot water flow rate. Lignin also dissolved at 200 ºC.	Bobleter et al. (1976, 1994)
Various woody and herbaceous biomasses	200-230 ºC 15 min	Complete recovery of hemicelluloses (90% as monomers) with partial solubilisation of lignin (35-60%).	Mok and Antal (1992)
Softwood (Japan cedar) biomass	180 °C 20 min	Most of the hemicelluloses was dissolved and their degradation was detected, some lignin also dissolved.	Ando et al. (2000)
Wheat straw	200 °C 0-40 min	The recovery of hemicellulose- derived sugars decreased from 53% to 7% of content in straw due to degradation.	Pérez et al. (2007)
Wheat straw	184 °C 24 min	The recovery of hemicellulose- derived sugars was 71% of the content in straw at these optimum conditions.	Pérez et al. (2008)
Corn fibre	215 °C 2 min	Pentose recovery mainly as oligomers 82%.	Allén et al. (2001)
Birch	180 and 240 °C Batch reactor up to 180 min	Complete degradation of xylans to oligomers, monomers, furfural and acetic acid.	Borrega et al. (2011)
Pine	160- 190 °C Various times. Time-temperature effect described by the H-factor	Up to 50% of wood hemicellulose extracted. 160 °C, 65 min. Polymeric hemicellulose extracted (8% of wood weight) with 3% monosaccharides and a minor amount of lignin (0.5%).	Yoon et al. (2008)

The extraction of galactoglucomannans (GGMs) from spruce with hot water has been in focus in many studies, and examples on these are presented in **Table 2.3**. As shown in the earlier studies (**Table 2.2)**, **Table 2.3** reveals that the extraction of GGMs from spruce wood could be performed at different operating conditions. However, the feedstock has been the same, so that the comparison of the results is a little easier. There are several parameters which influence the yield of GGMs in the PHWE process. The main parameters are the particle size of the feedstock, the extraction temperature, and time. Moreover, the structure of the extraction equipment has a significant effect on the yield, because it can either enhance or delay the mass transfer between the feedstock and the extraction solvent. Furthermore, the yield of GGMs with PHWE can also be influenced by introducing additives such as compounds buffering the pH change during the extraction (Hartonen, 1999; Teo et al. 2010; Song et al. 2011 b).

In PHWE, temperature is used to modify the dissolving power (polarity) of water. The key factor that presents the polarity effect and the interaction between the solute and the solvent is the dielectric constant. This factor decreases with the increasing temperature. The dielectric constant of water drops from 80 at room temperature to 27 at 250 ºC and 50 bar (Teo et al. 2010). A solvent with a high dielectric constant is able to dissolve high polar and ionic compounds, while a solvent with a low dielectric constant is able to dissolve low polarity compounds. At critical conditions of pressure and temperature, the dielectric constants and the densities of gaseous and liquid water are the same. In these conditions, the solubility of non-polar gases and organic compounds in water become high. When the temperature increases, the hydrogen bonds between the water molecules decrease, and that causes a drop in the solubility of inorganic (polar) compounds in water (Teo et al. 2010). When the temperature of water increases, the solubility of wood compounds, especially the low-polar ones, e.g. hemicellulose and lignin, increases. Therefore, in general the yield of extracted GGMs increases steadily with the operating temperature. However, the higher the extraction temperature, the more the GGM chains degrade. Thus, when the goal is to isolate GGMs from spruce, the temperature at which a reasonable yield of GGMs can be produced without a significant loss in their chain length, has to be found. The losses in chain length at a high temperature can also be decreased by decreasing the extraction time. Thus, when the desired molar mass of the target GGMs is known, the extraction temperature and time can be optimized accordingly.

Table 2.3 Examples of studies on hot water extraction of GGMs from spruce wood.

Study	Operating conditions	Observations
Batch extraction system		
Song et al. (2008)	Ground spruce wood 160 – 180 °C, up to 100 min Accelerated solvent extractor (ASE)	80 – 90% of GGMs extracted at 170-180 °C and 1h. pH decreased to 3.6-3.8 with extraction time, the lower pH was at 180 °C extraction. Highest M_w of 35 kDa at 160 °C and 5 min.
Song et al. (2011 b)	Ground spruce (<1 mm) pH levels (3.8, 4.0, 4.2 and 4.4) adjusted by phthalate 170 °C 20, 60 and 100 min ASE	The highest molar mass of hemicelluloses (14 kDa) extracted when the pH was the highest (4.4) and the extraction time the lowest (20 min). The advantage of extraction with a phthalate solution (pH ~ 4) over extraction with only water is a lower degradation and deacetylation of GGMs.
Pranovich et al. (2016)	Ground spruce (0.25–1.0 mm) 170 °C 60 min extraction in two steps using ASE	Regardless the time ratios between the 1st and the 2nd extractions, the total yield of the dissolved material was the same (25% of the wood). The highest yield of hemicellulose having highest molar mass (10 kDa) was 7% on dry wood basis at 20 min. GGMs were ~ 80% of the precipitated polymeric material.
Krogell et al. (2013)	Ground spruce sapwood with particle size between 0.5 and 12.5 mm 170 °C, up to 120 min Autoclave extractor setup	After 10 min of extraction, hemicellulose molar mass decreased rapidly from 30 kDa. The optimal time to achieve the maximum yield (50%) of high molar mass hemicelluloses (10 kDa) was 20 min. With smaller particle sizes, faster extraction and a higher yield were observed (at 20 min, the yield was 3 times higher than with the largest particles).
Lundqvist et al. (2003)	Milled spruce chips 180-190 ºC (5 min) 200 ºC (2 min) Water extraction in a microwave oven	At 190 ºC the yield of poly-and oligosaccharides was 78% (2 times higher than in other trials). Weight-average molar mass (M_w) of 3.8 kDa (190 ºC), 3.3 kDa (200 ºC) and 6.5 kDa (180 ºC).

Flow-through/ cascade reactor extraction system		
Leppänen et al. (2011)	Sawdust 120–240 °C. Extract collection time 30 min Flow rate 1 ml water/min	160 °C: 50% of hemicelluloses extracted 220 °C: most of the hemicelluloses and 15% of lignin extracted. 170 °C: the highest measured molar mass of hemicellulose ~31 kDa. Monosaccharides content was between 4-22%. pH decreased from 5.3 to ~ 4 during extraction (160-240 ºC).
Grénman et al. (2011)	1.25- 2 mm spruce sapwood chips 150-170 °C, up to 120 min Solid load = 6.25 g of dry wood/L	GGMs yield was 60% and increased with time and temperature up to 80%. pH decreased from 5.8 to 3.7. The degradation of hemicelluloses was 17%. The reaction rate increased considerably with the temperature.

As **Table 2.3** shows, an extraction temperature above 200 °C leads to high degradation of hemicelluloses. Therefore, although the yield of total carbohydrates might be high, the yield of polymeric compounds is low. This can be proved by the increase in the amount of degradation products, mainly monosaccharides, in the extract. In many cases, the extraction of GGMs from spruce has been studied in temperatures between 160 and 180 °C **(Table 2.3)**. At this temperature range, a high yield of GGMs (up to 90%) can be obtained. A low extraction temperature might be feasible in decreasing the amount of impurities in the produced extract and in maintaining the hemicellulose chain length. During PHWE of hemicelluloses presented by Leppänen et al. (2011), the dissolution of lignin increased from about 5% at 160 °C to 15% at 220 °C. Thus, extraction with water also at low temperature has been studied. For instance, Örså et al. (1997) and Willför et al. (2003 b) have done extractions with water at the temperature range between 20 and 90 °C. In their experiments, only a minor amount, about 1– 5 % of AcGGM in spruce wood was dissolved to the extract. Willför and Holmbom (2004) isolated GGMs from Norway spruce at room temperature for 1.5 h and the yield was about 5.2 mg hemicellulose/ g wood (average molar mass of 21 kDa).

A high extraction temperature could be applied in the PHWE process for a short time without too significant hemicellulose losses. For instance, as can be seen in **Table 2.3**, a short time (less than 10 min) was enough to extract hemicelluloses with molar mass of 30 kDa at 170 ºC using both the

batch (Krogell et al. 2013) and flow-through (Leppänen et al. 2011) extraction system. In the study of Krogell et al. (2013), the molar mass decreased rapidly to 10 kDa after 20 min of extraction. Lundqvist et al. (2003) obtained a considerable amount of poly- and oligosaccharides (78%) in a short extraction time (5 min, 190 ºC). They used a microwave oven to improve the heat treatment of wood, which probably facilitated the dissolution of hemicelluloses in the extraction process. Regardless the particle size of the wood chips and sawdust, Krogell et al. (2013) found that the extraction time of 20 min at 170 ℃ was the best for the isolation of polymeric materials (~ 10 kDa), mainly GGMs, with less formation of monomeric sugars. A yield of high molar mass hemicelluloses (7-8% on dry wood basis) was obtained at almost the same conditions by Song et al. (2011 b) and Pranovich et al. (2016).

In hydrothermal treatment of biomass, water acts simultaneously as a solvent and a reactant (Liu and Wyman, 2005). At a high temperature, water tends to have acidic features, for example at 220 ℃ the pH of water decreases from neutral (7.0 at 25 ℃) to acidic pH ~5.5 (Marshall and Franck, 1981). Presence of acidic conditions during extraction enhances the ionization of water especially at high temperatures (Kim and Lee, 1987). Water auto-ionization leading to generating hydronium ions liberates organic acids and releases other anionic compounds (Zumdahl and Zumdahl, 2007). These sequences eventually cause cleavage of the glycosidic bonds between the monosaccharide units, which causes the degradation of hemicelluloses to compounds with a lower molar mass, for instance to organic acids (Antal, 1996; Lai, 2001). If a pH buffer is not used, pH decreases during the PHWE due to the formation of organic acids. Typically, without buffering, the pH of a pressurized hot water extract is between the values 3 and 4 (Brasch and Free, 1965). When the feedstock contains mainly GGM, the hydrolysis of the acetyl groups in the mannose units of the GGM molecule leads to the formation of acetic acid. This decreases the pH further and promotes the degradation of hemicelluloses. For instance, the *O*-acetyl and *D*-galactosyl side groups in GGMs are sensitive to cleavage at acid conditions. At neutral and alkaline conditions, only cleavage of the *O*-acetyl side-groups on GGM would occur. The deacetylation contributes also to GGM precipitation usually as aggregation bundles. The solubility of these GGM aggregates is rather low (Hannuksela et al. 2002). However, compared to temperature, pH has a minor effect on the actual extraction kinetics, including the dissolution rate, even though its effect is clear on the degradation of wood compounds (Grénman et al. 2011).

In order to prevent the degradation of GGMs caused by the pH decrease during PHWE, Song et al. (2011 b) have tested the effect of a phthalate $NaHCO_3$ buffer on controlling the pH. With the buffer, the formation of monosaccharides from hemicelluloses was decreased by 70%. The hydrolysis of acetyl groups was decreased by 40% so that the water solubility of GGMs was maintained. Due to the inhibition of hydrolytic cleavage of polysaccharide chains and acetyl groups, the extracted GGMs had higher molar mass than the plain water extraction (Song et al. 2011 a). Krogell et al. (2014) developed in line system using stabilized Zr/ZrO_2 pH electrode to measure the pH during hot water extraction of hemicelluloses from spruce. The measured pH value in line at 170 ºC was 0.35 pH units higher than when the pH of extract was measured at room temperature. They explained this difference by higher dissociation of acetic acid at higher temperatures. This system was tested by Krogell et al. (2015; 2016) to enable the extraction of high molar mass hemicelluloses. The system was combined with a controller and HPLC pump to pump alkali solution (0.5 M NaOH) when the pH dropped. Comparing with no pH controlled extraction they found that higher molar mass hemicelluloses could be extracted at higher pH values (4.85 at 170 ºC and 5.15 at 180 ºC) where the formation of monomeric sugars could be avoided. At these conditions, the loss in the yield of the hemicelluloses could be low.

The particle size of the feedstock has significant influence on the extraction yield of hemicelluloses, because it affects the mass transfer between the feedstock and the extraction solvent. In general, the yield of GGMs has been found to be highest from the finest spruce particles (< 0.1mm), especially during the initial stage of extraction (Song et al. 2012; Krogell et al. 2013). Song et al. (2008) found that the yield of hemicelluloses from chips was about 60% lower than that from ground wood. The size of the wood particles affects the average molar masses of the extracted hemicelluloses slightly. Song et al. (2012) and Krogell et al. (2013) found that GGMs with high molar mass could be extracted easier from small wood particles. Moreover, the isolation of other wood components, such as lignin varied by about 10% with the wood particle sizes (0.5 to 12.5 mm). The release of acetic acid was slightly higher with the smallest particle size.

The mass transfer during the PHWE process is also greatly influenced by the structure of the extraction equipment used. Various extraction systems operating in the static and dynamic modes have been employed in the extraction of GGMs (Leppänen et al. 2011; Krogell et al. 2013). The differences in the extraction procedures and sampling in the studies presented in **Table 2.3** could

partly explain the variation in the extraction results of using the same extraction system at similar conditions. In general, a higher yield of GGMs was obtained with a static batch extractor. Among the extraction systems utilized, continuous flow-through mode extraction was found more efficient in avoiding degradation of the extracted GGM molecules because of their short time exposure to severe conditions (Leppänen et al. 2011). At the same extraction conditions (170 ℃, 60 min), utilization of different batch extraction setups leads to variation in the extraction yield. Krogell et al. (2013) obtained a yield of hemicelluloses (molar mass > 5 kDa) ~180 mg of hemicelluloses/g wood when using an autoclave batch extractor. This was higher than the yield of hemicelluloses (~120 mg of hemicelluloses/g wood) obtained by Song et al. (2012) when using an accelerated solvent batch extractor. The design of the extraction system had an effect on the extraction yield. For example, Leppänen et al. (2011) obtained a lower hemicellulose yield (70 mg of hemicelluloses/g wood) with flow-through extraction than the yield of 125 mg of hemicelluloses/g wood obtained by Song et al. (2008) with ASE batch extraction at 170 ℃ for 20 min. The two-step PHWE extraction using the ASE system was found a suitable approach to achieve better fractionation of the wood hemicelluloses (170 ℃ for 60 min) where the first fraction (after 20 min) contained hemicelluloses with weight average molar mass of 8–10 kDa and the second one with weight average molar mass of 6-2 kDa (Pranovich et al. 2016).

2.3 Extraction of phenolic compounds from lignocellulosic biomass

Phenolic compounds can be isolated from biomass using different extraction methods. Different types of phenolic compounds have been extracted from wood and agro-food residues, such as olive, grape and carob residues. The yields and types of phenolic compounds and their antioxidant activity are the main parameters that have been considered when evaluating the performance of extraction methods, and they have been found to depend strongly on the operating conditions of the extraction processes, like the type of solvent.

2.3.1 Extraction of phenolic compounds from wood

In addition to hemicelluloses, wood contains phenolic compounds, many of which are valuable in different food and health applications due to their antioxidant properties. Different types of phenolic compounds with different portions have been recognized in the wood of trees, e.g. pine, birch, spruce, and aspen. Specific wood parts like knots, leaves needles, cork and bark have been identified as rich sources of natural phenolic antioxidants (Kähkönen et al. 1999; Pietarinen et al.

2006). Especially in knotwood, the amount of extractable phenolic compounds with antioxidant activity has been found greater than in other parts of tree wood (Willför et al. 2003 d; Pietarinen et al. 2006). Lignans, oligolignans, stilbenes like pinosylvins and flavonoids like catechins and their derivatives are examples of hydrophilic phenolic compounds that have been found in several wood species (Willför et al. 2003 c, d). For instance, flavan-3-ols, catechin, quercetin, isorhamnetin, lignans, coumarins, hydroxybenzoic acids, hydroxycinnamic acids, and kaempferol have been reported to be present in Norway spruce residues (Strack et al. 1989; Kähkönen et al. 1999; Willför et al. 2003 c, d).

Different solvents have been utilized to extract phenolic compounds from wood materials (Kähkönen et al. 1999; Moure et al. 2001). Polar organic solvents like alcohol are frequently employed to extract phenolic compounds. For example, aqueous methanol and aqueous acetone were tested by Kähkönen et al. (1999) to isolate phenolic compounds from various types of wood residues. In their study, the highest content of phenolic compounds with antioxidant activity was found in spruce needles (~155 mg $_{GAE}$ (Gallic acid equivalent)/g of dry matter), where flavan-3-ols, (+)-catechin and (+)-gallocatechin were detected in the extract. Extraction with aqueous methanol (50%), followed by liquid-liquid extraction with ethyl acetate and diethyl ether was performed by Fernãndez de Simõn et al. (1996) to extract low molar mass phenolic compounds like gallic, ellagic, vanillic, syringic, and ferulic acids from oak wood. Pinelo et al. (2004) found that methanol and ethanol had superior performance (the yields were 11% and 8%, respectively, 4-5 times higher) compared to acidified water (pH=4) in the extraction of phenolic compounds with antioxidant activity (inhibition on DPPH free radicals) from pine sawdust. Ebringerová et al. (2008) extracted xylans from corncobs using alkali extraction and used it as a reference to test the antioxidant and DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activities of galactoglucomannan from thermomechanical pulping (TMP) process water of Norway spruce. In their study, due to their immuno-potentiating and antioxidant properties, AcGGM and GGM were proposed as additives for food and pharmaceutical products.

In addition to organic solvent extraction, water extraction has recently been used for the isolation of phenolic compounds with antioxidant activity from wood. Unlike organic solvents, water is a green solvent and offers a promising alternative due to several advantages, such as its abundance, low cost, non-hazardousness and less expense at disposal. Prudêncio et al. (2012) optimised

aqueous extraction of phenolic compounds from mate tree barks. They found that the optimum conditions to obtain the highest phenolic compound yield (1 g solids in 100 mL water) was 85± 5 °C for 1.5 min. The extract produced at these conditions contained ~1.6 mg chlorogenic acid equivalent/mL. Diouf et al. (2009) obtained a water extract (prepared at 80-100°C) rich in oligomeric proanthocyanidin and polymeric proanthocyanidin with antioxidant and anti-inflammatory activity from *Picea mariana* (black spruce) bark. Hartonen et al. (2007) propose PHWE to recover naringenin and other major flavonoids (dihydrokaempferol and naringin) from knotwood of aspen. In their study, the operating conditions of PHWE were optimized at 150 °C, 35 min and 220 bar. They claim that compared to methanol Soxhlet extraction (sonication or reflux for 24 h and over, yield of 11.5 mg flavonoids /g dry matters), PHWE proved to be a cheap, fast and effective extraction method with good recovery (10 mg /g dry matters) of bio-functional flavonoids from aspen knotwood. It can be concluded that hot water extraction of phenolic compounds is affordable and could achieve as a good yield of bioactive compounds as other common solvents. Compared with toxic solvents, water-based extracts are hygienic and could be utilized safely for food applications.

Isolation of phenolic compounds from wood has been performed using various apparatuses and systems. Soxhlet-, ultrasonic- or accelerated solvent extraction with methanol (Pietarinen et al. 2006; Hartonen et al. 2007) and CO_2 supercritical fluid extraction (Peng et al. 2006) have been employed for the extraction of various phenolic compounds from wood. Willför et al. (2003 c) used an accelerated solvent extractor (ASE) equipped with acetone: water (95:5, v/v) mixture for the isolation of phenolic compounds from Norway spruce knots and stemwood. In their study, lignans were identified as the main phenolic compounds in the extract. In addition, the knots contained less lipophilic than hydrophilic extractives. The ASE was also used by Willför et al. (2003 d) as extraction apparatus for extractives and phenolic compounds, i.e. lignans and the flavonoid taxifolin from knotwoods of several tree species like Norway spruce, birch and Scots pine. They found that the content of hydrophilic extractives with high antioxidant potency was 10-20% of the dry knotwood in most of the species under investigation. Peng et al. (2006) employed CO_2 supercritical fluid extraction for the extraction of antioxidant-active flavonoids from wood prior further recovery with high-speed counter-current chromatography.

Extraction of phenolic compounds from wood that is utilized in pulping manufacturing or is subjected to acid hydrolysis of hemicelluloses for fermentation processes has been considered in some studies. Extraction of phenolic compounds with antioxidant activity from wood prior to pulping is proposed in the review by Huang et al. (2008). They claim that this pre-extraction might enhance the profitability of the pulp mill when the extracted phenolic compounds could be used as renewable food additives. Holmbom et al. (2002) suggest extraction with pure water or an aqueous alcohol mixture to separate phenolic compounds from wood by-products, mainly knots. They claim that combining the extraction of phenolic compounds in connection with the manufacturing of pulp could be economically feasible in a pulp mill. González et al. (2004) have applied a process for the production of both monosaccharides (degradation from hemicelluloses) and antioxidant phenolic compounds from *eucalyptus globule* wood chips. Their process (**Fig. 2.1**) included acid hydrolysis of wood using sulphuric acid and separation of the solid phase from wood hydrolysates by filtration, followed by ethyl acetate extraction of phenolic compounds from the hydrolysates. Vacuum evaporation was used for the recovery of the solvent from the antioxidant extract. The ethyl acetate extract contained about 0.43 g of gallic acid equivalents/100 g dry wood with antioxidant activity of 61% of that presented by BHA (reference antioxidants in butylated hydroxyanisole). Thus, the extracted hydrolysates had better fermentation ability.

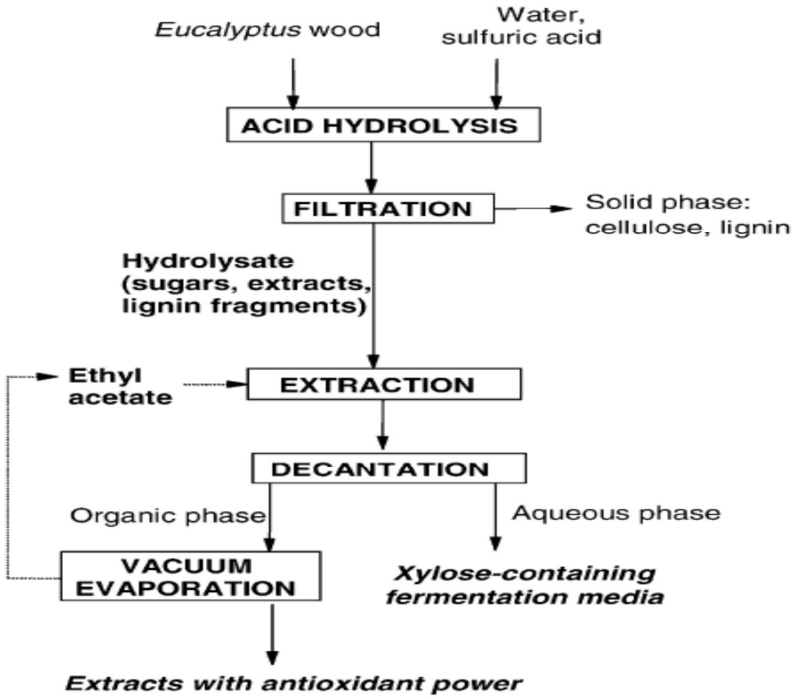


Figure 2.1 Scheme of the extraction process of hemicelluloses and antioxidants from eucalyptus wood (González et al. 2004, p.245).

2.3.2 Extraction of phenolic compounds from agro-food residues

Phenolic compounds with antioxidant activity extracted from agro-food industry wastes could be used in several food and pharmaceutical products (Scalbert et al. 2005). These compounds have been identified in a number of agro-industrial by-products, including grape seeds (Nawaz et al. 2006; Casazza et al. 2011), olive mill wastes (Paraskeva and Diamadopoulos, 2006; Marco et al. 2007), potato peel (Singh and Saldaña, 2011) and carob residues (Turhan et al. 2006), as well as various other agro-biomass residues (Sakakibara et al. 2003; Makris et al. 2007). Kähkönen et al. (1999) studied the phenolic compound content and activity in a wide range of edible and nonedible plant materials. Of various vegetable residues, the phenolic compounds in the peels of beetroot and sugar beet (4.3 mg $_{GAE}$/g dry materials) had the highest antioxidant activity. They also report that the highest phenolic compound content with remarkable antioxidant activity is present in wood residues like spruce needles and pine bark. Moure et al. (2001) have reviewed various extraction methods of phenolic compounds from agro-industrial residues, including the effect of different operating conditions. They point out that the effects of pH and temperature are variable. This might

be due to the fact that the phenolic compounds are a group of very diverse chemicals and they are present in a wide range of natural resources having different chemical structures. In general, the risk of decomposition of phenolic compounds increases at high temperatures, but in some cases, this degradation may produce compounds that are more active. Different pH values (acidic or alkaline conditions) have been found to have an effect on the antioxidant activity and yield of phenolic compounds. For example, during aqueous extraction of oat fibre, Lehtinen and Laakso (1998) obtained the highest yield of phenolic compounds at a pH of 6, while the highest antioxidant activity was noticed at a pH of 10. At these alkaline conditions, the phenolic compound content in the aqueous extract was even twice that of the methanol extract. They also observed that lowering the pH to acidic conditions led to precipitation of phenolic compounds.

Table 2.4 contains several examples of studies on the extraction of phenolic compounds from different biomass by-products, like plant and agro-food residues. In general, diverse groups of phenolic compounds, including gallic acid and its derivatives, catechin and its derivatives, as well as quercetin, are the most common bioactive compounds extracted from plant and agro-food residues. Other co-extraction compounds, such as carbohydrates, lipids and protein may also dissolve. Polar solvents, mainly ethanol and methanol are frequently utilized for carrying out this extraction. As **Table 2.4** shows, ethanol and methanol were better (2-3 times higher yield) than acidified water to extract phenolic compounds from almond hulls. However, the methanolic extract from almond hulls had higher antioxidant activity than the other extracts. Subcritical water was found to be favourable as a good, harmless substitute for alcoholic solvents to extract a higher amount of phenolic compounds (~81.8 mg $_{GAE}$/100 g) from potato residues (Singh and Saldaña, 2011). They found that the solubility of different phenolic compound groups was dependent on the extraction solvent. Moreover, the degradation of phenolic compounds occurred at a water extraction temperature higher than 180 ºC. Ethyl acetate has been commonly used to extract phenolic compounds from the liquid streams. It was found to be quite selective to extract low and medium size phenolic compounds from olive mill wastewater (Visioli et al. 1999). Drosou et al. (2015) obtained water extracts with generally higher antioxidant activity than ethanol extracts from grape pomace. It can be seen in **Table 2.4** that the extraction solvent has an important effect on the yield of phenolic compounds. Besides the extraction solvent, precipitation of phenolic compounds at acidic conditions (Marco et al. 2007) or utilization of liquid-liquid extraction

(Takeoka and Dao 2003) before the extraction of phenolic compounds could to some extent explain the variations in the extraction yield form the same types of residues.

Particularly nonedible carob residues are recognized as one of the biomass residues containing a high content of different phenolic compounds. Owen et al. (2003) quantified 24 individual phenolic compounds in carob kibbles. **Table 2.5** contains examples of studies on the extraction of phenolic compounds from carob residues. As can be seen, the solvent, temperature and time are the main parameters that need to be taken into account in the extraction of phenolic compounds. Hot water extraction (temperature ~ 100 ºC) achieved a high yield of phenolic compounds with remarkable antioxidant activity from carob residues in a short time. For instance, Roseiro et al. (2013) obtained a water extract containing 4% of phenolic compounds with 85% and 90% inhibition of DPPH and ABTS (2, 2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) radicals, respectively, at 17 min. Moreover, a 19% yield of phenolic compounds was achieved by Kumazawa et al. (2002) in 10 min extraction of carob pods. These values are much higher than that achieved by methanol Soxhlet extraction (phenolic compound yield 0.4%, 3-5 hr extraction time) of carob fibre by Owen et al. (2003). Regardless the extraction solvents, carob extracts usually show high antioxidant activity, where they contain different forms of gallic acid (most abundantly 42%) including the free form and its derivatives: gallotannins and methyl gallate, as well as flavonoids (~11%), mainly glycosides myricetin- and quercetin-3-O--l-rhamnoside. Catechins, quercetin and its derivatives (~10%), kaempferol and its derivatives (~0.5%) and cinnamic acid have also been recognized (Owen et al. 2003; Papagiannopoulos et al. 2004; Roseiro et al. 2013). According to the literature, compounds with high antioxidant capacity extracted from carob residues can be utilized in the development of functional beverages (Roseiro et al. 2013), functional food or food ingredients (Kumazawa et al. 2002), or chemo-preventive drugs (Corsi et al. 2002).

Table 2.4 Examples of studies on the extraction of phenolic compounds from various agro-industrial wastes.

Residue	Solvent	Yield of phenolic compounds (% dry weight)	Example of identified phenolic compounds	Reference
Wheat bran	Ethanol	3	Gallic, vanillic, chlorogenic acids	Onyeneho and Hettiarachchy (1992)
Lentil seed coat	Water	5.2	Quercetin, protocatechuic, caffeic acid	Muanza et al. (1998)
Carob	70% acetone	1.9	Gallic acid, catechin derivatives	Avallone et al. (1997)
Grape pomace	Water	9.6	Anthocyanins and flavonol derivatives	Drosou et al. (2015)
	Ethanol	10.3		
Grape pomace	80% Ethanol	4	oligomeric procyanidins, gallic acid, flavan-3-ols like catechin	Lu and Foo (1999)
Grape seeds	Ethanol	10.5	Catechin, quercetin, flavonoids	Casazza et al. (2011)
Olive mill wastewater	Ethyl acetate	16	Elenolic acid, cinamic derivatives, quercetin, hydroxytyrosol	Visioli et al. (1999)
Olive mill wastewater	Ethyl acetate	0.27	Hydroxytyrosol, tyrosol, caffeic acid	Marco et al. (2007)
Almond hulls	Methanol	4.1	-	Pinelo et al. (2004)
	96% Ethanol	4.6		
	Acidified water (pH= 4)	1.8		
Almond hulls	Diethyl ether then methanol	0.043	Chlorogenic acid	Takeoka and Dao (2003)
Potato peel	Subcritical water	0.081*	Gallic acid, chlorogenic acid, caffeic acid…etc	Singh and Saldaña, (2011)
	Methanol	0.047*		
	Ethanol	0.030*		

*: Wet basis

Table 2.5 Examples of studies on the extraction of phenolic compounds from carob residues, including operating conditions and main observations, as well as the major identified phenolic compounds.

Study	Operating conditions	Yield of phenolic compounds	Identified compounds
Kumazawa et al. (2002)	Sugar removal with cold water followed by 10 min boiling water extraction	19.2% TP with high antioxidant activity from carob pods	Flavanols as catechins, proanthocyanidins
Bernardo-Gil et al. (2011)	Supercritical CO_2 extraction co-solvent 80% ethanol 15–22 MPa, 40–70 °C	0.016 g $_{GAE}$/100 g carob pulp with highest antioxidant capacity at 22 MPa, 40 °C	4-Hydroxybenzoic acid and cinnamic acid groups
Corsi et al. (2002)	Infusion of 1 g of carob pod or leaf powder with 0.1 L of boiling water for 15 min	Polyphenols as anti-proliferative agents 0.63 and 0.14 g /100 g of carob leaves and pods	Gallic acid ,(_) epicatechin-3-gallate, (_)epigallocatechin -3-gallate
Balaban (2004)	Aqueous methanol extraction from carob heartwood and sapwood	5 g TP/100 g dry weight of heartwood	Gallotannins, pro-anthocyanidins
Makris and Kefalas, (2004)	Various solvents 20 min, 30 °C	Maximum amount of total phenols 0.93 g $_{GAE}$/100 g dry matter in 80 % acetone extract	Proanthocyanidins, catechin, gallic acid
Roseiro et al. (2013)	Various water decoction times (8–20 min) and temperatures (80–100 °C)	Highest polyphenol yield 4 $_{GAE}$/ 100 g dry kibbles at 98.5 °C, 17 min	Gallic acid, (−)-epigallo catechin gallate, (−)-gallocatechin gallate, (−)-epicatechin
Papagiannopo - ulos et al. (2004)	Pressurized liquid extraction with water and organic solvents, and solid-phase extraction	With 50% acetone, max. yield ~ 0.4 g TP/100 g carob kibbles	Gallic acid, isoflavonoids flavonolglycosides

TP: Total phenoic compounds, GAE: Gallic acid equivalent

In addition to phenolic compounds, carob kibbles are rich in easily fermentable sugars, which can reach up to 50% on a dry basis (Roseiro et al. 1991 b; Avallone et al. 1997; Batlle and Tous, 1997). They also contain a special chemical pinitol suitable as an anti-diabetic agent (Macias Camero and Sanjuan Merino, 2003). The soluble sugars in carob residues can be used in a wide range of applications, including the production of ethanol, citric acid (Roukas, 1998), xanthan (Roseiro, 1991 a), and mannitol (Carvalheiro et al. 2011). Water extraction at 25 ºC has been utilized in sugar removal for the production of carob fibre (Haber, 2002), or before further recovery of other valuable compounds like polyphenols (Kumazawa et al. 2002). The multiple-column water extraction process (25 ºC at liquid to solid ratio, LSR of 2), combined with the lime milk purification treatment technique was used by Petit and Pinilla (1995) to extract sugars from carob pods. In their study, a sugar fraction (580 g/kg, 62° Brix with 93% purity) suitable for commercial utilization in the food industry was obtained. Roseiro et al. (1991 b) obtained a sugar yield of 60 % (with concentration of 200 g/L) from carob pods with a multistage water extraction system. Manso et al. (2010) report that the optimal sugar extraction from carob pulp with a yield of 94% was achieved at a LSR of 10 (25 °C, for 1 h). As shown by the studies presented above, carob residues are a valuable source of phenolic compounds and sugars, and thus the development of their extraction with a green solvent like water needs more investigation.

3 Membrane filtration in the recovery of galactoglucomannans and phenolic compounds from aqueous extracts of biomass

The hemicelluloses in biomass extracts need to be recovered and purified before utilization in various value-added products like hydrogels (Söderqvist-Lindblad et al. 2001) and barrier films (Hansen and Plackett, 2008). For this purpose, pressure-driven membrane processes play a key role. These processes could provide good concentration and fractionation capabilities with no usage of chemicals and rather low use of energy. Therefore, Novalin and Zweckmair (2009) propose membrane operations as promising separation techniques for several valuable substances, such as oligosaccharides and phenolic compounds in green biorefineries. **Table 3.1** presents the main applications of microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), and reverse osmosis (RO) in biorefineries. These applications include concentration and purification of target compounds in hydrolysates, recovery of hydrolysis solvents or fermentation enzymes, and removal of compounds that might limit the further processing of hydrolysates, such as fermentation inhibitors.

Table 3.1 Overview of the utilization of the main membrane processes in biorefinery (based on He et al. 2012)

MF	UF	NF / RO
• Removal of solid particles and turbidity	• Concentration of oligo-and poly-saccharides	• Separation of acetic acids
• Recovery of extractives	• Recovery of lignin	• Reuse of water or solvents
• Separation and purification in production of bio-oil and biodiesel	• Separation and purification in production of biofuels and bio-chemicals	• Removal of fermentation inhibitors like furfural
• Recovery of enzymes	• Recovery of enzymes	• Concentration of monosaccharides

Fig. 3.1 depicts a pressure-driven membrane process that can be suitable for the separation of individual or groups of biomass compounds. In general, the recovery of hemicelluloses from

lignocellulosic liquor can be achieved with UF. The suspended and colloidal matters and extractives comprising a large group of substances, e.g. fatty acids, resin acids and tannins, are mostly separated from such streams with MF. Lignin and its complexes with hemicelluloses are removed on the basis of their molar mass during UF and NF. Concentration of monosaccharides occurs mainly by NF or RO. Moreover, NF and RO could be employed for recycling and reuse purposes of either water or extraction solvents.

The separation of hemicelluloses has been performed using different membrane materials, configurations and modules. Polymeric membranes with various pore sizes and active layer characteristics have been tested in several studies investigating the recovery of hemicelluloses (Hartman et al. 2006 a, b; Persson et al. 2007; Zeitoun et al. 2010; Krawczyk et al. 2013 a, b). Ceramic membranes have also been tested (Persson et al. 2010; Hasan et al. 2011). Various configurations of membrane modules, including tubular (Colyar et al. 2008), flat sheet/plate-and frame (Almanasrah et al. 2012), spiral wound (Krawczyk et al. 2013 a) and hollow fibre ones (Zeitoun et al. 2010) have been utilized in processing different types of biomass hydrolysates.

The performance of the membrane processes employed for hemicellulose recovery can be evaluated by various parameters like permeate flux and retention, purity, and concentration of hemicelluloses. He et al. (2012) address in their review the applications of membrane processes in biorefinery e.g. for the recovery of hemicellulose. As reported in their review, the permeate flux during the separation of hemicelluloses was in the range of 7-380 L/m^2h, and the retentions of hemicelluloses varied between 53%-100%. This wide range of permeate flux and retention values is due to some extent to the differences in the membrane processes utilized. However, the performance of hemicellulose recovery was influenced by not only the specification of the membrane process, e.g. membrane active material, cut-off and module, but also by other factors, such as the operating conditions and parameters, feed specifications, and the interaction between the membranes and the feed liquors.

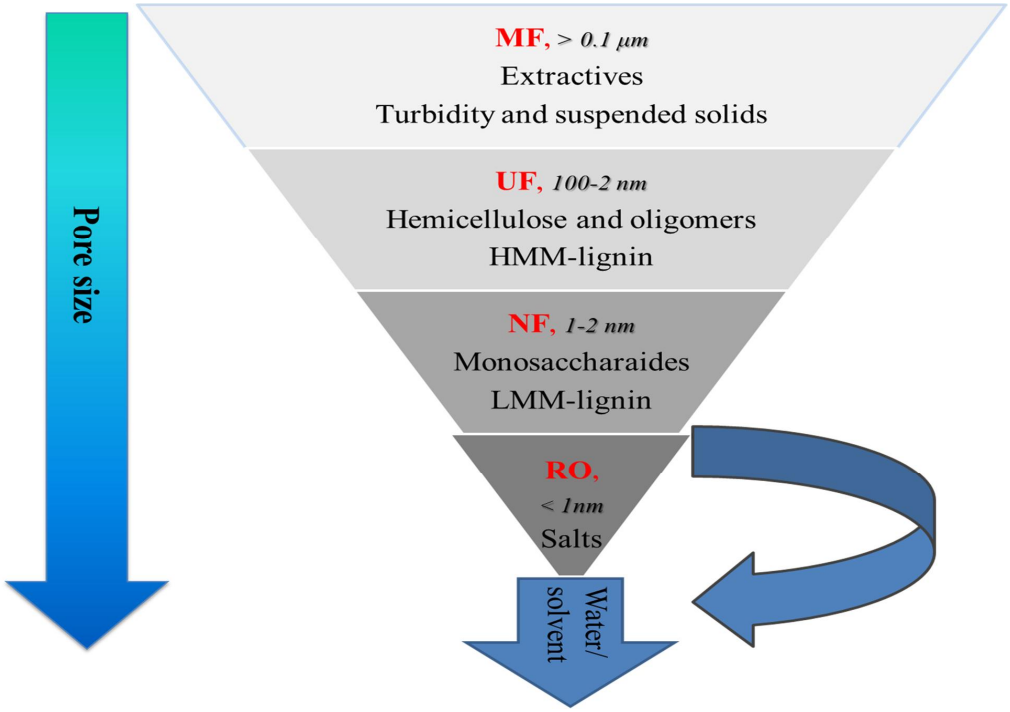


Figure 3.1 Separation of lignocellulosic components by membrane processes.
(LMM: low-molar-mass, HMM: high-molar-mass)

Today, membrane separation processes have also an important role in treatment of industrial agro-food effluents e.g. wastewater of olive mill and grape winery operations (Paraskeva et al. 2007 a; Giacobbo et al. 2013 a; Conde et al. 2013). The most common application of membrane processes mainly UF and NF aimed at valorization of theses waste streams by recovery of phenolic compounds and to some extent sugars. UF and NF were also employed for recovery of bioactive natural products, particularly phenolic compounds, sugars, and pectins from plant extracts (Díaz-Reinoso et al. 2010; Li and Chase, 2010). The concentration of the value-added compounds mainly phenolic compounds, the fractionation and separation of macromolecules (polyphenols or polysaccharides) from smaller compounds (i.e. low molar mass phenolic compounds and sugars), or their purification from other impurities could be achieved using appropriate membrane filtration process. In general, the phenolic compounds content and/or the antioxidant activity of either the permeate or the concentrate stream are one of the main parameters utilized to evaluate the efficiency of the membrane process applied.

3.1 Recovery of galactoglucomannans with a membrane

Microfiltration, ultrafiltration with or without diafiltration and nanofiltration have been studied in the recovery of GGMs from various aqueous streams, including the process water from thermo-mechanical pulping (TMP), Masonite production as well as, spruce autohydrolysates. MF has been primarily employed for the removal of suspended solids and colloids from GGMs, UF with DF has been applied for fractionation, concentration and purification of GGMs, and NF has been performed to recycle water or solvents for reuse purposes in e.g. extraction, but it can also be used to concentrate GGM degradation derivatives i.e. monosaccharides. Examples of studies investigating the utilization of MF, UF, DF and NF in the recovery of GGMs are presented in **Table 3.2**.

3.1.1 Microfiltration

Microfiltration has been found a feasible technique to clarify and decrease the turbidity of wood hydrolysates (Hasan et al. 2011) and TMP process water (Andersson et al. 2007). MF has been mainly used for the removal of suspended matters, dispersed substances and particulate materials from galactoglucomannan (GGM)-containing solutions (Persson et al. 2007; 2010; Krawczyk et al. 2011). This may contribute in reduction of fouling when the GGMs are concentrated with UF (Persson and Jönsson, (2010; Krawczyk and Jönsson 2011). As **Table 3.2** shows, MF processes were carried out with either polymeric (made from polytetrafluoroethylene) or ceramic membranes where the pore size was usually from ~ 0.2 to 1 μm.

Sundberg et al. (2002) and Willför et al. (2003 a) propose MF as an alternative pretreatment technique, before the recovery of hemicellulose with UF, to overcome drawbacks like low removal efficiency of impurities and high loss of hemicellulose. They report about 30% losses in hemicelluloses when other methods, like microflotation and adsorption, were utilized to separate dispersed substances from TMP process water. However, hemicellulose losses cannot be avoided during purification of GGMs from dispersed substances. Persson et al. (2010) measured about 25% loss of GGMs with MF when TMP process water was fractionated with membranes (**Table 3.2)**. They report that in addition to suspended matters, considerable amounts of extractives (0.3 kg/ 1 ton of pulp produced) like fatty and resin acids and sterols can be recovered in the MF retentate. As reported by Krawczyk and Jönsson (2011, **Table 3.2**), pore plugging of the membrane could be responsible for high retention of hemicelluloses in MF. In their study, even though the operating

parameters of MF, i.e. increase in cross-flow velocity and the use of techniques like back-pulsing enhanced the permeate flux, but their influence on the loss of GGMs (high retention during MF) was minor. While MF is frequently used as efficient pretreatment step to remove suspended matters and colloids before recovery of hemicelluloses, the exact value of hemicellulose losses is not often reported.

Table 3.2 Examples of membrane processes (MF, UF, DF and NF) studied for the recovery of GGMs.

Study	Description	Membrane processes	Results and observations
Persson et al. (2007)	Economic evaluation of a full-scale process (MF→UF) to recover hemicelluloses from TMP process water	MF: PTFE (10 μm) UF: PVDF (1, 10 kDa)	**MF:** suspended matter in the process water was removed. **UF:** the flux was higher with the 10 kDa membrane. Hemicelluloses were concentrated from 1-2 g/L to 66 g/L. Purity was increased from 27 to 79%.
Andersson et al. (2007)	DF compared with SEC to purify hemicellulose from TMP	MF: PTFE (1 μm) UF→DF: PVDF (1 kDa)	**UF:** average flux ~ 69 L/m^{2}h at 80% VR. **DF:** purity increased from 57 to 77%, which was comparable to SEC (82%). Lower recovery (87%) with DF than SEC (99%).
Persson and Jönsson, (2009)	Fouling of membranes when wastewater of masonite production is treated by UF	UF: PES (1,5 kDa) PVDF (1, 10 kDa) RC (5 kDa)	Fouling was less severe with RC membranes. Activated carbon treatment gave higher flux and low fouling for both PES and PVDF membranes. Hemicelluloses were highly concentrated to about 60 g/L.
Persson and Jönsson, (2010)	Effect of operating conditions on UF performance of TMP process water	MF: PTFE (0.2 μm) UF:PVDF (1,10 kDa), H-PS (5 kDa)	Retention of hemicellulose (15 g/L) was > 90% with 5 kDa, PES membrane regardless of flux and pressure. > 90% retention of hemicelluloses with 10 kDa, PVDF membrane could be achieved when a gel layer was formed at high transmembrane pressures (flux > the critical flux)

Persson et al. (2010)	Fractionation of spruce TMP process water into several fractions by MF→UF→NF scheme	MF: CE (0.2 μm) UF: H-PS (5 kDa) NF: PA (R_{MgSO4}> 98%)	**MF**: extractives were mostly removed. 75% of hemicelluloses and 90% of lignin permeated. **UF**: at a flux of 58 L/m^2 h, hemicelluloses were concentrated to 64 g/L and purified. Only 25% of lignin was recovered. **NF**: Permeate quality was as fresh water.
Krawczyk and Jönsson (2011)	Optimizing MF operating conditions, i.e. membrane pore size, cross-flow velocity and back-pulsing for highest recovery of GGM into the permeate stream.	MF: CE (0.2,0.4,0.8 μm)	Cross-flow velocity and back-pulsing affected the flux positively. Pore plugging of MF membrane led to over 50% retention of GGM. Reduction in the membrane flux was higher with a larger pore size membrane.
Song et al. (2013)	Separation of GGMs in polymeric and oligomeric forms from a hot water extract of spruce wood through a scheme of UF membranes with different cut-offs	UF: PES (300, 100, 30, 10 and 3 kDa)	40% of polymeric GGMs in the feed were found in the 30 kDa concentrated fraction. Their molar mass range was ~ 4–15 kDa. Fraction (>300 kDa) had the lowest yield of polymeric GGMs with average molar mass~ 22 kDa.
Krawczyk et al. (2013 a)	Production of a high molar mass hemicellulose fraction from CTMP process water using MF,UF and enzymatic treatment	MF: CE (0.2 μm) UF: H-PS (5,10 kDa)	When the aromatic moieties of hemicelluloses were cross-linked using enzymatic treatment prior UF, a fraction (~54 g/L, ~27 mPa s) containing most of the high molar mass hemicelluloses ~12 kDa was obtained with a 10 kDa membrane. High average flux and low fouling were observed in MF (260 L/m^2h) and UF (~120 L/m^2h).

(PTFE: polytetrafluoroethylene, PVDF: polyvinylidene fluoride, H-PS: hydrophilized polysulphone, PES: polyethersulphone, RC: regenerated cellulose, PA: polyamide, CE: ceramic, DF: diafiltration, R: retention, SEC: size exclusion chromatography).

3.1.2 Ultrafiltration

The separation mechanism of ultrafiltration is selective exclusion of particles based mainly on their size (molar mass).The molar mass of hemicelluloses is usually in the range of UF membrane cut-off (number expressed in dalton indicating that most of the species with a molar mass larger than this value could be retained). Therefore, UF has been found to be a potential technology for the recovery of hemicelluloses from high-viscosity biomass alkali extracts (Krawczyk et al. 2011), wood hot water extracts (Westerberg et al. 2012), kraft black liquor (Wallberg et al. 2006), lignocellulosic hydrolysates from corn stover (Colyar et al. 2008), and pulp and paper effluents (Nuortila-Jokinen and Nyström, 1996; Maartens et al. 2002). It is also used for fractionation of heterogeneous solutions containing GGMs (Persson et al. 2010; Song et al. 2013). In practice, the concentration of hemicelluloses by UF prior to hemicellulose precipitation is beneficial to reduce the consumption of the antisolvent (ethanol) (Swennen et al. 2005; Zeitoun et al. 2010).

UF membranes made from regenerated cellulose (RC), surface-modified polyvinylidene fluoride (PVDF), modified polyethersulphone (PES), and hydrophilized polysulphone (H-PS) with cut-off range from 1- 300 kDa have been investigated for GGM recovery (**Table 3.2**). Ceramic membranes made from TiO_2 have also been applied in the separation of GGMs (Westerberg et al. 2012). Hydrophilic membranes have been found more suitable for the processing of wood-based liquor like pulping process water than hydrophobic membranes, since their fouling by wood compounds is lower (Persson and Jönsson, 2009; Puro et al 2011).

As presented in **Table 3.2**, Persson and Jönsson (2009; 2010) tested 5 kDa, RC, PES and H-PS membranes and a 10 kDa PVDF membrane for the recovery of hemicelluloses from TMP and Masonite process water. According to them the RC membrane was favorable, as a rather high retention of GGM (85%) and a high permeate flux with a low fouling tendency were obtained. However, the maximum operating temperature of the RC membrane (up to 55 °C) was much lower than the temperature of the TMP process water (75–85 °C), so cooling was needed before filtration. The high temperature tolerance of the H-PS membranes (up to 90 °C) makes them an alternative for the filtration of TMP process water directly if their rather strong fouling tendency can be limited.

In general, the UF of dilute aqueous streams of GGMs to obtain concentrated hemicellulose streams requires a high VR (> 90%). For instance, Persson et al. (2009; 2010) were able to increase the GGM concentration from 1-2 g/L to ~ 64g/L with UF (VR ~ 99%). Furthermore, they found that high-volume reduction of GGM liquors could be useful for increasing the purity of the hemicelluloses due to the fact that the recovery rate of GGM was much higher than that of lignin, the most abundant undesired component. UF can also be used to fractionate a biomass-based extract into different fractions based on the molar masses. Song et al. (2013) applied UF scheme to separate GGMs from a spruce hot water extract into fractions according to their molar mass (**Table 3.2**). The filtrations were started with the highest cut-off membrane, and the permeate of each step was processed with the lower cut-off membrane. In their study, a small amount of the highest average molar mass of GGMs (7%, ~ 22 kDa) was found in the 300 kDa membrane concentrate. The concentrate fraction of the 30 kDa and 10 kDa membranes contained, respectively, about 40% and 15% of the GGMs in the feed. Most of the lignin, monosaccharides and free acetic acids permeated through the membrane with the lowest cut-off.

An economic evaluation of a large-scale recovery of GGM from TMP process water with UF by Persson et al. (2007) demonstrated that the production cost of 1 ton of hemicellulose solution containing ~30 g /L (purity 80%, TDS basis) could be several times lower than the cost of ethylene vinyl alcohol, which is now used for manufacturing oxygen barriers. Willför et al. (2003 a) obtained 5 kg GGM / ton pulp and a molar purity of 95% using the large laboratory scale process depicted in **Fig. 3.2**. Their multi-stage process consists of coagulation, filtration of suspended substances, and removal of lignin with resins from TMP-water before the concentration of the GGMs with UF. Based on a pilot scale study performed by Persson et al. (2010), 11 kg of hemicelluloses, mainly GGMs, could be recovered from process water used for the production of 1 ton of TMP. In their work, the purity of the hemicellulose (TS basis) increased from 21 to 59% with a hydrophilized polyethersulphone membrane (cut-off = 5 kDa).

Several researchers (Hartman et al. 2006 a, b; Persson et al. 2009; Edlund et al. 2010) have used diafiltration (DF) with UF membranes as a post purification method for GGMs. It improves the purity of GGMs by enhancing the removal of various impurities like lignin, monosaccharides and extractives. For similar purpose, Nabarlatz et al. (2007) have studied continuous DF on the purification of xylo-oligosaccharide from lignin-related low molar mass compounds in an almond

shell extract. Depending on the purity required, several dia-volumes can be needed. Therefore, water consumption can be a significant drawback in diafiltration. Furthermore, the yield of target compounds (e.g. GGMs) has decreased typically with dia-volumes. The efficiency of DF depends obviously on the retention of impurities and target compounds e.g. GGMs, as well as the dia-volumes used.

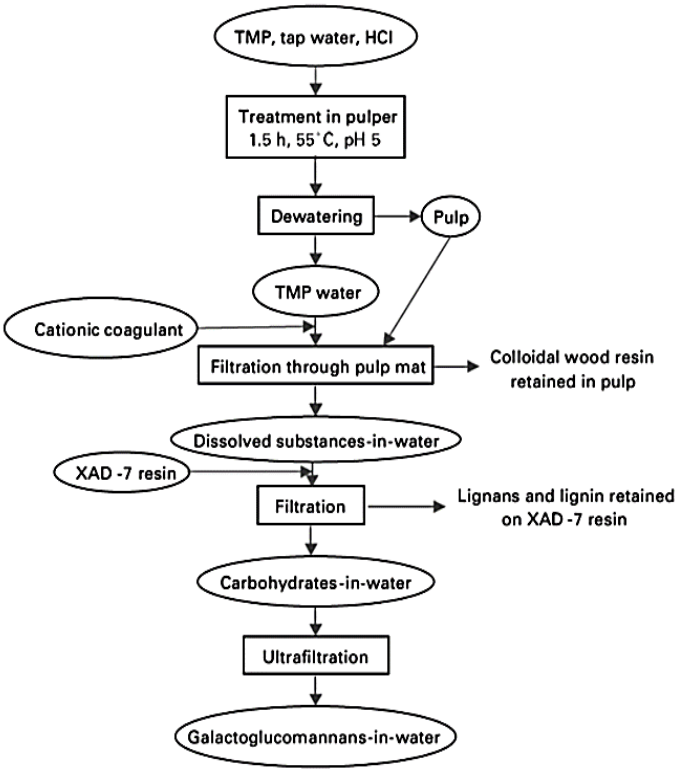


Figure 3.2 Recovery process of GGMs from TMP-water in a large scale (Willför et al. 2003 a, p.28).

DF is useful in reducing the concentration of salts and monosaccharides in hemicellulose fractions (Persson et al. 2006; Persson and Jönsson, 2009). Hartman et al. (2006 b) obtained about 90% purity for the GGM fraction when DF followed the UF of TMP process water (1 kDa, RC membrane). The produced fraction was evaluated to be pure enough for making of oxygen barrier materials. Andersson et al. (2007) managed to increase the purity of oligo- and polysaccharides (based on the total dissolved solids) from 57 to 77 % in diafiltration where four dia-volumes of

water was used. The loss in the yield of oligo- and polysaccharides could not be avoided, and about 16% of their content in the UF concentrate was lost. According to their study, DF is a cost-efficient purification method of GGMs compared to size-exclusion chromatography.

Goulas et al. (2003) performed discontinuous mode DF (1 kDa membrane) for the purification of oligosaccharides. Their study showed that the permeate flux was lower during the filtration of the first dia-volume compared to subsequent ones at the same VRF. This was due to the decrease in the osmotic pressure caused by the removal of sugars. They also report that the UF required less dia-volumes but exhibited higher losses than NF to obtain the same purity of oligosaccharides.

3.1.3 Nanofiltration

Nanofiltration is applied for the concentration of low molar mass compounds and purification of water for reuse. Monosaccharides and free lignin (low molar mass ones) can be recovered from UF permeates with NF. Persson et al. (2010) (**Table 3.2)**, recovered about 8 kg of aromatic compounds (lignin)/ ton of pulp from TMP process water by NF. Moreover, 40% of the process water was recovered as pure enough for reuse. In their study, rather high flux (63 L/m^2h at VR= 50%, 50º C and 20 bar) and lignin retention (85-94%) with the NF99HF membrane were obtained.

NF has been proposed as a technology for the concentration and purification of wood hydrolysates. Amidon and Liu (2009) propose the utilization of a 100 Da membrane to retain most of the platform chemicals, mainly hemicellulosic sugars in the concentrate stream, while furfural, hydroxymethylfurfural (HMF), acetic acid, methanol, low molar mass phenolic compounds, and formic acid will be preferentially passed to the permeate stream. Weng et al. (2010) have employed NF to remove fermentation inhibitors such as acetic acid and furans from biomass hydrolysates.

Fouling of NF membranes utilized for the filtration of biomass hydrolysates has not been often reported. Goulas et al. (2002) investigated the performance of NF in the purification of a galacto-oligosaccharide mixture. In their study, no irreversible fouling was observed. In several investigations, NF has usually been employed after the processing of biomass hydrolysates with other membrane processes like MF and UF, where most of the membrane foulants have been already removed. To avoid possible fouling in NF of a wood hydrolysate, Hasan et al. (2011) propose the utilization of ceramic micro-filters prior further filtration with NF**.** As most colloidal

and particulate materials are removed with MF, NF of the micro-filtered hydrolysate is assumed to be operated with lower fouling problems than with direct NF.

3.1.4. Challenges in the recovery of hemicelluloses with a membrane

Pressure-driven membrane filtration processes are widely applied in the separation of various dissolved materials from liquid streams. One of the difficulties that have emerged in the utilization of these techniques is the decline of the permeate flux. The reduction in the permeate flux is mainly caused by two phenomena: concentration polarization and fouling. Furthermore, osmotic pressure may be significant when the solute concentration increases, and viscosity may also change remarkably at the same time. These phenomena decrease the flux and reduce the membrane lifetime, as well as increase the operating cost.

Concentration polarization occurs due to the formation of a concentrated layer of particles on the membrane surface. Fouling is caused by reversible or irreversible adsorption and deposition of unwanted molecules on the membrane surface, or the pore plugging mechanism. Concentration polarization, as well as fouling, can be controlled by adjusting the operating conditions, such as flow velocity, feed concentration and transmembrane pressure. Moreover, frequent cleaning of the membrane with chemicals and/or back-flushing with water or a permeate are typically required to manage fouling problems (Mulder, 1996; Cheryan, 1998).

Membrane fouling is one of the major challenges in the utilization of membrane processes for the treatment of wood and biomass processing streams. A biomass-based liquor is a complex mixture containing various kinds of dissolved and colloidal substances, like lipophilic extractives, hemicelluloses and lignin. Therefore, it is highly likely to cause membrane fouling. In the ultrafiltration of wood pulping effluents, colloidal particles, for instance lipophilic extractives (i.e. wood resin) are considered potential foulants of the membrane (Ramamurthy et al. 1995; Dal-Cin et al. 1996). Puro et al. (2011) studied the fouling behaviour of extractives in the ultrafiltration of two pulp mill process waters with RC and PES membranes. They report that the membranes were fouled due to the adsorption of dissolved and colloidal extractives (mostly fatty and resin acids) on the membrane surface. The fouling of the RC membrane was mainly caused by sterols, even though their amount was very small. The PES membrane was significantly fouled because of its high hydrophobicity.

In addition to extractives, fouling is caused by dissolved lignin macromolecules, as reported by Dal-Cin et al. (1995) and Ramamurthy et al. (1995). The presence of lignin in wood hydrolysates makes its filtration by membrane processes quite challenging, as the fouling risk is being high, particularly with hydrophobic membranes (Koivula et al. 2011). Polymeric lignin has been reported to cause fouling of the membrane during the filtration of a hot water wood extract (Chen et al. 2014). Beside lignin, the presence of high molar mass dissolved polysaccharides like hemicelluloses may enhance the fouling tendency. In addition to flux decline, membrane fouling can cause higher retention of impurities. This can occur by the formation of fouling and/or a gel layer promoted by foulants like fatty and resin acids and lignin (Ramamurthy et al. 1995; Puro et al. 2011), or by pore plugging (Hasan et al. 2011; Krawczyk and Jönsson, 2011).

Another challenge appearing during the concentration of hemicelluloses is the increase of viscosity. This can be explained by the increase of the concentration and molar mass of hemicelluloses during filtration. High viscosity leads to a decline in the filtration capacity, i.e. permeate flux (Pritchard et al. 1995). This means that a larger filtration area and more energy for pumping the liquid are required to treat the same amount of solution as when the viscosity is lower. The key to efficient ultrafiltration processes is operation at low viscosity conditions, and various strategies and techniques can be used to control the viscosity effect. Viscosity can be reduced by carrying out the filtration at a higher temperature and applying high cross-flow velocity (higher mass transfer coefficient) (Krawczyk, and Jönsson, 2011; Krawczyk et al. 2011). In addition, pretreatment to remove or degrade the compounds responsible for high viscosity can be used to decrease the viscosity of hydrolysates. Krawczyk et al. (2013 b) report a decrease in the viscosity of hemicellulose extracts from wheat bran by 80% after MF (> 90% hemicellulose losses), and about 80% after dead-end filtration (< 5% hemicellulose losses).

The basic methods for flux-enhancing are based on increasing the turbulence of the flow at the membrane surface. Special module constructions, such as helical baffles and rotational or vibrating membrane modules have shown potential in enhancing the flux and avoiding concentration polarization, due to their ability to perform in high turbulence and shear rate conditions (Nuortila-Jokinen and Nyström, 1996; Jaffrin, 2008). Back-pulsing was tested in MF to remove that cake layer that reduce the flux (Krawczyk and Jönsson 2011). Another promising way to improve the efficiency of membrane processes is the removal or degradation of foulants prior to filtration. Various pretreatment methods for wood hydrolysates, such as activated carbon adsorption, pH

adjustment, oxidation by pulsed corona discharge (Koivula et al. 2011), and microfiltration have been studied (Hasan et al. 2011). Persson and Jönsson (2009) obtained higher flux and lower fouling of a hydrophilic RC membrane and a hydrophobic PVDF membrane when activated carbon pretreatment of wastewater from Masonite production was applied prior the recovery of hemicelluloses with UF. Persson and Jönsson (2009) also studied the effect of pH decreased from 4.5 to 3 on the performance of both membranes. They found that the permeate flux through the hydrophilic RC membrane was more stable and increased, and due to increase in the solubility of inorganic compounds, less fouling was observed. On the other hand, reduction in the permeate flux was observed with the PVDF membrane. Moreover, lowering the pH seemed to decrease the surface charge of the PVDF membrane, so its hydrophobicity and thus the fouling tendency increased.

Complete purification of hemicelluloses during their recovery by only UF is quite challenging. When hemicelluloses are extracted from solid lignocellulosic materials, release of other compounds, including dissolved lignin, monosaccharides and extractives, occurs (Örså et al. 1997; Amidon and Liu, 2009). In wood species, lignin molecules is attached to hemicellulose by covalent linkages in lignin-carbohydrate complexes. Formation more of these complexes could occur during wood processing like pulping (Lawoko et al. 2005). Overlapping of hemicellulose and lignin molar masses and the presence of lignin-hemicellulose complexes make complete separation of lignin from hemicelluloses directly by UF unrealistic. Therefore, combined membrane filtration and enzymatic treatment (Krawczyk et al. 2013 a) and adsorption chromatography (Westerberg et al. 2012) have been recently tested to improve the separation of lignin from hemicelluloses before or during filtration. Polyelectrolyte flocculation has been proposed by Duarte et al. (2010) to purify the hardwood hot water extract from lignin before MF. Chen et al. (2014) suggest polyaluminium chloride (PAC) precipitation as a specific removal method of large molecular lignin from a hot water wood extract before further purification with such as membrane filtration. The loss of oligosaccharide seemed to be avoided with this method. According to Koivula et al. (2013), even though polymeric resin pretreatment of wood hydrolysates before UF enhanced the filtration capacity and lowered the fouling, it was not selective for the removal of lignin, and some loss of hemicelluloses occurred.

3.2 Recovery of phenolic compounds with a membrane

Numerous features and applications of phenolic compounds and sugars have promoted the interest in their recovery from various natural resources, like biomass residues. Resin adsorption (Li et al. 2011; Soto et al. 2011), capillary electrophoresis (Ignat et al. 2011), and various chromatographic techniques like anion-exchange chromatography (Labarbe et al. 1999; (Vitrac et al. 2001; Vidal et al. 2003) have been applied in order to fractionate and separate phenolic compounds and mono- or polysaccharides from their sources. Challenges like scale-up of such separation techniques make their utilization for the recovery of phenolic compounds and sugars rather difficult. Membrane processes exhibit several advantages like operating at mild conditions, good separation efficiency and scale-up flexibility. Moreover, as no phase transition occurs, they could be compatible techniques for the recovery of heat-sensitive compounds. Based on that, membrane separation processes could play an important role in the recovery of phenolic compounds with antioxidants features from biomass liquid streams (Díaz-Reinoso et al. 2010; Li and Chase 2010).

Table 3.3 includes examples of studies employing membrane processes, mainly UF and NF, in the recovery of phenolic compounds from various plant extracts, fruit juices and agro-food industrial waste streams. In general, differences in membrane specifications such as active materials, cut-offs and configurations, as well as operating conditions make direct comparison of these studies rather difficult. As **Table 3.3** shows, a wide group of phenolic compounds, including e.g. gallic and chlorogenic acids, flavonoids, monomers, and oligomers of flavanol and flavanone glycosides have been separated, fractionated and concentrated with UF and NF. The main role of UF is to fractionate polyphenols into low and high molar mass fractions. Moreover, UF can be used to remove high molar mass compounds like proteins from phenolic compounds. NF has been utilized to concentrate phenolic compounds where high retention of phenolic compounds (> 90%) is usually achieved. Based on **Table 3.3**, when the purpose of NF is concentrating the phenolic compounds, the biomass-based streams are typically concentrated by 4-6 times. In addition to recovery, phenolic compounds could be purified from other low molar mass compounds like sugars, pectins, and ions during UF/NF. As an advantage over other methods like evaporation, the antioxidant activity of bioactive compounds is often preserved with membrane filtration.

3.2.1 Recovery of phenolic compounds from agro-industrial effluents

Membrane separation is a promising technique that could be used not only for purifying water from agro-industrial effluents, but also for the recovery of several valuable compounds, like phenolic compounds. The main agro-industrial streams containing antioxidants, i.e. phenolic compounds, are olive mill wastewater and grape winery effluents. Mainly UF and NF have been investigated in the separation of bioactive phenolic compounds from these particular streams (Paraskeva et al. 2007 a, b; Giacobbo et al. 2013 a, b; Conde et al. 2013). In addition, tannins have been recovered from cork processing wastewater (Bernardo et al. 2011).

3.2.1.1 Recovery of phenolic compounds from grape winery effluents

UF and NF have been studied for the recovery of polysaccharides and polyphenols from winery effluents. Giacobbo et al. (2013 a, b) used a 2 kDa (7.6 kDa according to their determination) PES membrane to treat winery effluents. The effluent was subjected to two sedimentation operations, one for the effluent at a pH of 3.6 to recover phenolic compounds and polysaccharides with UF, and the second at a pH of 5.4 for the UF concentrate clarified from the sedimentation (volume reduction factor, VRF =2). In their study, the concentration of high molar mass phenolic compounds and polysaccharides in the sediment increased by 6 and 5 times, respectively. When the UF permeate was nano-filtered, the NF270 membrane retained about 93.8% and 99% of low molar mass phenolic compounds and polysaccharides, respectively. Partial fractionation of the phenolic compounds and polysaccharides in the NF270 concentrate was achieved with another NF step using a more open laboratory-made cellulose acetate membrane. Versari et al. (2003) tested different NF membranes for enriching the sugar and polyphenol content of grape must in wine production. High retentions of sugars (77–97%) and polyphenols (range 70–94%) were obtained, whereas the retention of impurities, e.g. malic acid, was low (2–14%).

UF and NF have also been proposed for the fractionation of polysaccharides and/or polyphenols from extracts of winery wastes. Galanakis et al. (2013) studied the possibility to fractionate several phenolic compounds from ethanol extracts produced from winery sludge using PS membranes (100 and 20 kDa) and 1 kDa PVDF membranes. In their investigation, both PS membranes separated polymeric anthocyanins from monomeric ones. Purification of phenolic compounds and sugars from pectin and hydrolyzed derivatives was achieved by a 100 kDa membrane. With the 20 kDa membrane, the retentions of phenolic compounds and sugars were already over 60%. The 1

kDa membrane separated different phenolic groups like hydro-xycinnamic acids (in enriched fraction), flavonols and anthocyanins successfully, mainly based on their polarity.

3.2.1.2 Recovery of phenolic compounds from olive mill wastewater

The UF and NF processes of olive mill wastewater (OMWW) have been studied for the clarification and recovery of several valuable compounds like pectin and phenolic compounds. Turano et al. (2002) utilized a 17 kDa polysulphone membrane in order to reduce the chemical oxygen demand (COD) and separate the valuable compounds, e.g. sugars and phenolic compounds, from centrifuged OMWW. Diafiltration with PS membranes (cut-offs 8, 25 and 100 kDa) was tested by Gkoutsidis et al. (2011) to collect the major phenolic compounds from OMWW in the permeate side before further purification with absorbent resin. In their study, the permeate fluxes during DF were higher than in direct UF. They also propose a process with no waste streams involving diafiltration (UF membrane) and reverse osmosis for the treatment of olive mill wastewater.

Galanakis et al. (2010) **(**see **Table 3.3**) found that the permeate of the PS membrane (25 kDa) was rich in phenolic compounds with high antioxidant properties. When the permeate was treated with NF (120 Da) to purify phenolic compounds from salts, the retention of phenolic compounds (99%) was higher than the one of salts (~50%). However, loss in the yield of antioxidant compounds due to lower retention of *o*-diphenols (85%) was observed. Garcia-Castello et al. (2010) applied NF using a membrane with a higher cut-off (600 Da) on the MF permeate from OMWW. They recovered most of the phenolic compounds in the NF permeate before their concentration with osmotic distillation. The final product was almost free from low molar mass phenolic compounds and contained 0.5 g/L of polyphenols (60% as hydroxytyrosol), so it could be appropriate for food and pharmaceutical products.

Paraskeva et al. (2007 a, b) applied UF in combination with NF and/or RO to valorize olive mill wastewater (OMWW). The concentrates of NF and RO were rich fractions of phenolic compounds (up to 10 g/L). Russo (2007) combined MF, UF and RO stages to separate phenolic compounds, mainly low molar mass ones, from OMWW. In his study, severe fouling was observed during MF. UF (1 or 6 kDa cut-off) was effective for the separation of phenolic compounds, mainly hydroxytyrosol, into the permeate, and the yield of the phenolic compounds increased by diafiltration. Phenolic compounds (0.06 g/L in the OMWW) were mainly present in the MF and

UF permeates (0.3-0.5 g/L) or RO concentrate (> 90% retention). Theses streams were proposed as functional ingredients for food, pharmaceutical or cosmetic industries.

Table 3.3 Examples of membrane processes used in the recovery of phenolic compounds.

Raw material	Membrane process	Observations	Reference
Mate leaves	Pilot scale NF (TF, 150- 300 Da)	Retentions (at VRF of 4): gallic acid (95%), 4, 5-dicaffeoylquinic acid (100%). Retentions of high antioxidant capacity compounds i.e. chlorogenic acids and 3, 4-dihydroxybenzoic acid > 98%.	Murakami et al. (2011)
Bergamot juice	UF: PS (100 kDa), PVDF (1 kDa) NF: CE (450 and 750 Da)	450 Da membrane demonstrated best separation of polyphenols from sugars. The permeate was enriched in sugar and organic acids. The retentate was enriched in phenolic compounds with antioxidant activity (flavonoid retention ~91–99%).	Conidi et al. (2011)
Almond skins	UF→DF : (10, 30 and 50 kDa)	The best fractionation was achieved with the tightest 10 kDa membrane. The permeate contained low molar mass phenols, e.g. benzoic acids, monomers and oligomers of flavanol and flavanone glycosides. The concentrate contained high molar mass ones e.g. proanthocyanidin oligomers.	Prodanov et al. (2008)
Grape pomace	UF: TF (1 kDa), CE (1kDa). NF: PA (250 and 350 Da), TF (150–300 Da).	The antioxidant capacity of retentates was increased by 3.5–6.6 times. The highest yield and antioxidant activity recovered by ethyl acetate extraction was obtained with 1 kDa CE membrane.	Díaz-Reinoso et al. (2009)
Olive mill wastewater	UF: PS (25, 100 kDa), PES (2 and 10 kDa). NF: PPA (120 Da)	The lowest permeate flux was observed with PES membranes. With 25 and 100 kDa membranes, pectins were concentrated and separated from ions and phenols. 25 kDa membranes separated phenols with antioxidant properties in the permeate stream.	Galanakis et al. (2010)

Table 3.3 continues……..

Root cortices of mulberry	MF: (0.45 μm) UF: PS (10,50 and 200 kDa), PA (20 kDa)	At VRF= 4, antioxidant capacity from e.g. chlorogenoic acid and p-hydroxybenzoic acid in permeates was 2-3 times higher than those of the feed.	Yu et al. (2007)
Propolis	NF: PA/PS (R_{MgSO4} > 98%)	At VRF= 4, higher retentions of phenolic compounds (94%) and flavonoids (99%) were achieved from the aqueous extract compared to the ethanol extract.	Mello et al. (2010)

(TF: thin film, PVDF: polyvinylidene fluoride, PS: polysulphone, PES: polyethersulphone, RC: regenerated cellulose, PPA: Polypiperazine, PA: polyamide, CE: ceramic, DF: diafiltration, R: retention, VRF: volume reduction factor).

3.2.2. Recovery of phenolic compounds from plant extracts

The clarification and concentration of fruit juice are typical applications of membrane filtration techniques in the food industry. In general, juice clarification (MF or UF) aims at enhancing the product quality and fractionating the juice by retaining high molar mass macromolecules (pectin-suspended solids or protein) in the concentrate stream and allowing permeation of small solutes (sucrose, acids, salts, and aroma or flavor compounds) through the membrane. The purpose of the concentration process is usually to remove water from the juices before transportation. The valuable phenolic compounds are not commonly recovered from the juices, although it can be done. This is because the juice itself is already a valuable product. However, flavonoids, hydroxycinnamic acid derivatives and anthocyanin could be retained in the concentrated juice fraction (Mangas et al. 1997; Kalbasi and Cisneros-Zevallos, 2007; Conidi et al. 2011).

More attractive raw material for recovering phenolic compounds are plant leaves and residues like mulberry root cortices, grape seeds, castanea leaves, almond skin, and bergamot juice, which are not food as such (see **Table 3.3**). Several researchers have studied the recovery of phenolic compounds from these residues using membrane-based processes. For instance, Todisco et al. (2002) recovered catechins from black tea in the permeate side by retaining the proteins with a 40 kDa ceramic membrane. The quality of the clarified tea in term of polyphenol stability was preserved after UF. When Yu et al. (2007) used UF (10-200 kDa) to separate phenolic compounds from extracts of mulberry root cortices**,** more phenolic compounds with high antioxidant capacity were present in the permeate than in the retentate**.** The concentration of phenolic compounds from a grape seed extract with UF was studied by Nawaz et al. (2006), and 11.4% of the polyphenols in

the seeds were recovered. Kalbasi and Cisneros-Zevallos (2007) investigated fractionation of anthocyanin from grape juice. In their study, 36-66% of polymeric anthocyanin in the feed were recovered using membranes with 10- 100 kDa cut-offs. For the purpose of chemical analysis, UF was utilized by Prodanov et al. (2008) as an efficient pretreatment procedure to separate low molar mass phenolic compounds from high molar mass ones in almond skin extracts. In their study, the best fractionation between low molar mass phenolics, e.g. benzoic acids, monomers and oligomeric flavanol, and high molar mass ones, e.g. proanthocyanidin oligomers, was achieved with a 10 kDa membrane. UF was also coupled with other membrane separation techniques in a hybrid process to recover phenolic compounds. Díaz-Reinoso et al. (2011) used UF followed by DF to concentrate the phenolic compounds and increase the antioxidant content of aqueous extracts of *Castanea sativa* leaves.

NF has been studied (see **Table 3.3**) for concentrating and purifying bioactive phenolic compounds from aqueous extracts of mate leaves (Murakami et al. 2011), propolis (Mello et al. 2010), and distilled grape pomace (Díaz-Reinoso et al. 2009). Prudêncio et al. (2012) nano-filtered an aqueous mate residue extract to recover phenolic compounds with high antioxidant activity. They observed that the concentration of phenolic compounds with high antioxidant activity increased from 1.6 to 11 g/L when the extract concentrated to the VRF of 6. The utilization of NF alone or in combination with UF and diafiltration for the separation and fractionation of phenolic compounds from plant extracts has been reviewed by Tsibranska and Saykova (2013). They conclude that coupling NF with other processes, like DF and UF, extends the application of NF in the fractionation and purification of polyphenols and flavonoids from natural extracts by decreasing the membrane fouling and exhibiting better selectivity.

A membrane-based process consisting of UF and NF was investigated by Conidi et al. (2011) for the separation and concentration of polyphenols in bergamot juice (see **Table 3.3**). UF with a 100 kDa membrane removed suspended solids and foulants efficiently before NF. Most phenolic compounds were recovered in the concentrate of 450 Da membrane concentrate, and partially separated from sugars (52% of the sugars were permeated). Díaz-Reinoso et al. (2009) also applied UF and NF processes for the recovery of bioactive phenolic compounds from an aqueous extract of grape pomace. In their study, the content of phenolic compounds and ABTS radical scavenging capacity in the concentrates of the tested membranes were 3–6.6 higher compared to the feed.

When ethyl acetate extraction was applied in these concentrates, the antioxidant capacity of the extracts increased by 2–6 times. An UF-DF-RO scheme was applied by Xu et al. (2004) for the concentration of isoflavones from the waste stream of soy processing. They concentrated about 50% of the total amount of isoflavones in the soybeans with RO, and the retentate quality was suitable for utilization as an isoflavone supplement. A fractionation sequence containing four different membrane processes was developed by Santamaría et al. (2002) in order to recover proanthocyanic fractions (gallic acid, catechin and dimer- and trimer-gallates) with different degrees of polymerization (molar mass range of 170-882 Da) from defatted milled grape seeds. In their study, NF followed by diafiltration was employed to purify proanthocyanidins from low molar mass acids and aldehydes. Partial fractionation of dimeric and trimeric proanthocyanidins (retention 70-100%) from monomeric ones (retention 30%) was achieved by UF (8 kDa membrane) of the NF concentrate.

Coupled membrane filtration and adsorption has been proposed for recovering and removing phenolic compounds from a green tea aqueous extract (Li et al. 2005) or alfalfa protein concentrates (D'Alvise et al. 2000). In these studies, the phenolic compounds in the extracts were concentrated by UF and then recovered and purified by resin adsorption. Tsibranska and Saykova (2013) conclude that utilization of separation techniques like adsorption, precipitation, and crystallization as a pretreatment step of the plant extracts prior NF could play a role in improving the purity of the filtered streams and reducing the membrane fouling.

Día-Reinoso et al. (2010) propose and validate multistage recovery approaches (see **Fig. 3.3)** for the concentration and fractionation of antioxidant compounds from an aqueous grape pomace extract (4.4 g_{GAE}/L). The produced fractions were rich of phenolic compounds by direct UF/NF and by direct polymeric resin adsorption–desorption (product 3). Moreover, the phenolic compounds in the concentrate were enriched with ethyl acetate extraction (product 1), and in the permeate by resin adsorption–ethanol desorption (product 2). The concentration of phenolic compounds in the UF/NF concentrates was 1.25 to 2 times higher than in the feed (VRF of 3). The antioxidant capacity increased by 5 times with the ethyl acetate extraction of the concentrates (product 1). The antioxidant capacities of the permeates were 17-32 % of the feed capacity, and were increased to 40-60% after adsorption-desorption (product 2). The phenolic compound contents and antioxidant capacities for products 2 and 3 were rather similar (50 g_{GAE}/100 g product,

Trolox Equivalent Antioxidant Capacity (TEAC) ~ 10 g Trolox/g product). Compared to the other fractions, the contents of phenolic compounds in products 2 and 3 were slightly higher than in product 1, and 4-5 times higher than in direct UF/NF permeates. Furthermore, their antioxidant capacities were about 5 and 25 times higher than in product 1 and the direct UF/NF permeates, respectively.

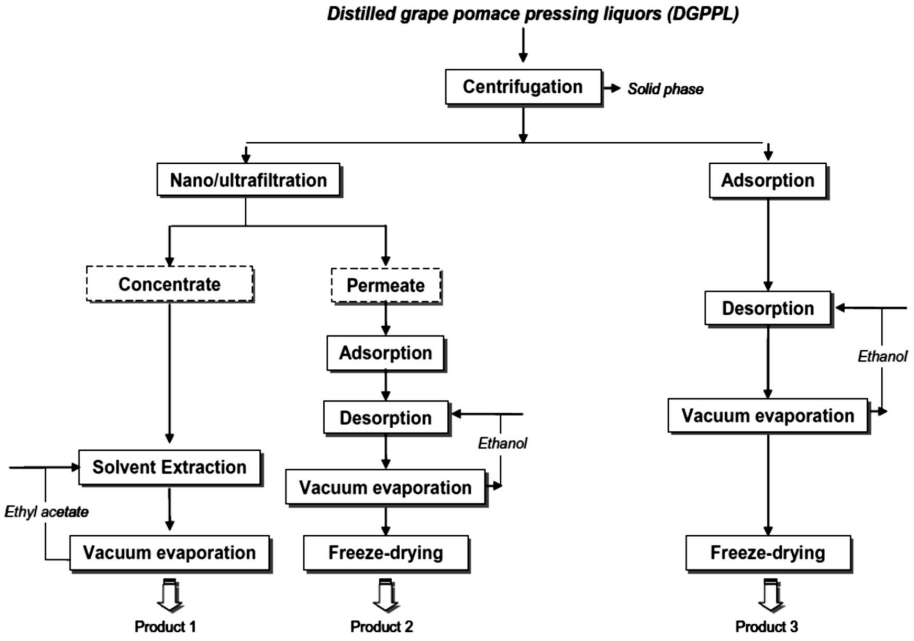


Figure 3.3 Processes involving UF and NF tested for recovery and concentration of bioactive phenolic compounds from aqueous extract of grape pomace (Díaz-Reinoso et al. (2010, p.128).

During the filtration of extracts containing phenolic compounds, reversible and irreversible fouling of the membranes has been commonly observed (Cassano et al. 2008; Susanto et al. 2009). These compounds seem to modify the membrane surface. Susanto et al. (2009) studied membrane–solute interactions and solute–solute interactions during UF of phenolic compounds and observed that the hydrophilic phenolic components may hydrophilize the polyethersulphone membrane. Xu and Wang (2005) claim that the formation of a hydrogen bond between flavonoids and membrane materials improved their separation during NF of a ginkgo biloba aqueous extract.

As a summary, various raw materials, like agro-food effluents and plant residue extracts have been refined to fractions where phenolic compounds are enriched and suitable for nutraceutical purposes. Even though plant residues like carob kibbles are recognized as a rich source of phenolic compounds, in our knowledge there are no studies on membrane-based separation processes of phenolic compounds from carob residue extracts.

4 Materials and methods

4.1 Raw materials

4.1.1 Spruce autohydrolysates

The autohydrolysates were obtained from pressurized hot water extraction (PHWE) of spruce saw dusts. These autohydrolysates could be described as a light brown liquid. Their pH was around 3.6- 4 and conductivity in the range of 200-300 µS/cm. The extraction solutions and the solid samples were prepared by Metla, the Finnish Forest Research Institute (Nowadays it is known as Luke, Finnish Natural Resources Institute). In addition to hemicelluloses, the spruce wood autohydrolysate (SWaH) contained different amounts of other co-extraction compounds, such as lignin, extractives and monosaccharides. The dominating hemicellulose in spruce is galactoglucomannan (GGM) (typically 14-20 % of dry wood), and xylans (4-11 % of dry wood) is the second abundant hemicellulose (Sjöström 1993). **Table 4.1** presents the main specifications of the spruce autohydrolysates that were utilized as raw material for the recovery of GGMs.

Table 4.1 Specification of the extraction liquors (spruce wood autohydrolysate -SWaH).

Spruce wood auto-hydrolysates	Extraction temperature °C	Mass (kg)	TOC, g/L	GGMs[1], g/L	Lignin, g/L	Turbidity, NTU	Molar mass Mw, (kDa)
$SWaH_1$	160	820	2.7	3.0[2]	0.4	1700/590[3]	5.6
$SWaH_2$	170	3	4.0	4.9	1.0	1350	-
$SWaH_3$	170	800	2.6	3.8[2]	0.6	444	7.0
$SWaH_4$	160	720	3.2	3.5	1.6	2000/594[3]	6.8
$SWaH_5$	180	660	8.9	11.1	0.8	8700/1090[3]	6.0

1: The GGM content is based on the assumption that all analysed mannose, glucose and galactose units are from GGM molecules; **2**: total hemicellulose concentration; **3**: turbidity measurements after centrifugation. TOC: total organic carbon, Mw: average molar mass.

4.1.2 Carob kibbles

Carob (*Ceratonia siliqua L.)* kibbles (chopped carob pods) were obtained from a local factory in Portugal. Before extraction, the carob pods were de-seeded into kibbles and then stored at room temperature in a dark and dry place. Screening of the kibbles showed that 27% were larger than 8 mm, the size of 40 % of the material was between 8 and 4 mm, 21% between 2 and 4 mm, and only 11 % were smaller than 2 mm. The average particle size of the carob kibbles utilized as raw material was about 4.3 mm and their moisture content was 10–13%. In this study, Soxhlet extraction (4g kibbles in 200 mL water, 16 h with 5–6 cycles/h) was carried out in order to estimate

the total content of extractable sugars and phenolic compounds in the carob kibbles. After Soxhlet extraction, the dry solid residues were about 40 % of their initial mass.

4.2 Extraction methods

4.2.1 Extraction of spruce hemicelluloses

The autohydrolysates were made by Metla (Luke nowadays) using pressurized hot water extraction (PHWE). The laboratory scale apparatus utilized to produce **$SWaH_2$** is described in Leppänen et al. (2011). The flow-through equipment for pilot-scale PHWE (for the production of **$SWaH_{1, 3, 4, and 5}$)** has been described in detail by Kilpeläinen et al. (2014). The autohydrolysates were produced at operating temperatures between 160 and 180 °C. After extraction, the autohydrolysates were cooled and then stored in a freezer before further use in the filtration experiments.

4.2.2 Aqueous extraction of carob kibbles

The experiments to optimize the operating conditions for one-step aqueous extraction are presented in **Table 4.2 (a).** The procedure scheme of this extraction is depicted in **Fig. 4.1 (a).** The carob kibbles (25 g - 4 kg) were exposed to aqueous extraction by mixing with water at a defined liquid-to-solid ratio (LSR) in the range of 2-50. The extractions were carried out using a temperature-controlled orbital incubator (Infors Unitron HT, Switzerland) or a water bath (for high temperature). The range of the extraction conditions, i.e. temperature and time were 30-100 ºC and 20-300 min, respectively.

Table 4.2 (b) presents the two-step extraction experiments**.** The scheme of the two-step aqueous extraction is shown in **Fig. 4.1 (b)**. The temperature of the first step was set at 30 °C to ensure significant isolation of sugars (mainly glucose, fructose and sucrose), together with low removal of phenolic compounds. For the determination of the LSR and time of the first extraction step, different LSRs (10 and 20) and times (220 minutes and 300 minutes) were tested. The second extraction step (hot water extraction) was carried out on the solid residues of the first step. The operating conditions of the one-step extraction experiments that were optimized to achieve a high phenolic compound yield were used in the second step extraction.

A pilot scale trial was done using 4 kg of carob kibbles, and the first step was performed with 4 batches (1 kg of kibbles/ batch) at 30 °C and determined LSR and time based on the tested ranges explained above. The solids from all batches were collected as one batch in the autoclave to perform the second, hot water extraction.

Table 4.2 Description of aqueous extraction experiments of carob kibbles.

Experiment	Description	Operating conditions
a) One -step aqueous extraction		
Optimization of LSR	Experiments at 50 °C for 5 h	LSR : 2,3,4,9 and 50
Optimization of temperature and time	Experiments at optimized LSR	Temperature range: 30-100 °C Time range : 20-300 min
b) Two -step aqueous extraction		
Determination of LSR and time of the first aqueous extraction step	Experiments at 30 °C	LSR : 10 and 20 Time : 220 and 300 min
Two- step aqueous extraction	Experiments using different solid loads (0.3 and 1.2 kg of carob kibbles) Pilot scale using 4 kg of carob kibbles	1st step : 30 °C and determined LSR and time (highest sugars removal and lowest phenolic compounds removal) 2nd step: Optimized conditions of one-step aqueous extraction

In all extraction experiments, the residual liquid inside the solids was taken out using a hydraulic press with a pressure up to 200 bars, to enhance the recovery of the solutes. Prior to the analysis, the suspended solids in the extract were removed by centrifugation with a Heraeus Sepatech centrifuge (12000 rpm, 10 minutes at 4 °C). Finally, the liquid extracts were stored in a freezer for further use. To recover phenolic compounds and sugars, distinct streams from the extraction processes were operated as feed for dia-nanofiltration (Di-NF), nanofiltration and reverse osmosis experiments.

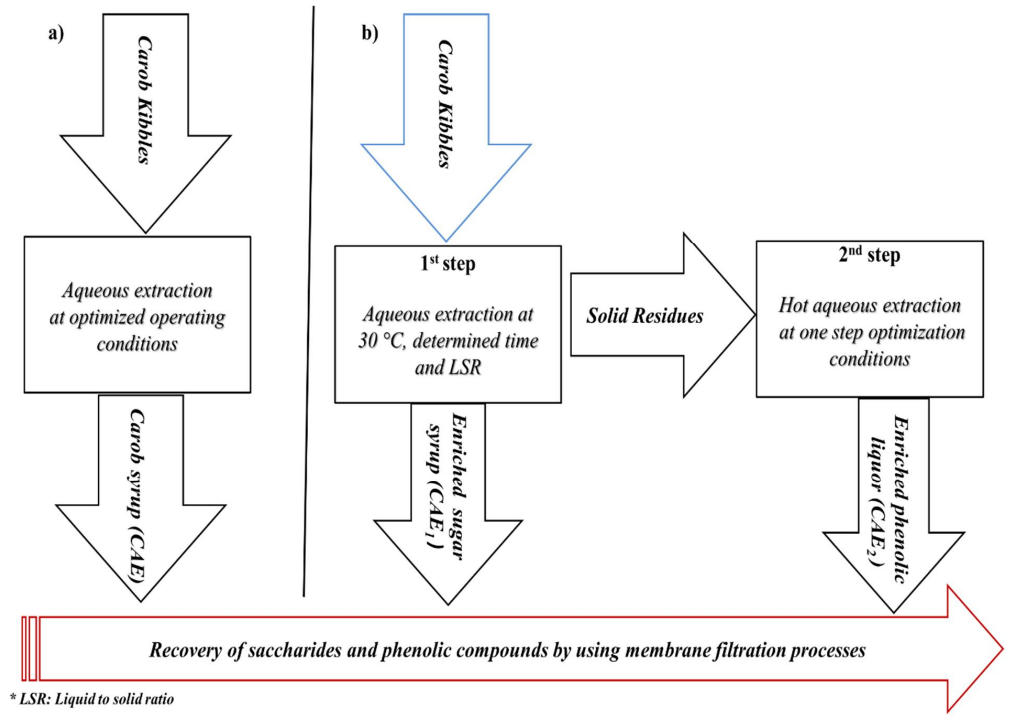


Figure 4.1 Scheme of a) one- and b) two- step aqueous extractions.

4.3 Membranes

UF membranes with different cut-offs and materials were tested in the treatment of spruce autohydrolysate. The performance of hydrophilic and hydrophobic membranes for the filtration of this type of liquor was evaluated. The specification of the tested membranes is shown in **Table 4.3**. The selection of the membranes was based on earlier investigations where the separation, fractionation and concentration of hemicelluloses from various autohydrolysates and pulping process water had been studied (Persson, 2009; Persson and Jönsson, 2010; Persson et al. 2010; Song et al. 2013).

In the treatment of the extracts from carob kibbles, NF and RO membranes (**Table 4.4)** were examined for purification and fractionation, as well as concentration of phenolic compounds and sugars. The NF membranes were selected on the basis of a related survey aimed at concentrating the phenolic compounds present in a biomass-derived extract (Versari et al. 2003). An RO membrane was utilized for concentrating the sugars. Ferrarini et al. (2001) have used this membrane for the same purpose.

Table 4.3 Main specifications of the membranes tested in the ultrafiltration of spruce hydrolysates (SWaHs)

Membrane	Manufacturer	Material	Cut-off, kDa
UP010	Microdyn-Nadir	Polyether sulphone	10
UFX010	Alfa Laval	Hydrophilized polysulphone	10
UC010	Microdyn-Nadir	Regenerated cellulose	10
RC70PP	Alfa Laval	Regenerated cellulose acetate	10
Desal GM	GE Water	Thin film composite	8
Desal GK	GE Water	Thin film composite	3.5
Desal GH	GE Water	Thin film composite	2.5
Desal GE	GE Water	Thin film composite	1
ETNA01PP	Alfa Laval	Composite fluoro polymer	1
NP010	Microdyn-Nadir	Polyether sulphone	1
UC030	Microdyn-Nadir	Regenerated cellulose	30
UC005	Microdyn-Nadir	Regenerated cellulose	5

Table 4.4 Main specifications of the NF and RO membranes tested in the treatment of carob aqueous extracts (CAEs)

Membrane	Manufacturer	Material	Cut-off, Da/ Salt retention
Desal 5-DK, DK2540	GE Water	Polyamide-TFM (thin film membrane) composite	150-300
NF270	Dow Chemicals	Semi-aromatic piperazine-based polyamide	200-400
SW30-2540	Dow Chemicals	Polyamide-TFM composite	99.4% salt rejection

4.4 Membrane filtration experiments

4.4.1 Filtration of spruce autohydrolysates

The filtrations of the spruce wood autohydrolysates (SWaHs) at laboratory scale were carried out using a cross-flow flat sheet membrane module. A DSS Labstak M20- filter was used for the screening of various membranes and evaluation of their performance in the filtration of autohydrolysates. Membrane concentration and fractionation experiments in pilot scale were performed by a cross-rotational (CR) filter (Metso Paper Inc.). The filtration equipment is presented in **Table 4.5.**

Table 4.5 Membrane filtration units operated in the treatment of spruce autohydrolysates

Membrane filtration unit	Active area, m^2	Operating parameters
Rectangular cross-flow-flat sheet	0.01	Max. pressure 40 bar
DSS Labstak M20	5×0.036	Max. pressure 70 bar
CR200	0.06	Max. pressure 4 bar Rotor peripheral velocity 9 m/s
CR250	2×0.045	Max. pressure 10 bar Rotor peripheral velocity 9 m/s

Prior to utilization, the membranes were treated according to the manufacturer's recommendations. The membranes in the flat sheet module were washed 3×15 min with water in an ultrasonic bath. In the beginning of the DSS Labstak M20 and CR pilot scale experiments, the membranes were cleaned for 20 min with an alkaline Ultrasil 110 solution (the operating conditions were 0.05-0.1%, 0.5-1 bar, 35 °C, pH= 10-11).

Pure water flux (PWF) measurements were carried out before and after each filtration of the spruce autohydrolysates as a parameter to estimate the fouling tendency of the tested membrane (**Papers I-II**). In several trials, the membranes were washed with a cleaning agent (Ultrasil 110 solution, 0.1%, 0.5-1 bar, 50 °C, and 15 min) after filtration to remove foulants and to estimate the ability to retrieve the permeability of the tested membranes.

The selection of the proper membranes was based on filtration capacity measurements, i.e. autohydrolysate permeability, membrane fouling tendency and the capability to fractionate and concentrate GGMs. In the membrane selection experiments (see **Table 4.6 (a)**), total recirculation mode filtrations were performed by circulating the concentrate and permeate streams back to the feed vessel (keeping the volume constant in the feed tank). The concentration mode filtrations (**Table 4.6 (b)**) were done by recycling the concentrate back to the feed tank and collecting the permeate to a separate vessel until the desired volume reduction ratio (VR, %) was achieved (VR is defined as the ratio of the collected permeate volume to the initial feed volume).

The two-step UF scheme (**Fig. 4.2)** was carried out to fractionate GGMs into two concentrated fractions suitable for different value-added applications. The effect of pulsed corona discharge oxidation (PCD) for 45 min (**Paper II**) and high volume reduction (VR > 99%) on the fractionation efficiency were studied. **Table 4.6 (b)** presents the major two-step filtration experiments.

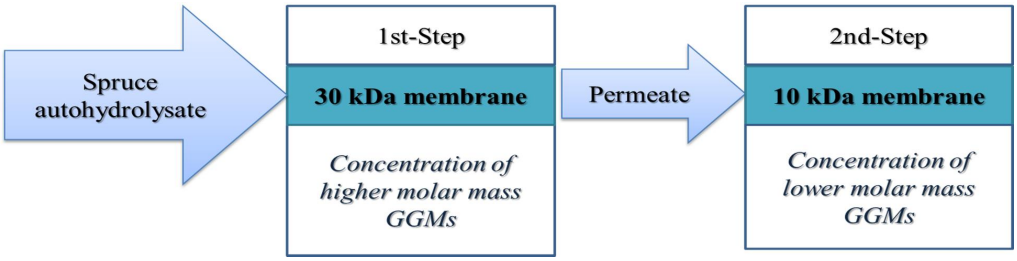


Figure 4.2 Schematic illustration of the spruce autohydrolysate fractionation approach.

The batch diafiltration (DF) technique was applied after concentration mode filtration to purify the retained hemicelluloses. During DF, a specific volume of RO-filtered water (κ = 0.054 µS/cm) was added at intervals to the concentrate in the feed tank, and a similar volume of permeate was removed. The DF volume is defined as the ratio of the DF water volume to the concentrate/feed volume.

Table 4.6 UF experiments performed for the recovery of hemicelluloses from SWaHs

Spruce autohydrolysates	Experiments	Membranes
a) Membrane selection and one-step UF		
$SWaH_1$	Membrane evaluation experiments, 8-10 kDa	GM, RC70PP,UC010, UFX010,UP010
	Membrane evaluation experiments, 1-3.5 kDa	GK,GH,GE, ETNA01PP, NP010
	Direct concentration filtration	RC70PP
$SWaH_2$	Lab-scale filtration for the evaluation of the performance of RC membranes	UC030 (30 kDa), UC010 (10 kDa), UC005 (5 kDa)
	Diafiltration	UC030
b) Two-step UF		
$SWaH_3$	Hybrid process: PCD oxidation treatment followed by membrane fractionation as descriped in Paper II	UC030, RC70PP
$SWaH_4$	Fractionation and concentration of GGMs (VR > 99%) using the scheme in **Fig 4.2**	UC030, RC70PP
	Diafiltration	UC030
$SWaH_5$	Fractionation and concentration of GGMs (VR > 99%) using the scheme in **Fig 4.2**	UC030, RC70PP
	Diafiltration	

4.4.2 Filtration of carob aqueous extracts

In the dia-nanofiltration (Di-NF) and NF of the one-step carob extract (**CAE**, **Fig. 4.3(a))**, the laboratory scale experiments were done with Desal 5-DK and NF270 membranes in a dead-end Met-cell filtration unit (active membrane area of 51.4 cm^2, 50 ºC, 12 bar). This extract was diluted with water (dilution ratio 3.9) before processing, due to its high viscosity. After one dia-volume, the extract was concentrated with NF to the highest possible volume reduction factor (VRF).

The extraction liquors from the two-step extraction were fractionated and concentrated in pilot scale with spiral wound modules. Reverse osmosis (RO) process was done using a SW30-2540 spiral wound module with the active area of 2.8 m^2 to concentrate the sugars in the extract produced in the first extraction step of the two-step extraction approach (**CAE_1**, **Fig. 4.3(b))**. RO was carried out at 25 ºC and 30 bar until the highest possible VRF was reached.

Pilot-scale Di-NF and NF experiments (50 ºC, 8 bar) were carried out for the fractionation of the second extraction stage liquor (**CAE_2**, **Fig. 4.3(b)**). A Desal 5 DK 2540 spiral wound module with the active area of 2.6 m^2 was employed. Di-NF was continued until 3 dia-volumes were reached, and then concentration mode NF was done to the highest possible VRF. In both scales, Di-NF was performed in a continuous mode where the volume in the feed tank was kept constant by compensating the amount of extract passing through the membrane with the same amount of fresh water. More details about membrane processing for the filtration of carob kibbles can be found in **Paper IV**.

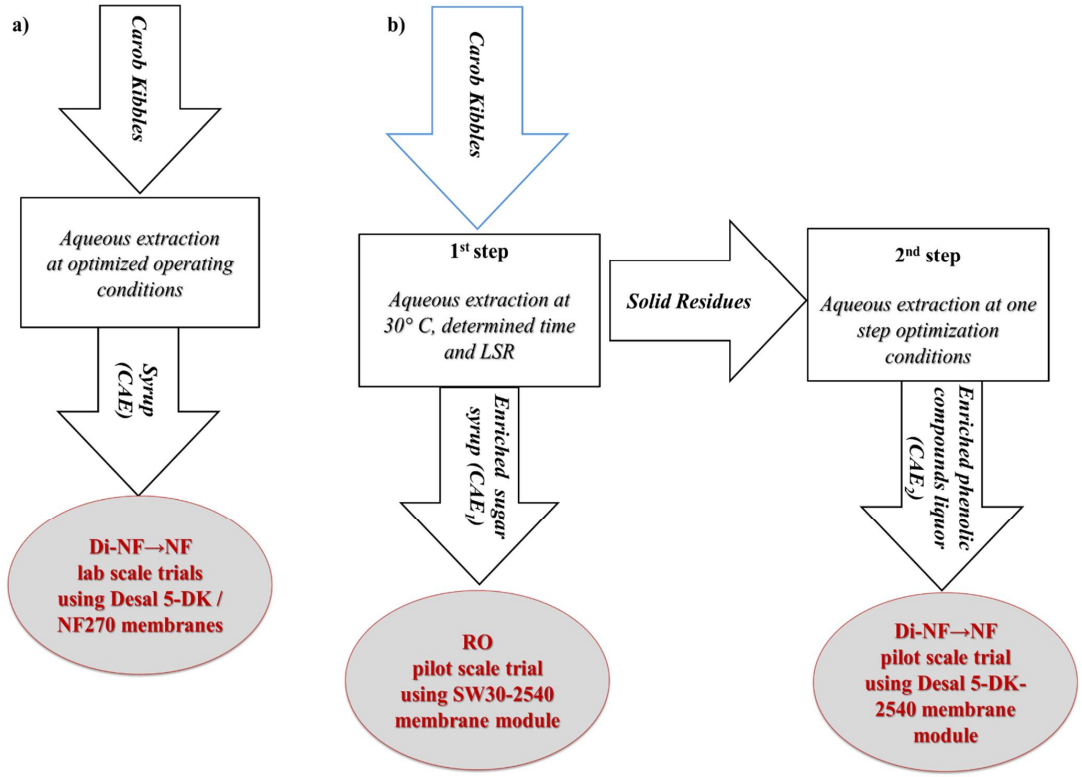


Figure 4.3 Scheme of experiments performed for the filtration of carob aqueous extracts

4.5 Analytical methods

The analysis methods and equipment utilized to characterize the various liquid streams produced in the filtration and extraction experiments are presented in **Table 4.7**. Detailed description of the analysis methods utilized in this study can be found in **Papers I – IV**.

Table 4.7 Analysis methods and equipment employed in this study.

Analysis method / equipment	Use/Purpose
TOC-5050 analyzer	Estimation of total carbon (TC) content in spruce autohydrolysates (Paper I and II).
Acid methanolysis + gas chromatography	Estimation of hemicellulose and monosaccharide content according to Sundberg et al. (1996) (Papers I and II)
Absorbance of ultraviolet light at 280 nm	Estimation of lignin content with correlation coefficient of 17.8 L/ (g cm) with absorbance measurement (Örså et al. 1997) (Papers I and II).
pH and conductivity measurements	Determination the ionic content of the samples. Conductivity measured with a digital conductivity meter (Knick Konduktometer 703)
Hach 2100AN IS turbidimeter	Turbidity measurements as a parameter to detect the presence of suspended solids and possible extractives in spruce autohydrolysates
COMECTA I viscometer	Measurements of the kinetic viscosity of carob extracts (Papers III and IV).
Size-exclusion chromatography (SEC) in on-line combination with a multi-angle-laser-light-scattering (MALLS) instrument	Determination of molar mass distribution (Paper II).
HPLC (Waters, Milford, USA) using a Sugar Pak-1 column, RI detector ,80 °C, flow rate of 0.5 mL/min.	Quantification of sucrose, glucose and fructose in carob aqueous extracts (Papers III and IV).
Aminex HPX-87H HPLC column (Bio-Rad, Hercules, CA, USA) 50 °C, flow rate of 0.6 mL/min.	Quantification of acetic and gallic acids in carob aqueous extracts (Papers III and IV).
Folin-Ciocalteu method using gallic acid as standard (Singleton et al. 1999)	Quantification of total phenolic compounds content in carob aqueous extracts (Papers III and IV).
Capillary zone electrophoresis (CZE) (Agilent Technologies CE system) with a diode array detector (DAD)	Analysis of the phenolic compound profile and identification of various phenolic compounds in carob aqueous extracts, based on the procedure of Roseiro et al. (2013) (Papers III and IV).

4.6. Calculations

Mass flux is the rate of permeate mass flow across a membrane surface area. In this study, it has been calculated with equation (1):

$$J_m = \frac{m_p}{A_m} \quad (1)$$

where J_m is the mass flux (kg/(m^2h)), m_p is the mass flow rate (kg/h) and A_m is the membrane surface area (m^2).

Permeability (kg/ (m^2 h bar)) through the membranes at different filtration stages have been calculated with equation (2):

$$\text{Permeability (P)} = \frac{J_m}{\Delta p} \quad (2)$$

$$\text{Permeability corrected for the osmotic pressure effect } (P_{corr}) = \frac{J_m}{\Delta p - \Delta \pi} \quad (3)$$

where Δp is the applied operating pressure (bar) and $\Delta\pi$ is the osmotic pressure difference (bar).

The osmotic pressure difference ($\Delta\pi$) between the feed and permeate side of the membrane was estimated using the Van't Hoff equation (4):

$$\Delta\pi = R\,\Delta Ci\,T \quad (4)$$

where R is the ideal gas constant = 0.083145 L bar/mole °K , ΔCi is the difference in the molar concentrate of solutes (mole/L) through the membrane (between the feed and permeate sides) and T is the absolute temperature in Kelvin, °K = (T(°C) +273.15). The ionic strength for sugars =1.

The estimation of osmotic pressure using Van't Hoff equation gives approximate values for osmotic pressure at the sugar concentration levels in this study. As shown in Timkin and Lazarev (2015) study, the experimental data of osmotic pressure at 20 ºC was almost equal to the values calculated from the van't Hoff equation for glucose and sucrose solutions with concentrations up to 200 g/L.

For the evaluation of the fouling of membranes, the reduction of the pure water flux, *FR (PWF)*, has been used. Fouling has been calculated according to equation (5).

$$FR(PWF) = \left(1 - \frac{PWF_a}{PWF_b}\right) \cdot 100\% \quad (5)$$

where PWF_a is the pure water flux after filtration of the spruce autohydrolysates or carob extracts, and PWF_b is the pure water flux before the filtration.

The purity of the GGMs has been defined as the ratio between the carbon content in the hemicelluloses (about 40%) and the total organic carbon (TOC) in the final concentrate, and calculated with:

$$\text{Purity}(\%) = \frac{0.4 \times C_{GGM}}{TOC} \cdot 100\% \quad (6)$$

where $C_{GGMs(C)}$ is the concentration of GGMs and $TOC_{(C)}$ is the total organic carbon in the filtration stream.

The volume reduction ratio, VR (%), describing the concentration degree of the feed during the experiments has been calculated as follows:

$$VR(\%) = \frac{V_p}{V_{fb}} \times 100\% \quad (7)$$

where V_{fb} (L) is the feed volume at the beginning of filtration and V_p (L) is the volume of the accumulated permeate during and/or at the end of the filtration.

The volume reduction factor (VRF) is defined as the ratio of the initial feed volume to the concentrate volume during filtration; it can be calculated as a function of volume reduction (VR), as in equation (8):

$$\text{VRF} = \frac{1}{1 - VR} \quad (8)$$

The increase of the concentration in any solute, like GGMs or phenolic compounds due to filtration is described with the concentration factor (CF), which has been calculated by equation (9):

$$CF = \frac{C_c}{Cf} \qquad (9)$$

where C_c (g/L) is the concentrate of GGMs in the concentrate, and C_f (g/L) is the concentration of solute in the feed.

D (-), the number of dia-volumes, is defined as the ratio of the volume of the diafiltration permeate (V_{perm}) to the volume of the feed at the beginning of diafiltration (V_{fb}, almost constant at diafiltration mode), as in equation (10):

$$D = \frac{V_p}{V_{fb}} \qquad (10)$$

R_o is the observed retention of the solute by the membrane, calculated through equation (11)

$$R_o = 1 - \frac{C_p}{C_f} \qquad (11)$$

where C_p is the observed concentration of solute in the permeate and C_f is the concentration of solute in the feed.

The observed membrane retention of the solute considering the effect of VR has been calculated **(Paper I)** by equation (12) (Strathmann et al. 2006):

$$R_o = 1 - \frac{\ln(1 - \frac{C_p \Delta}{C_f})}{\ln(1 - \Delta)} \qquad (12)$$

where R_o is the observed retention, C_p is the concentration of accumulated solute in the permeate at a specific recovery rate, C_f is the feed concentration, and Δ is the recovery rate (accumulated volume of permeate divided by feed volume, which corresponds to the final VR definition and value in this study).

5 Results and discussion

In the first part of this work, the separation of galactoglucomannans (GGMs) from spruce autohydrolysates using ultrafiltration (UF) was studied. The performance of UF was evaluated in terms of filterability parameters and the quality of the concentrate fractions of GGMs. To improve the performance of GGM recovery in UF, diafiltration (DF) and oxidation of the autohydrolysates were also investigated. In the second part of the work, the recovery of phenolic compounds and sugars from carob kibbles using aqueous extraction and membrane filtration processes was studied. The fractionation and concentration of these compounds from the extraction streams were investigated with nanofiltration (NF) and reverse osmosis (RO). The evaluation of the extraction and membrane processes was based on the yields of extractable compounds, the filterability of the carob extracts, and the quality of the concentrated streams.

5.1 Recovery of GGMs from spruce autohydrolysates

UF was investigated for recovering GGMs from spruce autohydrolysates. Membranes with different active materials were tested, and the one with the best performance was used in large-scale experiments. The efficiency of UF was evaluated in terms of the permeability of the autohydrolysates and the fouling of the membrane, as well as the purity and concentration of GGMs in the concentrate fractions. DF and pulsed corona discharge (PCD) oxidation were performed, and their effect on the purity of GGMs was evaluated. The results are summarized in this section, and they were discussed in detail in **Papers I-II**.

5.1.1 Membrane selection

Membranes with different active surface materials and cut-off values were tested in filtering spruce autohydrolysates. The comparison of the tested membranes was based on the permeability of the spruce wood autohydrolysates (SWaHs), i.e. the filtration capacity and the fouling tendency of the membrane. The pH of these autohydrolysates (3.8-3.9) was in the operating pH range of the tested membranes (2-11). Moreover, the tested membranes were found to withstand the filtration temperature (65 ºC). Of the tested membranes (**Table 5.1.1**), regenerated cellulose (RC), RC70PP and UC010 membranes had rather high filtration capacity in the filtration of **$SWaH_1$**. Moreover, they were only slightly fouled and their original pure-water permeability (PWP_o) was efficiently recovered (~ 95 % of PWP_o) by water flushing and alkaline cleaning. In a number of studies, RC

membranes have been found suitable for the recovery of GGMs from thermomechanical pulping (TMP) process water (Persson et al. 2005; Hartman et al. 2006 a, b), Masonite process water (Persson and Jönsson, 2009) and wood hydrolysates (Koivula et al. 2011).

Unlike RC membranes, the low filtration capacity and/or severe fouling of the other tested membranes (more hydrophobic membranes) make their utilization for GGM recovery quite challenging. Several studies have shown that the reduction in the permeate flux of hydrophobic membranes compared with hydrophilic ones is due to the higher fouling effect of lignin and other hydrophobic compounds like extractives existing in the wood pulping effluents (Puro et al. 2002; Maartens et al. 2002). Cellulose-based membranes are extremely hydrophilic compared to PS or PES membranes. This was found to lead to higher permeability during the filtration of autohydrolysates and lower fouling tendency in this study.

Table 5.1.1 Permeabilities and fouling (directly after filtration (FR) and after alkaline cleaning (FR_{ac})) of UF membranes when **$SWaH_1$** was filtered in the total circulation and concentration mode (DSS Labstak M20-filter at 65 °C, 2.5-3.5 bar).

Membrane	Surface material	Cut-off, kDa	Pure water permeability	Initial--> End Permeability, (kg/m^2h bar)		Fouling	
			PWP_o (kg/m^2h bar)	Circulation mode for 16 h	Concentration mode for 20 h	FR %	FR_{ac} %
RC70PP	RCA	10	61	39--> 27	26--> 19	18	4
UC010	RC	10	44	34--> 25	24--> 20	3	_
UP010	PES	10	60	3--> 1	0	96	60
UFX010	H-PS	10	60	13--> 2	2--> 0.2	93	46
Desal GM	TFC	8	17	13--> 10	10--> 8	35	8

RCA: Regenerated cellulose acetate, RC: Regenerated cellulose, PES: Polyether-sulphone, H-PS: Hydrophilized polysulphone, TFC: Thin film composite.

Ultrafiltration membranes having cut-off values from 1 to 3.5 kDa were also tested for the recovery of hemicelluloses from the permeate of the 10 kDa RC membrane**.** As **Table (5.1.2)** shows the permeabilities of all the tested membranes were around 1 kg/ (m^2h bar) after 36 hours of filtration. In addition, pure water permeabilities were 20-40% of the original values after the filtration and water flushing of the membrane. Alkaline cleaning with 0.1% Ultrasil 110 restored the capacity by 60-90% with the tested membranes. However, both the filtration capacity and fouling were too high to enable utilization of tight membranes (**Table 5.1.2**) for the concentration of hemicelluloses from the permeate of the 10 kDa membrane. High reduction in the permeate flux and significant

fouling with PVDF and PS membranes in the filtration of wood hydrolysates and extraction liquors have also been reported by for instance Persson and Jönsson (2009), Persson et al. (2010) and Koivula et al. (2011).

The results showed the importance of membrane materials on the filtration capacity, i.e. the permeability of the autohydrolysates and fouling. Therefore, in this study the fractionation and concentration of spruce autohydrolysates were mainly performed with membranes made from regenerated cellulose (10 and 30 kDa).

Table 5.1.2 Permeabilities and fouling (directly after filtration (FR) and after alkaline cleaning (FR_{ac})) of UF membranes (cut-off 1-3.5 kDa) when the permeate of the 10 kDa membrane was filtered (DSS Labstak M20-filter at 65 °C, 8-9 bar).

Membrane	Surface material	Cut-off, kDa	Pure water permeability	Initial--> End Permeability (kg/m^2h bar)		Fouling	
			PWP_o (kg/m^2h bar)	Circulation mode for 16 h	Concentration mode for 20 h	FR %	FR_{ac} %
Desal GK	TFC	3.5	15	9--> 2.6	2.6--> 1.3	75	8
Desal GH	TFC	2.5	10.5	5.7--> 2.2	2.2--> 1.3	71	28
Desal GE	TFC	1	8.2	5.3--> 1.2	1--> 0.5	87	20
ETNA01PP	PVDF	1	34.3	25--> 17	14--> 1.2	59	_
NP010	PES	1	11.7	1.8--> 0.7	2--> 0.7	82	19

TFC: Thin film composite, PVDF: Polyvinylidene difluoride, PES: Polyether-sulphone.

As the RC membranes were found to be the most efficient ones for the filtration of spruce autohydrolysates, the effect of their cut-off values on the filtration efficiency was studied (**Paper I)**. **Table 5.1.3** shows that the membrane with the highest cut-off had the highest permeability of the autohydrolysates, as could be expected. In all the tested RC membranes, when the autohydrolysate was concentrated about three times, the reduction in the permeability of the autohydrolysates was only about 5%. The fouling tendency of the tested membranes was rather low. The higher fouling (26%) of a more open structure, i.e. the 30 kDa membrane could be explained by the higher possibility of pore blocking with high molar mass compounds. The highest GGM retention and the greatest difference between the GGMs and lignin retentions (88% and 55%, respectively) were achieved with the 5 kDa membrane. Moreover, with this membrane, the purity of GGM was increased by 15 percentage units. The 10 and 30 kDa membranes also retained hemicelluloses, mainly GGMs, better than they retained lignin. Therefore, the purity of GGMs in their concentrates was somewhat higher than in the feed. It can be assumed that the production of

GGM fractions with certain specifications, like molar mass distribution, can be reached by selecting RC membranes with a proper cut-off. During the study, the 5 kDa RC membrane was not commercially available, so it was not used in the further experiments. However, obtaining GGM fractions having as high molar mass as possible could be achieved with the other tested RC membranes.

Table 5.1.3 Permeability of the autohydrolysate and fouling of the membrane, as well as the retention and purity of GGMs when **$SWaH_2$** was ultra-filtered (VRF= 3-4) with RC membranes having different cut-off values (flat sheet filter, 65 ºC).

Cut-off, kDa	Pressure, bar	Initial--> end **Permeability** (kg/m^{2}h bar)	Fouling	Retentions, %		Purity of GGMs ,% (TOC basis)
			FR,%	**$R_{o\ GGMs}$**	**$R_{o\ Lignin}$**	**Feed --> Concentrate**
30	1	260--> 247	26	38	32	48-->51
10	3	46--> 43	16	70	42	48-->57
5	3.5	37--> 35	18	88	55	48-->63

5.1.2 Permeability and fouling during the fractionation of GGMs

The pilot-scale UF scheme presented in **Fig. 4.2** was carried out to recover GGMs from spruce wood autohydrolysates (SWaHs) into concentrated fractions with narrower molar mass distribution. In this scheme, **$SWaH_4$** and **$SWaH_5$** were first filtered to high VR values with 30 kDa membranes in a high shear rate CR-filter. When the filtrations were started, the achieved permeabilities of the **$SWaH_4$** and **$SWaH_5$** autohydrolysates were higher than 200 kg/m^{2}h bar. As **Fig. 5.1.1** shows, the decline in the permeability with the 30 kDa membrane could be divided to 3 periods where various phenomena had an influence. In the beginning of filtration ($P1_a$, $P1_b$), the decline in the permeability compared to pure water permeability was 42% at VR= 15% (**$SWaH_4$**) and 48% at VR= 16% (**$SWaH_5$**). Furthermore, at these VR values, the reduction of permeability was quite close to the measured membrane fouling (30-35%). This behaviour may have been due to the pore blocking phenomenon, as well as the higher osmotic pressure and viscosity of the autohydrolysate than those of pure water. In the beginning of the UF, the effect of osmotic pressure corresponded to less than 5% in the flux decline. The experiments were made at high shear rate conditions, and therefore the effect of concentration polarization could be assumed to be low. The TOC content of **$SWaH_5$** (9 g/L) was about three times higher than the one of **$SWaH_4$**. This difference in their content, as well as in their average molar masses (7 kDa and 6 kDa, respectively)

might explain the variance in their permeability values. Generally, the more dilute autohydrolysate (**$SWaH_4$**) had higher permeability.

After the fast initial decrease of permeability, a slighter reduction ($P2_a$, $P2_b$ in **Fig. 5.1.1**) could be observed due to the increase of osmotic pressure and viscosity. The increase of osmotic pressure reduced the effective pressure by about 15% at this stage.

A stronger decline in autohydrolysate permeability was seen ($P3_a$, $P3_b$ in **Fig. 5.1.1**) when GGMs were concentrated to a concentration associated with the formation of a gel layer on the membrane surface. The drastic decline in the permeability of **$SWaH_4$** and **$SWaH_5$** started at VR of 77% and 56%, respectively, till the end of the filtration. At these VR values the concentrations of GGMs were 9 g/L (**$SWaH_4$**) and 15 g/L (**$SWaH_5$**) and increased to ~100 g/L (VR~ 99%). Albuquerque et al. (2014) indicate that galactomannan solutions could reach a gel-like state at the concentration of 20 g/L. Based on the studies of Xu et al. (2007; 2009), the viscosity of GGM solutions increases rapidly with GGM concentration. As the concentration of the feed increases during filtration, its content of high molar mass compounds, i.e. the polymeric fraction, becomes more purified because small molecules permeate through the 30 kDa membrane. This enhances the formation of a gel layer that disturbs the permeate flux through the membrane (Persson and Jönsson, 2010). Therefore, it can be assumed that the permeability decrease was mostly due to a viscous gel layer formed on the membrane surface. Compaction and the high operating temperature may also have contributed to the decline of the permeate flux and pure water flux after the filtration. When the **$SWaH_4$** and **$SWaH_5$** autohydrolysates were filtered with the 30 kDa membranes, the operating pressure range was 1-4 bar and 65 ºC. Kallioinen (2008) reports that the compaction of this membrane could occur at operating trans-membrane pressure higher than 3 bar. However, in our study the compaction of the membrane could not explain the fast decrease of the permeability completely, because the applied pressure was 1.6 bar at maximum when the fast decrease in the permeability started. However, compaction had definitely an effect on the decrease of the pure water permeability, but it was not possible to define its effect on fouling. The permeability behaviour (**Fig. 5.1.1**) observed in this study was somewhat dissimilar to the behaviour shown in other related studies aiming at concentrating GGMs from e.g. TMP and Masonite process water (Persson and Jönsson, 2009; Persson et al. 2010, Krawczyk et al. 2013 a). Pre-filtration of the feed

by MF and differences in the feed origin, membrane material and operating conditions may be the reason for such variation.

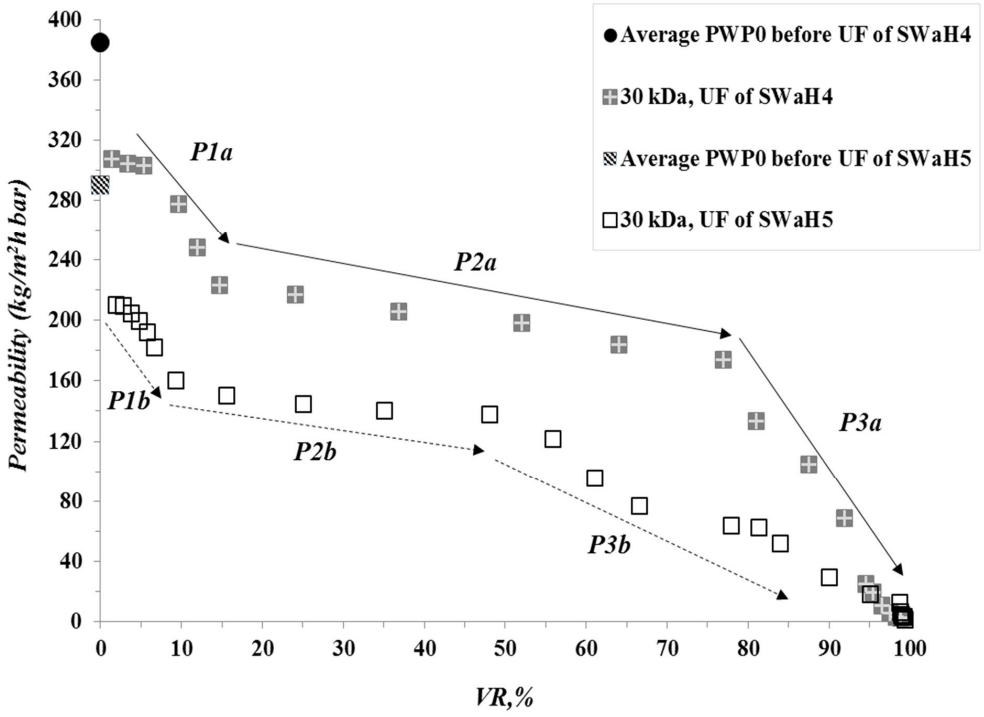


Figure 5.1.1 Permeability of **$SWaH_4$** and **$SWaH_5$** through the UC030 (30 kDa) RC membrane (pressure 1-4 bar, temperature 65 °C, CR250-filter, rotor speed 9 m/s). PWP0 is the initial pure water permeability (average).

In the second step of the scheme (**Fig.4.2),** the permeates of the 30 kDa membrane were filtered with 10 kDa membranes. The filtrations were continued until a very high VR (> 99%) was reached. At that stage of the filtrations, the permeability was already very low. As **Fig. 5.1.2** shows, in the beginning of the filtrations the permeability values of these autohydrolysates (30 kDa permeates) were quite close to the pure water permeability of the membrane (PWP_0). When the filtrations progressed, significant declines in the permeabilities were observed. This was not caused by fouling of the 10 kDa membranes. Fouling was lower than 10%, although very high VR (>99.5%) was reached in the filtrations. During the concentration of GGMs with the 10 kDa membranes, the osmotic pressure (20-30% of the operating pressure) and the viscosity of autohydrolysates increased, and thus the permeability decreased.

In order to investigate the effect of the pre-filtration of the autohydrolysates with the 30 kDa membrane on the filtration behaviour of the 10 kDa membrane, **SWaH$_1$** was concentrated directly using high shear CR-filters equipped with the 10 kDa RC70PP membrane. The pre-filtration seemed to have a minor effect on the permeability of the 10 kDa membrane until a very high VR was reached (**Fig. 5.1.2**). For instance at a VRF of 70 (VR ~ 98.5%), the permeability of the pre-filtered **SWaH$_4$** through the 10 kDa membrane was still 30 kg/m^{2}h bar, being about 2.3 time higher than when the same membrane filtered **SWaH$_1$** directly. Therefore, pre-filtration with a 30 kDa membrane before the utilization of a 10 kDa membrane may enable filtration to extremely high volume reduction levels (> 99.5%, corresponding to a VRF >100).

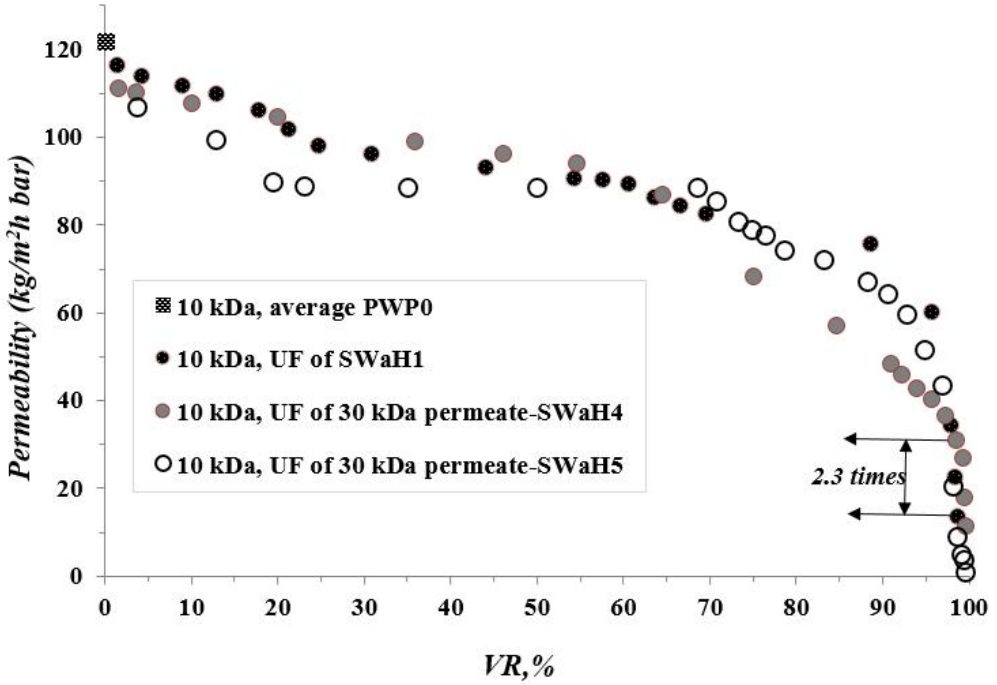


Figure 5.1.2 Permeability of the original spruce autohydrolysate (**SWaH$_1$**), and the 30 kDa permeates from **SWaH$_4$** and **SWaH$_5$** through the 10 kDa, RC70PP membrane (Pressure 2-5 bar, temperature 65 °C, CR200-filter, rotor speed 9 m/s). PWP0 is the initial pure water permeability (average).

In both filtration stages, the increase in the viscosity due to the increase in the concentration of high molar mass GGMs seemed to have a significant effect on the flux decline. Renaud et al. (2005) considered that the viscosity of a polysaccharide solution (as function of shear rate) depends

on the concentration of high molar mass polysaccharides in their study of the rheological behaviour of such solutions. Xu et al. (2007; 2009) observed viscous behaviour for a GGM solution (prepared by re-dissolving precipitated GGMs) with concentrations below 5%. Above this concentration, the viscosity of the GGM liquor increased sharply. Moreover, the elastic characteristics were more dominant than the viscous ones, as higher amounts of entanglements were formed. As shown in **Fig. 5.1.3** (Xu et al. (2009)), the viscosity of GGM solutions was shear rate -dependent, but it increased by roughly 10^2 when the concentration of GGMs in the solution increased from 0.5% to 10%. According to Pritchard et al. (1995), the increase in the viscosity during concentration of macromolecule solutions (i.e. wood autohydrolysates) affects the mass transfer coefficient, which decreases when the viscosity increases during filtration under turbulent conditions. Other parameters, like flocculation of deacetylated GGMs, formation of a gel-like layer due to high concentration, and pressure drop due to the osmotic effect may also have contributed to the flux decline (Xu et al. 2007; 2009; Albuquerque et al. 2014).

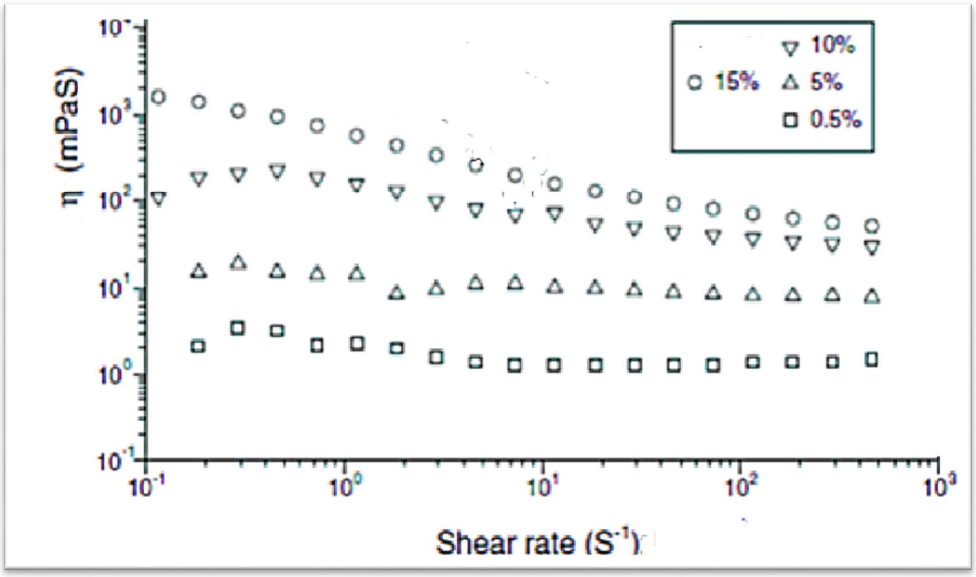


Figure 5.1.3 The dependency of shear rate on the viscosity of GGM solutions at different concentrations (25 ºC) (Xu et al. 2009, p.500).

The use of a high shear CR filter showed a high permeability of autohydrolysates compared with the other filters used in this study. For example, with the 10 kDa RC70PP membrane, the initial permeability of **SWaH$_1$** in the CR filter was about 3 times higher than that of the plate and frame

Labstak DSS-filter. A high shear rate leads to greater turbulence, which moves the particles away from the membrane surface, resulting in a decrease of concentration polarization.

The fouling of the tested membranes in the fractionation scheme was low. This was also due to the utilization of a high shear rate filtration module equipped with hydrophilic RC membranes. The fouling (reduction in pure water permeability) was ~10% when the 10 kDa RC70PP membranes were utilized for the filtration of either one of the original autohydrolysates or the pre-filtered one with the 30 kDa membrane. On the other hand, higher reductions, about 30%, in the pure water permeability were observed with the 30 kDa UC030 membranes during the filtration of **SWaH$_4$** and **SWaH$_5$**. This may have been partly due to the pore plugging phenomenon and membrane compaction. The fouling mechanisms by such macromolecule solutions i.e. wood autohydrolysates, are adsorption, pore blocking and formation of a gel layer (Puro et al. 2011; Krawczyk, and Jönsson, 2011; Koivula et al. 2011). In this study, the blocking of membrane pores with dissolved macromolecules and other potential foulants, mainly lignin and extractives, seemed to be more pronounced in the beginning of filtration, as the adsorption of these foulants with hydrophilic membrane is low. Moreover, the formation of the gel layer might have occurred at the end of the filtration, when high concentration is attained, causing diminishing in the effect of shear rate generated in the CR filter. In several other studies, pre-filtration of wood-based liquors using microfiltration has been applied to remove colloidal solids and extractives that may cause fouling during the UF concentration of GGMs (Persson et al. 2007, 2010; Andersson et al. 2007; Krawczyk et al. 2013 a). Therefore, from the cost-efficient point of view, the low fouling tendency of the tested RC membranes, especially the 10 kDa membrane, enable direct concentration of GGMs without pre-filtration. This simplifies the process and reduces the operating costs.

In this study, promising results for the recovery of GGMs from spruce autohydrolysates were achieved. Compared to the studies listed in **Table 5.1.4,** higher permeabilities of the autohydrolysates were measured even though higher GGM concentrations (100 g/L and above) were obtained. This was achieved with less operating costs, as the UF processes operated at a lower operating pressure and the autohydrolysates were not pre-treated before the concentration of the GGMs. Moreover, the fouling of the RC membranes was relatively low compared to the severe fouling reported by Persson and Jönsson (2009; 2010) with PVDF and PS membranes. The results

also showed that spruce autohydrolysates are promising materials for producing GGMs which are efficiently concentrated using UF with hydrophilic membranes.

Table 5.1.4 Comparison of the recovery efficiency of GGMs with other studies.

Study / Parameter	This study		Persson et al. (2007)	Persson et al. (2010)
Raw material	**Spruce autohydrolysate**		**TMP process water**	**TMP process water**
Pretreatment with MF	No		Yes	Yes
Membrane	RC (30 and 10 kDa)		PVDF (10 kDa)	H-PS (5 kDa)
Pressure and temperature	30 kDa 10 kDa	1-4 bar, 65 ºC 2-5 bar, 65 ºC	10 bar, 50 ºC	6 bar, 80 ºC
Average permeability (kg/m^2 h bar)	30 kDa 10 kDa	100-150 50-70	~6	~10
GGMs, g/L	30 kDa 10 kDa	~100 above 200	66	64

5.1.3. Separation and fractionation of GGMs

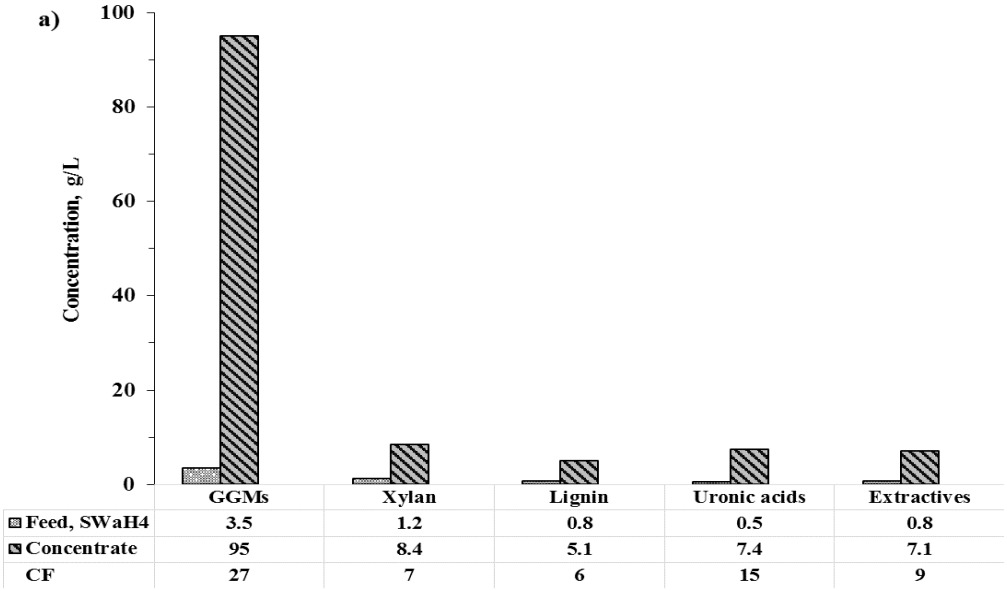
The separation efficiency of UF in the recovery and fractionation of GGMs was evaluated in terms of the concentration and purity of the GGM fractions. The differences in the specifications of these fractions depend on the composition and molar mass distribution of the original feeds, volume reduction (VR, %), and the characteristics of the tested membranes (particularly the cut-off).

GGMs were successfully concentrated and partially purified with one-step ultrafiltration. When **SWaH**$_1$ was directly ultra-filtered (concentrated ~70 times) with the 10 kDa RC membrane, a rich fraction of GGMs (112 g/L, 40% purity) having the average molar mass (Mw) of 18 kDa was obtained. This concentrate fraction had a lower polydispersity index, PDI ~1.5, than the original feed (~2.1), due to the removal of small-size molecules. During this filtration, GGMs were concentrated three times higher than the UV absorbance compounds, mainly lignin. This means that GGMs were partially purified from these compounds. However, only about 10% of turbidity was removed with this membrane. The GGM concentrate fraction also contained about 1.66 g/L of extractives, including lipophilic extractives (fatty and resin acids, sterols, steryl esters and triglycerides) and lignans. The dilute permeate contained lower molar mass GGMs (~ 2.4 g/L, 1.8 kDa, 32% purity).

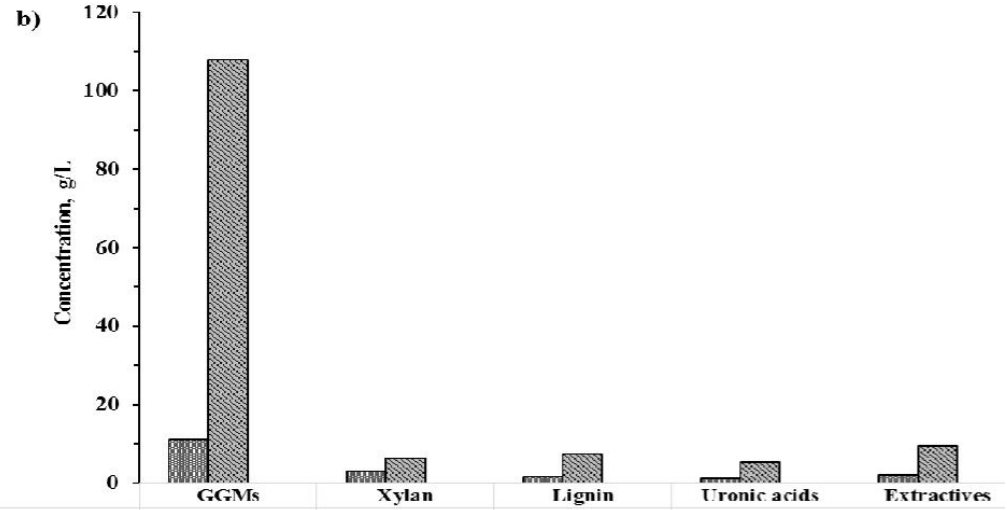
A two-stage UF process (30 kDa and 10 kDa membranes) was also studied to produce concentrated GGM fractions having as high average molar mass as possible. When **SWaH**$_4$ and **SWaH$_5$** were first filtered with the 30 kDa membrane to a VR of ~99 %, high molar mass GGMs were successfully concentrated (**Fig. 5.1.4).** The concentration factors (CF) of GGMs from **SWaH$_4$** and **SWaH$_5$** were ~27 (final concentration about 95 g/L, GGMs recovery ~30%) and 10 (110 g/L, GGMs recovery~ 10%), respectively. The average molar mass of the concentrated fraction from **SWaH$_4$** was 17.5 kDa. Due to the removal of low molar mass compounds, the polydispersity index (PDI) of this fraction (1.4) was lower than the PDI of the original **SWaH$_4$** (~2), which exhibited a narrower molar mass distribution.

Even though other main co-extraction compounds (including xylans, lignin, uronic acids, i.e. glucuronic and galacturonic acids, and extractives) were also concentrated, their contents in the concentrated streams were much less than those of GGMs. This means that the concentrate fractions of the 30 kDa membrane were mainly rich in GGMs as much more of them was recovered than of the other compounds in the autohydrolysates (the CF values of the GGMs were higher, **Fig. 5.1.4**).

The extraction temperature of **SWaH$_4$** was lower (160 ºC) than that of **SWaH$_5$** (180 ºC). As **Fig. 5.1.4** shows, at a lower extraction temperature the amount of dissolved substances was three times lower than at a higher temperature (180 ℃). Comparison of the final concentration factors with 30 kDa membranes showed that roughly three times higher concentration factor for GGMs was achieved when the **SWaH$_4$** solution was filtered. Due to the lower temperature of **SWaH$_4$**, the average molar mass of GGMs was higher because the degradation possibility of the hemicelluloses was lower. This could explain partly the high concentration factor of uronic acids when the **SWaH$_4$** solution was filtered, as their possibility to cleave into low molar mass molecules from either hemicellulose and/or pectin chains is lower at a lower extraction temperature. Molar mass analysis of the original autohydrolysates, i.e. **SWaH$_4$** (Mw = 6.8 kDa, PDI= 2.0, pH 3.8) and **SWaH$_5$** (Mw = 6 kDa, PDI= 1.7, pH 3.5), confirmed the difference in the average molar masses of the concentrated autohydrolysates. Moreover, the lower pH of the **SWaH$_5$** indicates that the degradation of GGMs was higher. This means that the concentrated GGMs from these autohydrolysates might have lower molar mass.



	GGMs	Xylan	Lignin	Uronic acids	Extractives
Feed, SWaH4	3.5	1.2	0.8	0.5	0.8
Concentrate	95	8.4	5.1	7.4	7.1
CF	27	7	6	15	9



	GGMs	Xylan	Lignin	Uronic acids	Extractives
Feed, SWaH5	11.1	3.1	1.6	1.1	1.9
Concentrate	108	6.3	7.3	5.3	9.5
CF	10	2	5	5	5

Figure 5.1.4 Contents of GGMs, xylans, lignin, uronic acids, and extractives in (a) **$SWaH_4$** (extraction temperature = 160 ºC) (b) **$SWaH_5$** (extraction temperature = 180 ºC), and in their 30 kDa membrane concentrate fractions when the VR was 99%. CF is the concentration factor for the analysed compounds.

In the second fractionation step, the permeates of the 30 kDa membrane from **SWaH$_4$** and **SWaH$_5$** (Mw were 3.4 kDa and 2.3 kDa, respectively) were concentrated using the 10 kDa RC membrane. Extremely high concentrations of GGMs, i.e. 200 g/L (**SWaH$_4$**: Mw= 9.1 kDa, PDI =1.3) and 400 g/L (**SWaH$_5$**: Mw= 5.3 kDa, PDI= 1.2) were obtained (VR about 99.5 %). These concentration values were significantly higher than the values reported in the literature. For example, a concentration of hemicelluloses of about 64 g/L at a VR of 99% was obtained by Persson et al. (2010) when they filtered TMP process water using a 5 kDa H-PS membrane. Persson et al. (2007) also used a 10 kDa PVDF membrane to concentrate hemicelluloses from TMP process water. They succeeded to increase the hemicellulose content from 1 g/L to 66 g/L at a VR of 99%. When a similar liquor, i.e. spruce autohydrolysate (PHWE at 170 °C) was fractionated by Song et al. (2013) through a UF scheme (PES membranes, 300-3 kDa), the average molar masses of the concentrated fractions were somewhat different than the ones in our study. This could be explained by the fact that their autohydrolysate was filtered first with a 300 kDa membrane and it was not filtered to an extremely high VR. In their study, the concentrated fraction of the 300 kDa membrane (6% of hemicelluloses in the feed) had the highest average molar mass of about 22 kDa. The 30 kDa and 10 kDa membrane concentrates contained 40% (Mw= 5.5 kDa) and 15% (Mw= 3 kDa) of GGMs present in the original feed, respectively. During the concentration of hemicelluloses with UF as the small size molecules (low molar mass ones) are permeated, the average molar mass of hemicelluloses in the concentrate will increase. Persson and Jönsson (2010) observed that when the hemicelluloses in TMP were concentrated from 0.7 to 5 g/L with 5 kDa H-PS membrane, the yields and molar mass of hemicelluloses were 45% with molar mass < 4 kDa and 96% with molar mass > 4 kDa, respectively.

In UF, GGMs were not only concentrated to high concentrations and fractionated based on the molar mass, but also purified with a two-stage fractionation scheme. The purification of GGMs in the concentrate fraction was proved by a decrease of the portions of co-extraction compounds, i.e. lignin, xylans and uronic acids in the total organic compounds (TOC), compared to the original autohydrolysates and feeds. As shown in **Figs 5.1.5 and 5.1.6,** the purity of GGMs increased in the concentrates during both filtration stages. The purity of high molar mass GGMs increased from 44 to 56% and from 50 to 61% when 30 kDa membranes were utilized to concentrate the **SWaH$_4$** and the **SWaH$_5$** solutions, respectively. With the 10 kDa membrane the purity values were almost doubled, being even 80% compared to the feeds. However, this increase in the purity of GGMs

(100%) was higher than in the case of direct filtration with the 10 kDa membrane (the purity increased by 66%).

In studies related to UF of TMP process water, the reported values of GGM purity (TDS basis) have varied. Hartman et al. (2006 b) claim 90% GGM purity with a 1 kDa RC membrane where lignin was the most abundant impurity. The purity of hemicelluloses increased from 27 to 79% (TDS basis) in a study by Persson et al. (2007) with a 10 kDa PVDF membrane. Willför et al. (2003) achieved 95 mol.% GGM purity by applying UF (20 kDa PES membrane) in their laboratory-scale recovery method of GGMs. A GGM fraction with the purity of 70–80 mol. % was produced by Willför et al. (2008) using a series of UF steps followed by spray drying or precipitation. It seems that the origins of the feed solution and the method of estimating the purity using either molar, TDS or TOC basis could explain the variation in the purity values in different studies to some extent.

Most of the monosaccharides (5-10% of TOC in feed streams) permeated through the 30 and 10 kDa membranes. Therefore, their contents in the concentrated fractions were only 60-90 mg/L. In addition, the ratio of wood extractives content to the content of GGMs decreased to less than 8% in the concentrates. This indicates that the GGMs were partly purified from the extractives. The proportion of lignin in the concentrated GGM fractions was about 10% or less (on TOC basis). A similar lignin proportion has been reported for wood autohydrolysates that have been utilized for the preparation of packaging barrier films (Edlund et al. 2010; Ryberg et al. 2011). Overlapping in the molar masses of hemicellulose and lignin and the presence of lignin-hemicellulose complexes makes their complete separation directly by UF unachievable. However, as shown in the study of Ryberg et al. (2011), higher purity of GGMs in the concentrated fractions is not needed for manufacturing barrier films. These barriers have much lower oxygen permeability than the ones produced from a highly purified *O*-acetyl galactoglucomannan (AcGGM) mixture.

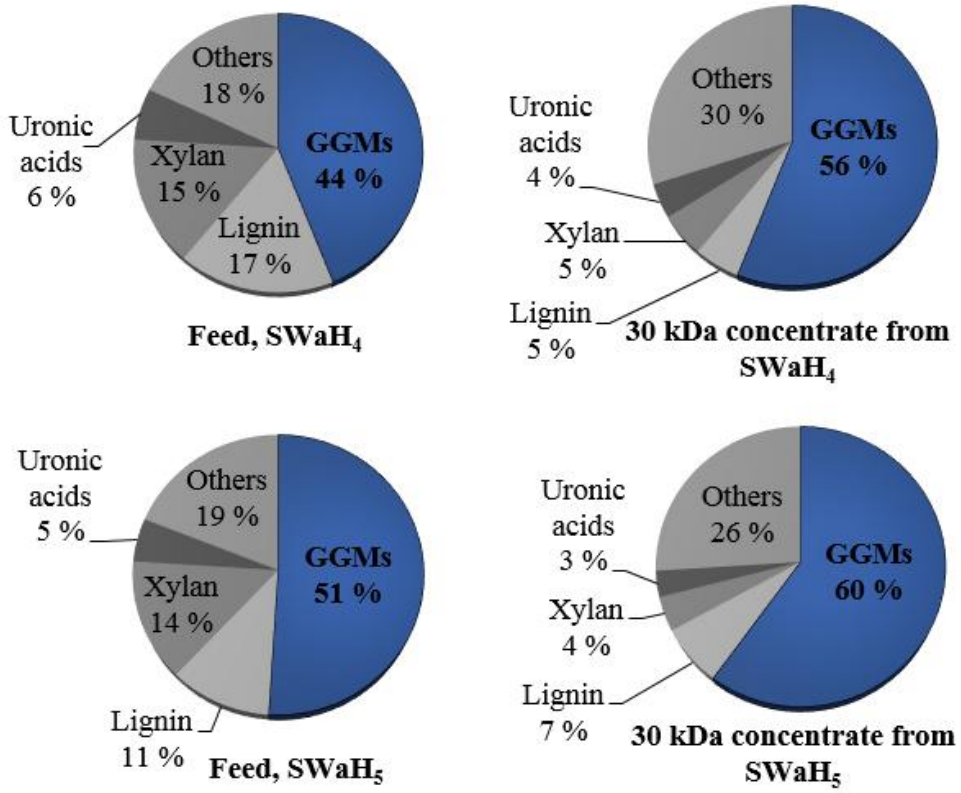


Figure 5.1.5 Compound composition of the total organic carbon (TOC) in 30 kDa membrane feeds **($SWaH_4$, $SWaH_5$)** and final concentrate streams. The main compounds are galactoglucomannans (GGMs), lignin, xylans and uronic acids. The others mainly include extractives and other wood organics.

* The calculations in **Figures 5.1.5 and 5.1.6** are based on the total organic carbon values (the main component contents were calculated on TOC basis by assuming the percentage of carbon in sugars ~40%, in softwood lignin ~64% and in uronic acids ~ 39%). The mass balance of each compound closed within less than 10%.

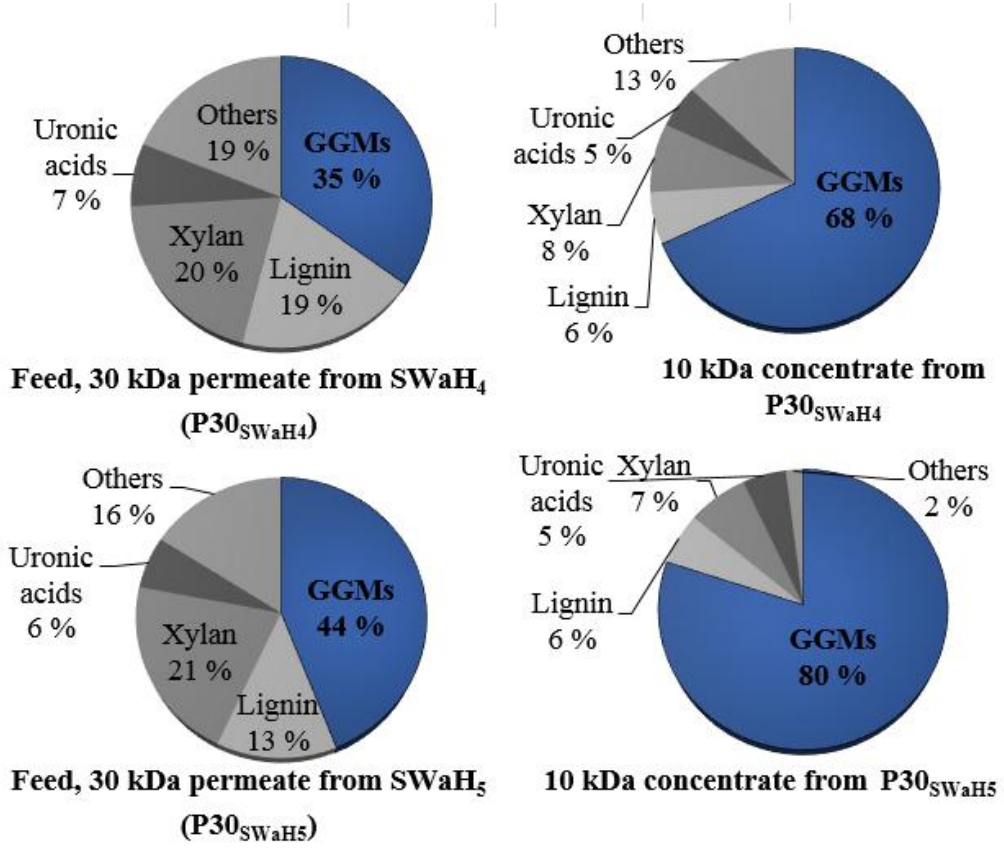


Figure 5.1.6 Compound composition of TOC in 10 kDa membrane feeds (permeates of 30 kDa) and final concentrate streams. The main compounds are galactoglucomannans (GGMs), lignin, xylans and uronic acids. The others mainly include extractives and other wood organics.

In this study, the produced fractions had extremely high concentration of GGMs (up to 400 g/L) with an average molar mass in the range of 5- 18 kDa. Therefore, further purification of these fractions by precipitation is possible as shown by Song et al. (2013) to produce high quality GGMs suitable for e.g. food applications. They found that the concentration of GGMs with UF could be advantageous to obtain the highest yield of precipitated materials (hemicelluloses with 4 kDa molar mass, 78% of total hemicelluloses in the extract and ~2 % lignin) with low ethanol consumption.

The most concentrated and purified fraction produced was the 10 kDa membrane concentrate, which contained 400 g/L GGMs (Mw ~ 5 kDa, PDI 1.2) with the purity of 80% and a low lignin content (6%), and therefore it can exhibit remarkable potential as a candidate for making renewable barrier materials for sustainable food packaging. For such an application, Edlund et al. (2010) utilized a wood autohydrolysate containing 88.8% of oligo- and polysaccharides, mainly GGMs, 2.1% of monosaccharides, 9.1% of lignin, and < 0.1 wt % of ashes (average molar mass 3.1 kDa). In their study, transparent, shiny and freestanding films with promising mechanical properties and very low oxygen permeabilities were produced.

In the literature, variations have been reported in the GGM content, average molar mass and purity of wood autohydrolysates utilized for the formation of packaging barrier films and hydrogel. Persson (2009) claims that a concentrated hemicellulose fraction having a molar mass higher than 5 kDa could be appropriate for making oxygen barriers for food packaging applications. A concentrated GGM fraction (15–20 wt % GGM with 10 kDa molar mass, PDI of 1.3) was tested by Hartman et al. (2006, a, b) for the formation of a barrier film. In their studies, food packaging films with high moisture resistance and low oxygen barrier properties were developed. For hydrogel production, fractions containing ~60 g/L hemicelluloses with around 80% purity and weight-average molar mass ~ 6.6 kDa were tested by Albertsson et al. (2010). The hydrogels they developed had good swelling properties and mechanical strength, and they were thus suitable for biomedical and agricultural applications. Based on the studies mentioned above, also the other concentrated fractions (100-200 g/L GGMs, purity 60-70% and Mw> 5 kDa) produced in this study had promising characteristics as feedstock for various bio-based products, e.g. packaging films and hydrogels.

In addition to the high potential of the concentrated fractions produced in this study, the dilute permeate streams of the 10 kDa membrane (2-4 g/L GGMs) could be concentrated further and utilized as raw material for biofuel production or as pulp and paper additives to increase their strength (Ragauskas et al. 2006; Hu et al. 2008).

5.1.4 Methods to enhance the efficiency of fractionation

In this study, diafiltration (DF) and pulsed corona discharge (PCD) oxidation were utilized to improve the performance of the GGM recovery. DF was applied after UF of the autohydrolysates and it was evaluated in terms of the permeability of the autohydrolysates and the removal of different components from the UF concentrate streams. The effect of autohydrolysate oxidation on their permeability through the 30 and 10 kDa membranes and on their compositions and purity after UF was investigated.

5.4.1.1 Diafiltration

Diafiltration was mainly employed to remove impurities like lignin and small components from high molar mass GGMs. Its effect on the contents of the 30 kDa membrane concentrates from **$SWaH_2$**, **$SWaH_4$ and $SWaH_5$** solutions was investigated. Generally, the permeabilities behaved similarly during the DF of these concentrates. **Fig. 5.1.7** presents the permeability during the DF of the 30 kDa concentrate from **$SWaH_5$**. The dia-volume in the first DF step was two times its volume in the second and third steps, so that the permeability values during the first DF (more diluted) were higher than during the further DFs (**Fig. 5.1.7**). The viscosity of the diafiltered fraction increased by 34% from 5.6 to 7.5 cP at 65 °C after the three DF stages. This could be explained by the fact that the average molar mass of the concentrated GGMs increased by the removal of low molar mass compounds in DF. This purification enhanced the steric interactions between the high molar mass macromolecules, resulting in high viscosity (Kök et al. 1999). As DF proceeded, an increase in the concentration of high molar mass GGMs might have led to formation of a gel-like structure on the membrane surface. Such gelation behaviour is strongly associated with the increase of viscosity (Richardson and Norton, 1998). These phenomena probably caused lower permeabilities in the second and third diafiltration stages, although the amount of dissolved solids was even 50% lower after the third diafltration stage.

Compared with the former UF step, the initial permeability of the 30 kDa concentrate from **$SWaH_5$** during DF was much lower, about seven times, than during the UF of **$SWaH_5$.** The higher content of GGMs in the DF feed (**$SWaH_5$** concentrate, ~ 60 g/L) than in the original **$SWaH_5$** (11 g/L) could be the reason for the lower permeability during DF. The viscosity of the DF feed was also 10 times higher than the water viscosity at 65 °C, which decreased the initial permeability

significantly. Moreover, the fouling of the membrane during DF (53%) was higher than during UF (~30%). However, pure water permeability was recovered by ~ 90% with alkaline cleaning.

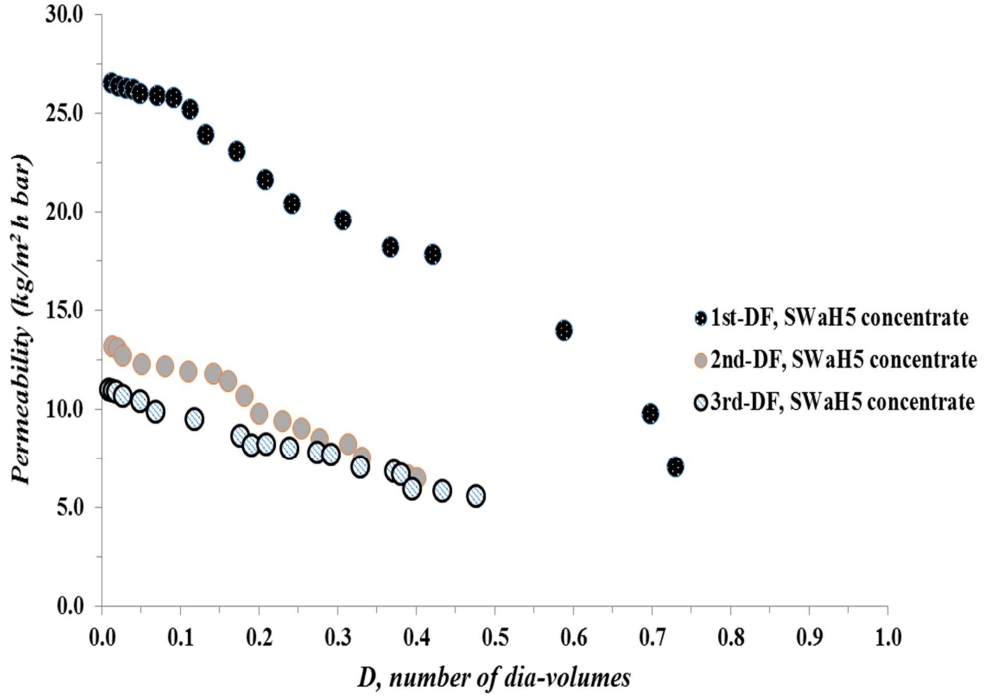


Figure 5.1.7 Permeability of the GGM concentrate fraction (30 kDa membrane) in three diafiltration steps of **$SWaH_5$** (Pressure 1-3 bar, temperature 65 °C, flat sheet membrane module, cross-flow velocity = 2 m/s).

In general, a narrower molar mass distribution (lower polydispersity) could be observed in the concentrated fractions after DF. The increase in the molar mass with DF was due to the removal of low molar mass GGMs. This removal caused a decrease in the TDS including the content of the total GGMs. For example when the resin-treated 30 kDa concentrate (from **$SWaH_4$**) was subjected to DF(2 dia-volumes), the average molar mass increased from 17 to 22 kDa, and the polydispersity decreased from 1.5 to 1.3. During this DF, the reduction in TDS was about 40%. Moreover, the average molar mass of the DF permeate (~8 kDa) was higher than that of the UF permeate (~3.5 kDa). This difference in the average molar mass of the permeates could confirm the higher possibility to form a gel layer by big macromolecules, resulting in lower permeability during DF than UF.

The effect of DF on the content of the 30 kDa concentrated fraction from **SWaH$_2$** is presented in **Table 5.1.5** and **Paper I.** The results showed DF-facilitated separation of xylans from high molar mass GGMs, where their content in the concentrate fraction declined by 80 %. Moreover, about 30 % of lignin, 55 % of uronic acids, 95 % of monosaccharides, and 11 % of turbidity were removed from this fraction during DF. It could be assumed that the uronic acids were removed with DF, including the free uronic acids as well as the ones are originally attached to either the hemicellulose and/or pectin chains. In general, the direct UV absorbance in the concentrate fractions decreased by 20-40 %. The overlapping of the GGMs and lignin molar mass distributions, and the fact that most of the free low molar mass lignin had already been removed in the concentration filtration step, could be the reasons for the limited removal of lignin by DF. Based on previous observations, DF enabled partial separation of various impurities from GGMs. It thus improved the purity of the hemicelluloses having the highest molar mass, even though the overall hemicellulose purity decreased by ~2%. Andersson et al. (2007) report that DF increased hemicellulose purity from 57 to 77 % with 1 kDa PVDF membranes. Higher purity values of GGMs (> 90%) have been reported when using ethanol precipitation and size-exclusion chromatography (Willför et al. 2003 a, b; Andersson et al. 2007), but difficulties related to high ethanol consumption or high expenses make DF more suitable and cost-efficient for the purification of hemicelluloses in a large scale. Several studies have shown that UF combined with DF allows concentration and purification of hemicelluloses, and as a result, concentrates containing large molecules with narrow molar mass distribution suitable for the production of packaging films and hydrogels are produced (Hartman et al. 2006 a, b; Persson, 2009; Edlund et al. 2010; Albertsson et al. 2010). As a conclusion in this study, the removal of low molar mass components from high molar mass GGMs was achieved with DF, but DF could only partially purify GGMs from lignin fragments. As shown in **Paper I**, the loss of GGM yield was higher with the membranes having higher cut-offs. High GGM concentration (also viscosity) in the DF feed, high fouling and increase in the viscosity (associated with the gel layer) during DF explain the lower permeability during DF than UF.

Table 5.1.5 Variation in the content of 30 kDa concentrated fractions (produced from **$SWaH_2$**) before and after DF (2 dia-volumes).

Analysed compounds	Content before DF	Content after DF
GGMs, mg/L	9000	4900
Xylans, mg/L	1600	360
Lignin*, mg/L	2200	1500
Uronic acids, mg/L	430	200
Monosaccharides, mg/L	600	30
Turbidity, NTU	5200	4650
Direct UV absorbance	70	40

*: MTBE-treated sample

5.4.1.2 Oxidative pre-and inter-treatment

Pulsed corona discharge (PCD) oxidation was combined with UF in a hybrid separation process to enhance the recovery efficiency of GGMs (**Paper II**). **Fig. 5.1.8** illustrates the effect of PCD oxidation treatment on the permeability of the 30 kDa RC membrane. The effect of oxidation pretreatment was rather significant, especially at high VR, where the permeability of the **$SWaH_3$** was almost doubled. However, the effect of PCD oxidation was negligible on the permeability of the 10 kDa membrane when the permeate of the 30 kDa membrane was filtered. Therefore, it can be concluded that the 30 kDa membrane removed substances that caused a decrease in the permeability of the 10 kDa membrane sufficiently. Koivula et al. (2011) achieved six-fold improvement in autohydrolysate permeability when a 5 kDa hydrophobic membrane was tested to concentrate hemicelluloses from PCD-treated spruce autohydrolysate, compared to the filtration of an untreated spruce autohydrolysate. In their study, the PCD treatment seemed to reduce fouling by the degradation of foulants.

The fouling of the tested RC membranes was low (~11%), and pure water permeability was efficiently recovered by alkaline cleaning. Therefore, other phenomena like concentration polarization, increase of the osmotic pressure and viscosity during concentration filtration, or formation of a gel layer on the membrane surface could explain the reduction in the permeability. Viscosity measurements showed a decrease of 20 % for oxidized spruce autohydrolysates (1.36 cP). This decrease led to about 30% higher in the average permeability of autohydrolysate than that of the untreated autohydrolysate. Therefore, the change in viscosity explained a significant part of the enhancement of the permeability of the **$SWaH_3$** autohydrolysate (~30%) after pre-oxidation of the autohydrolysate.

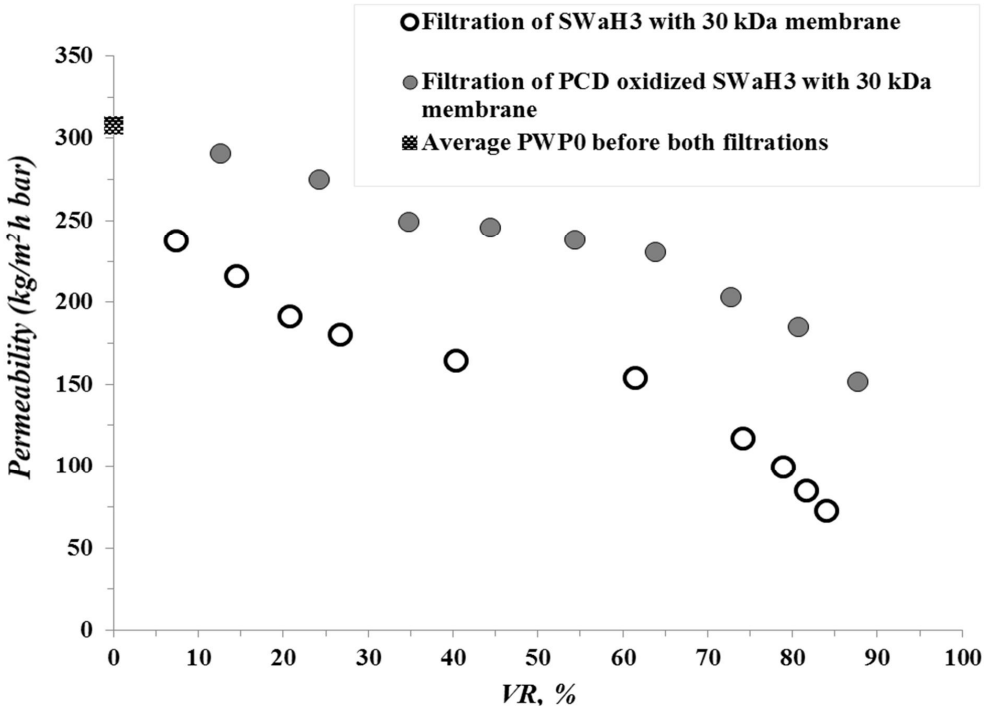


Figure 5.1.8 Permeability of a 30 kDa RC membrane during the filtration of the original and PCD oxidized **SWaH$_3$** liquor. (PWP_0: initial pure water permeability) (Pressure 1-2 bar, temperature 65 °C, CR200-filter, rotor speed 9 m/s).

The effect of PCD treatment on the composition of oxidized **SWaH$_3$** was analysed. Since the largest changes in the compositions of autohydrolysates were observed at 45 min of oxidation with little progress at a longer time, detailed analysis of the autohydrolysate collected at this treatment time was performed. **Table 5.1.4** presents the characteristics of the original spruce autohydrolysate (**SWaH$_3$**), as well as the autohydrolysate after 45 minutes of PCD treatment. During the oxidation process of this liquor, the decrease in pH from 4.1 to 3.2 indicated the formation of organic acids (e.g. acetic, formic, glycolic, oxalic, and glucoisosaccharinic (GISA) acids). The total amount of these organic acids was ~10% of the total organic carbon. The ionic content measured by conductivity increased with oxidation. Compared with the original **SWaH$_3$**, the hemicellulose content in the oxidized liquor was lower by 7%. The changes in the pentoses and hexoses and their monomer compositions were only slight. Thus, it can be assumed that the minor loss in carbohydrates was mostly due their oxidation to sugar-based acids like formic, glycolic and oxalic acids.

The effect of oxidation on the main impurity compound, i.e. lignin, were measured by UV absorbance at 280 nm. The presence of high portions of polymeric lignin and lignin-hemicellulose complexes in the concentrated fractions of GGM could probably limit their utilization in barrier film formation. Direct UV absorbance at 280 nm of **$SWaH_3$** decreased from 2200 m^{-1} to 1500 m^{-1} after 45 minutes of oxidation (**Table 5.1.6)**. Although the direct UV absorbance was decreased by 30% with oxidation, the UV absorbance was almost the same (**Table 5.1.6)** when it was measured by treating the samples first with a methyl *tert*-butyl ether (MTBE) extraction to remove lignans and other wood extractives selectively (Örså et al. 1997). This revealed that the oxidation intensity (360 kJ/m^3 min) was not sufficient to degrade a significant amount of polymeric lignin in the autohydrolysate.

The SEC/MALLS analysis with RI, UV and MALLS detectors showed that changes occurred in the lignin structure during the PCD treatment. The effects were obvious in the signal of the UV detector as it was spread over time (i.e. lignin concentration at different retention times). The change in the retention time range of the UV-absorbing compounds due to PCD oxidation indicated formation of new UV-absorbing compounds, the molar masses of which remained somewhat the same as in the untreated autohydrolysates. Quantitatively, lower lignin amounts were found in 30 kDa and 10 kDa concentrates compared to the concentrate of the original spruce autohydrolysate at the same VRF. For instance, at the VRF of 17, the lignin concentration in the concentrate of the 30 kDa membrane of the original and oxidized autohydrolysates were 2.4 and 1.9 g/L, respectively. The proportion of lignin of the total organic carbon decreased slightly from 15% in the oxidized autohydrolysate to 11% in the final concentrate. When the 30 kDa permeates were concentrated with the 10 kDa membrane, the proportion of lignin of the total organic carbon in the final concentrate was only 4%.

Turbidity as a measure of lipophilic wood extractives indicated efficient removal of these extractives during the PCD treatment. Detailed analysis of lipophilic extractives (including fatty and resin acids, sterols, steryl esters, and triglycerides) and lignans showed that their content decreased from 26 and 42 mg/L to 6 and 2 mg/L, respectively. As these compounds have been claimed to be responsible for the fouling of the membrane tested in the filtration of wood-based liquors (Puro et al. 2011), their degradation by oxidation could be useful for the enhancement of the permeate flux and the prevention of fouling in PES and PVDF hydrophobic membranes.

Degraded extractives by oxidation could be repolymerised (Widsten et al. 2004; Widsten and Kandelbauer, 2008). The presence of such repolymerised products in the extract was not investigated in this study.

Only minor changes in the concentration of total organic carbon were measured after oxidation (**Table 5.1.6**). Therefore, it can be concluded that significant mineralization of organic compounds did not occur, although changes in their structures were clear.

Table 5.1.6 Analysis characteristics of the original spruce autohydrolysate (**$SWaH_3$**) and the autohydrolysate after 45 minutes of PCD treatment.

Analysed compounds	**Original wood autohydrolysate**	**45 min PCD treatment**
Total acids, mg/L	**430**	**580**
▪ **Acetic acids**	270	280
▪ **Formic acids**	50	120
Hemicelluloses, mg/L	**3600**	**3340**
▪ **Pentoses**	725	690
▪ **Hexoses**	2700	2500
Monomeric carbohydrates, mg/L	**316**	**314**
▪ **Pentoses**	280	250
▪ **Hexoses**	36	60
UV absorbance, m^{-1}	2200	1500
UV absorbance (MTBE-treated), m^{-1}	1000	990
pH	4.1	3.2
Total carbon, mg/L	2500	2400
Conductivity, µS/cm	270	450
Turbidity, NTU	500	350

As stated above, the PCD oxidation removed the majority of wood lipophilic extractives and lignans from the wood autohydrolysates, but the degradation of lignin was only minor. The purification of hemicellulose from these extractives could be useful to avoid the formation of sticky deposits and spots on hemicellulosic-based films and hydrogels. The organic acids that were formed during PCD oxidation were permeated by the tested membranes. However, if further purification of hemicelluloses from low molar mass extractives and acids is needed, DF could be applied. The modification of the lignin structure with oxidation led to a slightly lower lignin content in the produced UF concentrates. The results showed that the filterability of the original wood autohydrolysates was significantly improved with the PCD oxidation when hydrophilic membranes were used. This was mainly due to reduced viscosity.

5.2 Recovery of bioactive compounds from carob residues

In this study, aqueous extraction processes were applied to recover phenolic compounds and sugars from carob kibbles. The yields of phenolic compounds and sugars were used to evaluate these processes. After extraction, membrane filtration processes, particularly dia-nanofiltration (Di-NF), nanofiltration (NF) and reverse osmosis (RO) were utilized to recover, fractionate and concentrate the phenolic compounds and sugars. The performance of the filtration processes was assessed based on the filterability of the extracts and their quality after the filtrations. The results of this work are summarized in this section, and details can be found in **Papers III-IV**.

5.2.1 Extraction of phenolic compounds and sugars from carob kibbles

The study of the extraction processes focused on the optimisation of the operating conditions, particularly temperature, time and the liquid-to-solid ratio (LSR). Evaluation of the performance of the extraction in one and two steps was based on the yield of phenolic compounds and sugars, and the fractionation of these compounds into different extracts.

In order to estimate the yield of the extraction processes, the amounts of total phenolic compounds (TP) and sugars in the carob kibbles were analysed. Soxhlet extraction is a commonly used method for analysis purposes, and it was applied in this study to estimate the amounts of total phenolic compounds and sugars in the carob kibbles. The assumption was that in Soxhlet extraction, practically most of the extractable phenolic compounds and sugars would be dissolved into water. The results showed that the concentration of total sugars and total phenolic compounds in the Soxhlet aqueous extract were 13.8 and 0.7 g/L, respectively, corresponding to 54.6 g sugars and 2.8 g_{GAE} / 100 g dry kibble. These values were used to estimate the efficiency of the extraction by calculating the percentage of the extracted components for each trial.

The analysis of the total dissolved solid content in the Soxhlet extract revealed that it contained mostly sugars and phenolic compounds (~97%). The measured values for the total phenolic compounds and sugars were rather identical with values presented in the literature. For instance, Owen et al. (2003) state that the total phenolic compounds content was 2.84 g_{GAE} / 100 g dry matter when they analysed the chemical composition of carob fibres. The reported values of sugar content in carob pods are in the range of 48-56% (Roseiro et al. 1991 b; Petit and Pinilla, 1995; Avallone et al. 1997; Batlle and Tous, 1997).

5.2.1.1 One-step aqueous extraction

The carob kibbles were first subjected to one-step aqueous extraction. The efficiency of this extraction process was evaluated on the basis of the yield of phenolic compounds. To obtain the highest yield, the effect of the key operating parameters, i.e. the liquid-to-solid ratio (LSR), temperature and time was investigated in **Paper III** to optimize the extraction conditions.

The LSR had an influence on the yield of phenolic compounds. About 11% of the phenolic compounds in the carob kibbles were extracted at the LSR of 4. At the extremely high LSR of 50, only about 20% of the phenolic compounds were extracted. These results showed that the LSR may not have been the key factor for the high yield of phenolic compounds. Experiments with different LSR values (2-50) revealed that the LSR of 4 seemed to be adequate, as the yield of phenolic compounds increased linearly with the LSR up to the value of 4 **(Paper III)**. Casazza et al. (2011) report that the yield of total phenolic compounds extracted from grape residues did not improve greatly at LSR > 5. However, the reported LSR values for the extraction of phenolic compounds from various biomass sources have been significantly diverse (Ignat et al. 2011).

In this study, the recovery of phenolic compounds was improved with the extraction temperature. Compared with other operating conditions, the effect of temperature on the extraction efficiency of phenolic compounds seemed to be greater. The highest yield of phenolic compounds ~0.55 g $_{GAE}$/ 100 g dry carob mass (~20% of phenolic compounds in the carob kibbles) was obtained at the highest operating temperature (100° C, LSR =4). At high extraction temperatures, the plant tissue was “softened” more than at lower temperatures, and therefore the interaction between the phenolic compounds and protein or mono/polysaccharides was diminished, leading to improvement in their solubility and diffusivity (Roseiro et al. 2013). A similar effect of the extraction temperature on the yield of phenolic compounds has been reported in studies on grape seeds (Bucic´-Kojic´et al. 2007; 2009) and carob residues (Turhan et al. 2006).

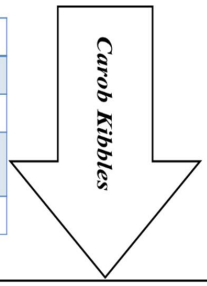
In general, the yield of phenolic compounds increased as the extraction time increased. In the present study, (0.47 g $_{GAE}$/ 100 g dry carob mass) of phenolic compounds was already extracted at 20 min and 82.5 ºC. At a longer extraction time (280 min, 82.5 ºC), the increase in the yield was only about 10%.

Identification and quantification of the effect of the main operating parameters of water extraction (temperature and time) was determined using the response surface model approach (**Paper III**). The results revealed that 100 °C and 220 minutes (at LSR = 4) were the optimal operational conditions to extract phenolic compounds from carob kibbles. The optimum extraction temperature was very close to the value reported by Roseiro et al. (2013) to achieve the highest yield of phenolic compounds. **Fig. 5.2.1** presents the content of the total phenolic compounds and sugars in the carob kibble (obtained by Soxhlet extraction) and in the one-step extract that was produced at optimum conditions. At these conditions, ~ 20% of the phenolic compounds and 90% of sugars in the carob kibble (54% of them was sucrose) were extracted.

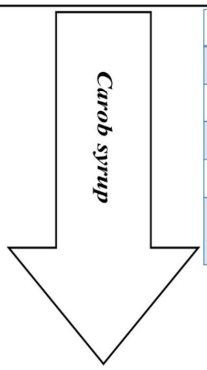
Roseiro et al. (2013) obtained ~ 4 g $_{GAE}$/ 100 g dry kibbles mass by aqueous decoction of carob pods performed at operating conditions of 98.5 °C, 17 min and LSR ~ 33.3. They did not report any degradation of phenolic compounds at these optimal conditions (high temperature). Moreover, the antioxidant activity of their extract was high (inhibition of DPPH 85%, ABTS radicals 90%), which would give it appropriate characteristics for antioxidant dietary products. However, the variation in the reported yield values of the phenolic compounds is probably a result of the differences in the extraction procedures and the methods of analysis used, although the quality and growth conditions of the carob plants also play a role.

Compounds	g/100 g dry mass
Total dissolved solids*	59.6
Total sugars*	54.6
Total phenols* (As GAE equivalent)	2.8
Organic acids*	1

* Obtained from Soxhlet extraction



Aqueous extraction at optimal conditions LSR=4, 100°C ,220 min



Compounds	g/100 g dry mass
Total sugars	49.1 (90%)
▪ Glucose	14.2
▪ Fructose	8.5
▪ Sucrose	26.4
Total phenols (As GAE equivalent)	0.6 (21%)

Figure 5.2.1 One-step aqueous extraction of carob kibbles: Total content of sugars and phenolic compounds in the kibbles (estimated by Soxhlet extraction) and their contents in the produced extract.

5.2.1.2 Two-step aqueous extraction

The extraction in two steps aimed at fractionating the sugars and phenolic compounds into different extracts. As observed in **Paper III,** a significant amount of sugars (76%) could be already isolated at 30 °C in the one-step aqueous extraction. At this temperature, the amount of extractable phenolic compounds was about 10-14% (corresponding mainly to gallic acid) at different values of time and LSR. Testing different extraction times (210 and 300 min) and LSRs (10 and 20) aimed at extracting the maximum amount of sugars with the lowest amounts of phenolic compounds. At 210 min, 70% of the sugars in the carob kibbles were extracted, and the yield of sugars was increased by ~11 % when a longer extraction time (300 min) was used. By doubling the LSR, the yield of sugars was not increased statistically. Based on this, rather high separation of sugars from

kibbles was obtained at LSR of 10 and time of 300 min. In these conditions, 80% of sugars (~ 55 % sucrose) with only 14% of phenolic compounds (mainly gallic acid) were dissolved. Manso et al. (2010) also report that a higher LSR in the water extraction was not beneficial to increase the yield of sugars. In their study, ~ 94% of the sugar content in carob residues was released by the LSR value of 10 at 25 °C for 60 min. Their utilization of milled carob particles and agitation could be the reason for the higher yield of sugars compared to the present study.

Fig. 5.2.2 presents the total content of phenolic compounds and sugars in carob kibbles (obtained by Soxhlet extraction) and their content in the first and second step extracts using two different solid loads. As shown**,** when the second aqueous extraction step was performed at optimal conditions for the extraction of phenolic compounds (LSR=4, 100°C and 220 min), only 6.5 g sugars/ 100 g dry mass were dissolved from the solid residues of the first step. A significantly higher amount of phenolic compounds (70% corresponding to 1.9 g $_{GAE}$/100 g dry mass) than in the first step (14%) was dissolved in the second extraction step. This yield value is comparable with the results of Avallone et al. (1997) who performed three-step extraction using 70% acetone and 70% methanol to obtain as high yields as 1.95 and 1.25 g $_{GAE}$/ 100 g, respectively. Corsi et al. (2002) report remarkably lower phenolic compound yield, ~ 0.14 g TP /100 g dry matter, with two times infusion in boiling water for 15 minutes. The utilization of Soxhlet extraction (5 hours) with methanol operated by Owen et al. (2003) also produced a lower amount of dissolved phenolic compounds (0.4 g/100 g) from the carob fibres.

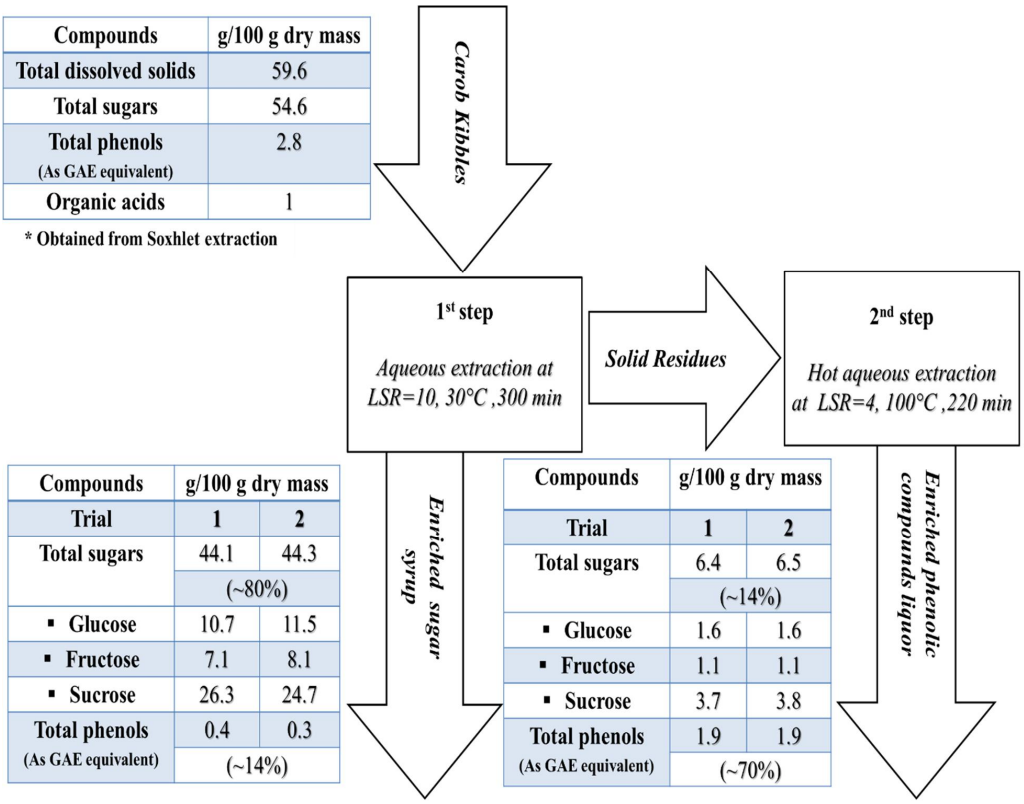


Figure 5.2.2 Two-step aqueous extraction of carob kibbles: specifications of the carob extract produced at different solid loads (trial 1 with 0.3 kg and trial 2 with 1.2 kg of carob kibbles).

To verify the lab-scale results, a pilot-scale trial of two-step aqueous extraction using 4 kg of carob kibbles was performed. As shown in **Table 5.2.1**, the yield of the phenolic compounds in the first extraction step was 0.5 g $_{GAE}$/100 g dry mass. Compared to the lab-scale trial, a lower yield of phenolic compounds (0.9 g $_{GAE}$/100 g dry mass, corresponding to 32%) was obtained in the second extraction step. This behaviour was probably due to a lack of mixing in the autoclave, which might have led to a decrease in the mass transfer of phenolic compounds to the liquid phase with time. Most of the sugars (88%) were removed in the first step also in the pilot-scale experiments. The remaining sugars in the solid residues were dissolved in the second step extract. Therefore, high sugar concentration at the interface (saturation concentration limit) might also decrease the extraction efficiency of phenolic compounds. As a result, almost total removal of sugars from carob kibble was achieved with the two-step aqueous extraction in this pilot-scale trial.

Table 5.2.1 Pilot-scale trial: yield values of phenolic compounds and sugars in the first and second aqueous extraction steps.

Compounds	1st step Yield (g/ 100 g dry mass carob)	2nd step Yield (g/ 100 g dry mass carob)
Total sugars	47.6 (87%)	6.5 (12%)
▪ **Glucose**	12.8	2.2
▪ **Fructose**	11.1	1.8
▪ **Sucrose**	24	2.5
Total phenolic compounds	0.5 (18%)	0.9 (32%)

5.2.1.3 Comparison between one- and two-step aqueous extraction

Fig. 5.2.3 presents the amounts of phenolic compounds and sugars isolated from carob kibbles through one and two-step aqueous extraction, as a percentage of their total contents in kibbles, estimated using Soxhlet extraction.

Due to the high sugar concentration (~ 125g/L), the one-step aqueous extract was like viscous sugar syrup (viscosity 2.2 cP) with a low content of phenolic compounds (1.5 g/L). It seems that this high content of sugar limited the isolation of more phenolic compounds. As the results showed, the two-step approach was successful to fractionate the sugars and phenolic compounds. The aqueous extract of the first step was enriched in sugars (~ 110 g/L). This corresponds to eight times its content in the extract of the second step. Meanwhile, in the second step, the extract content of phenolic compounds was 5 and 3.5 times higher than in the first step and the one-step extracts, respectively. However, these extracts may need to be processed and purified before further utilization. For example, a sugar-enriched extract can be concentrated by reverse osmosis (Ferrarini et al. 2001; Versari et al. 2003). In addition, the separation of phenolic compounds in their enriched fraction can be achieved by various methods, such as adsorption (Soto et al. 2011; Díaz-Reinoso et al. 2010) or membrane filtration (Díaz-Reinoso et al. 2009; Prudêncio et al. 2012).

The capillary zone electrophoresis (CZE) profiles of all the extracts **(as shown in Paper III)** illustrated that the amount and type of identified components varied clearly with the extraction temperature and time (all data not shown). The CZE and HPLC analysis techniques revealed that low molar mass phenolic compounds, in particular gallic acids (170 g/mol) were present in all the extracts (extraction temperature 30-100 ºC). At a high extraction temperature (above 80 ºC), high molar mass phenolic compounds, i.e. catechin derivatives, namely epi-catechin gallate (442.37 g/mol), (−)-epigallo catechin gallate (EGCG, 458.3 g/mol), (−)-gallocatechin gallate (GCG, 458.3 g/mol), and (−)-epicatechin (EC, 290.3 g/mol) were also identified. These compounds are found in e.g. green tea (catechins) and are recognized as some of the most effective antioxidants (Ananingsih et al. 2011). High antioxidant activities of catechin and its derivatives have been reported by Corsi et al. (2002) in the boiling water infusion liquor of carob pods, and more recently, in the aqueous decoction of carob kibble (Roseiro et al. 2013). Based on the literature, the antioxidant activity of phenolic compounds in carob residues is mostly preserved with water extraction (Kumazawa et al. 2002; Papagiannopoulos et al. 2004).

In the two-step extraction process, most of the low-value phenolic compounds, mainly gallic acids, were extracted with sugars in the first extraction stage at the temperature of 30 ℃. Electropherograms of the different extracts showed that the area representing the content of the identified compounds, mainly catechin derivatives, in the electropherograms of the second stage extract in the two-step extraction approach was larger than that in the one-step extract at the same temperature (100 ℃). It seems that the removal of sugars in the first extraction step facilitated the isolation of the high molar mass phenolic compounds from the carob solids. When most of the sugars were first extracted, the recovery of the phenolic compounds (mainly high molar mass ones) was enhanced by about 4 times (from 0.5 to ~ 2 g $_{GAE}$/ 100 g dry carob mass in the laboratory scale experiments). In the light of the results of this study, the two-step aqueous extraction approach could offer a green alternative for the recovery of phenolic compounds, instead of extraction with a common hazardous solvent like methanol.

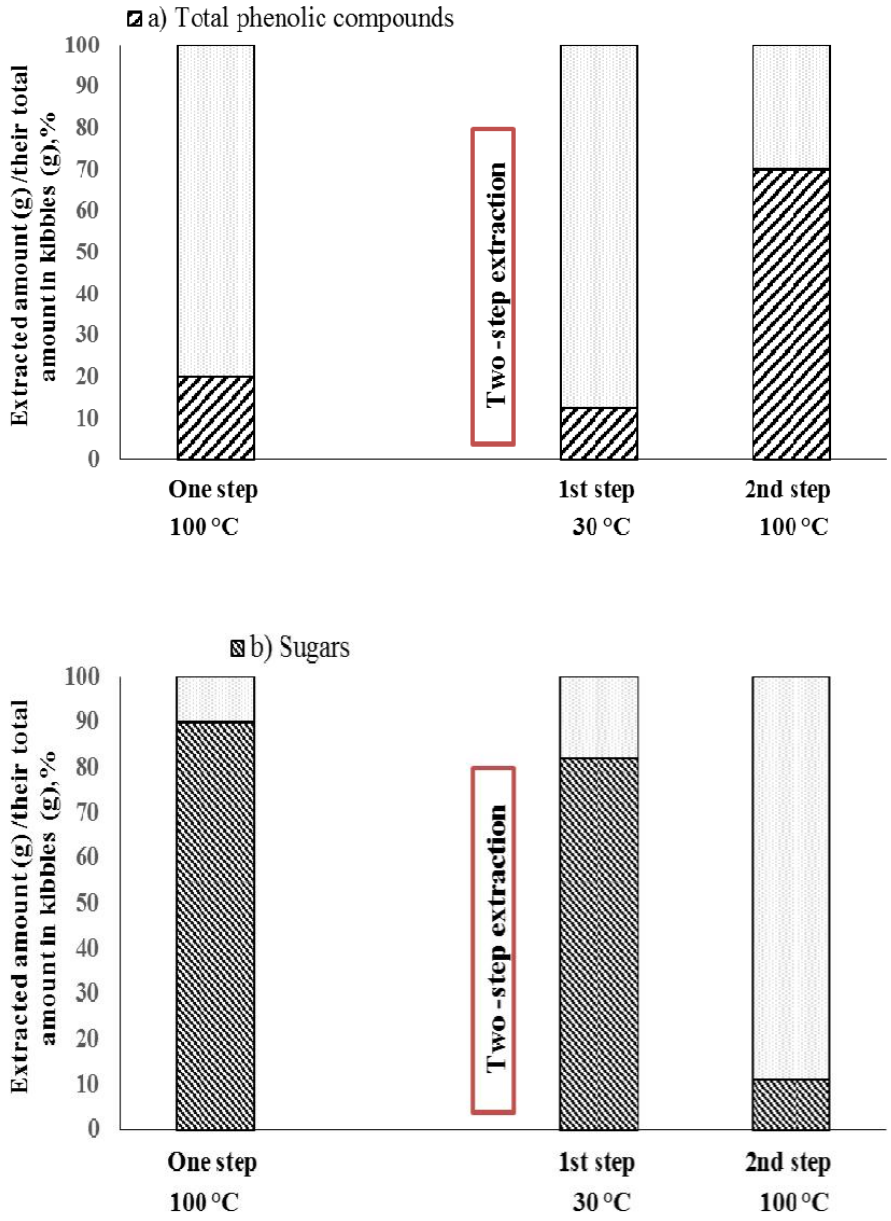


Figure 5.2.3 Fractions of a) phenolic compounds and b) sugars isolated from carob kibbles by one- and two-step aqueous extraction. Fraction is expressed as the percentage of the extractable content of each compound to their total content in the carob kibble.

5.2.2 Fractionation and concentration of phenolic compounds and sugars in carob aqueous extracts

Dia-nanofiltration (Di-NF), nanofiltration (NF) and reverse osmosis (RO) were studied for the recovery and fractionation of phenolic compounds and sugars from carob aqueous extracts. The evaluation of these processes focused on the permeability of the extracts during the filtration, fouling of the membranes, and the quality of the concentrated fractions in terms of phenolic compound and sugar concentration.

The utilization of Di-NF, NF and RO aimed at enabling the separation of sugars and gallic acid (low value phenolic compounds) from higher molecule catechin (valuable phenolic compound) and its derivatives. This fractionation is beneficial for producing a high-value phenolic compound (catechin and its derivatives) fraction free of sugars for the profitable nutraceuticals market, and other fractions rich in sugars containing some amount of low-value phenolic compounds, i.e. gallic acids, suitable for fermentation processes in the food industry.

5.2.2.1 Permeability of carob extracts during membrane fractionation

Desal 5-DK and NF270 membranes were tested in laboratory scale for the filtration of a one-step carob extract (**CAE**). Versari et al. (2003) and Giacobbo et al. (2013 b) used these membranes to concentrate phenolic compounds from biomass-derived extracts. The Desal 5-DK membrane offers almost 100% retention of phenolic compounds and higher difference between the phenolic compounds and sugar retentions (99% and (~90% in average, respectively) than NF270 membrane. The average permeability of the **CAE** (0.6 kg/m^{2}h bar) through this membrane was realistic with such a complex and concentrated mixture. Therefore, the Desal 5-DK membrane was used in further investigations to fractionate the carob extracts.

Figure 4.3 presents the scheme for the filtration experiments used to fractionate the carob aqueous extracts. First, the one-step extract (**CAE**) was processed to purify the phenolic compounds with Di-NF before concentrating with NF. Second, due to the high sugar content in the one-step extract, two-step aqueous extraction was performed to fractionate the sugars and phenolic compounds. The extract from the first step of the two-step extraction (enriched in sugars, **CAE$_1$**) was processed with RO to concentrate the sugars. The second extract of the two-step extraction process (enriched in phenolic compounds, **CAE$_2$**) was filtered with Di-NF and NF.

The viscosity of the **CAE** was 1.93 mPa.s (2×water viscosity at 25 °C), so the extract was heated to 50 ºC and diluted 3.9 times to obtain a viscosity value close to water viscosity at 20 ºC before Di-NF (one dia-volume). The observed permeability during Di-NF was between 1.4 and 0.7 kg/m^{2}h bar at 12 bar. When the diafiltered **CAE** was then concentrated with NF to VRF of 2.8, its permeability decreased from 1.1 to 0.4 kg/m^{2}h bar. Flushing the membrane with water prior to NF may explain the higher permeability in the beginning of NF compared to the end of Di-NF. It was observed that the the sticky layer on the membrane surface was removed with water.

In the present study, the permeability of the **CAE** in the beginning of Di-NF and NF was 11% and 9% of pure water permeability, respectively. This behaviour could be partly explained by the high sugar content (28 g/L after 3.9 dilution) corresponding to the osmotic pressure of 3.5 bar (i.e. 30% reduction in the operating pressure). According to several studies, low permeability has been observed during NF of biomass-derived extracts (Díaz-Reinoso et al. 2009; Conidi et al. 2011). The fouling (as reduction in the pure water permeability) of the Desal 5-DK membrane was ~26% at the end of the filtration processes. During Di-NF, the osmotic pressure decreased by about 10% due to the decrease of the total concentration of solutes in the retentate side. Thus, the flux decrease during Di-NF was mainly caused by membrane fouling. In the following concentration step with NF, other factors like an increase in the viscosity (~10%) and osmotic pressure contributed to the decline of permeability.

In a pilot-scale trial (**Paper IV**), **CAE$_2$**, the extract that enriched in phenolic compounds was subjected to Di-NF (3 dia-volumes). The observed permeability and the permeability corrected by osmotic pressure during Di-NF of the **CAE$_2$** solution are presented in **Fig. 5.2.4(a)**. As shown, the decline of permeability can be divided into three periods. The period in the beginning presents a rather rapid decrease in permeability, the middle period exhibits a slighter decrease in permeability, and in the last period, permeability reaches almost steady-state values at the end of filtration. A similar trend has been reported during NF of apple and pear juice at low operating pressures (8-12 bar) (Warczok et al. 2004), and in the recovery of polyphenols from bergamot juice by NF (Conidi et al. 2011). The observed permeability and the permeability corrected by osmotic pressure at the beginning of Di-NF was 60% and 80% of pure water permeability, respectively. Similarly, in the Di-NF of the one-step extract, the decrease in osmotic pressure was only ~6%, so that its effect on the changes of the observed permeability was almost negligible.

The permeabilities corrected by osmotic pressure exclude the effect of osmotic pressure on permeability but include mainly the membrane fouling effect. The osmotic pressure of sugar syrups is an important parameter affecting the behaviour of the permeate flux during membrane filtration, but it is not always considered. This effect has been investigated in some studies, e.g. in those by Scordino et al. (2007) and Zhao et al. (2012).

After Di-NF, diafiltered **CAE_2** was concentrated with NF to a VRF value of 6. During NF, the observed permeability through the water-flushed Desal 5-DK decreased gradually from 1.9 kg/m^2h bar to 0.30 kg/m^2h bar at 8 bar pressure, and the concentration of sugars increased from 16 to 75 g/L. Fouling of the Desal 5-DK membrane at the end of NF was 43%. As the membrane surface was coloured after filtration, phenomena like adsorption or gel layer formation by carob aqueous extract components may have occurred on the membrane surface. Interaction (adsorptive layer) between membrane materials and phenolic compounds has been suggested by Xu and Wang (2005) and Susanto et al. (2009). This adsorption of phenolic compounds on the membrane surface can have high contribution to membrane fouling, as reported by Doherty et al. (2010). In addition to fouling, the increase in the osmotic pressure and viscosity (by ~25%) due to concentration of sugars could clarify the decline in permeability.

Reverse osmosis was shown to be a promising method to concentrate the sugar-rich carob extract **CAE_1** produced by the first stage of the two-step extraction. This extract was subjected to RO using a spiral wound module equipped with a SW30 membrane. **Fig. 5.2.4 (b)** presents the decline in the observed permeability and the permeability corrected by osmotic pressure during the filtration of **CAE_1**. In the beginning of RO, the observed permeability was only 40% of pure water permeability. This could be explained by the effect of osmotic pressure (20% of the applied pressure) and viscosity, which was about 20% higher than pure water viscosity at 25 ºC. During RO, the viscosity increased by 40%. This may have enhanced the formation of gel layer on the membrane surface. As RO retained in practice all solutes, the increase in the total dissolved solid content was significant. This increase corresponded to the increase in the osmotic pressure from 6 to 20 bar during RO. The effect of osmotic pressure seemed to be higher than the fouling effect, as the fouling of the membrane was only 6%. Ferrarini et al. (2001) obtained rather similar permeability behaviour as in this study when the sugars from grape juice were concentrated with the same membrane, but they observed much higher fouling of the membrane (24%).

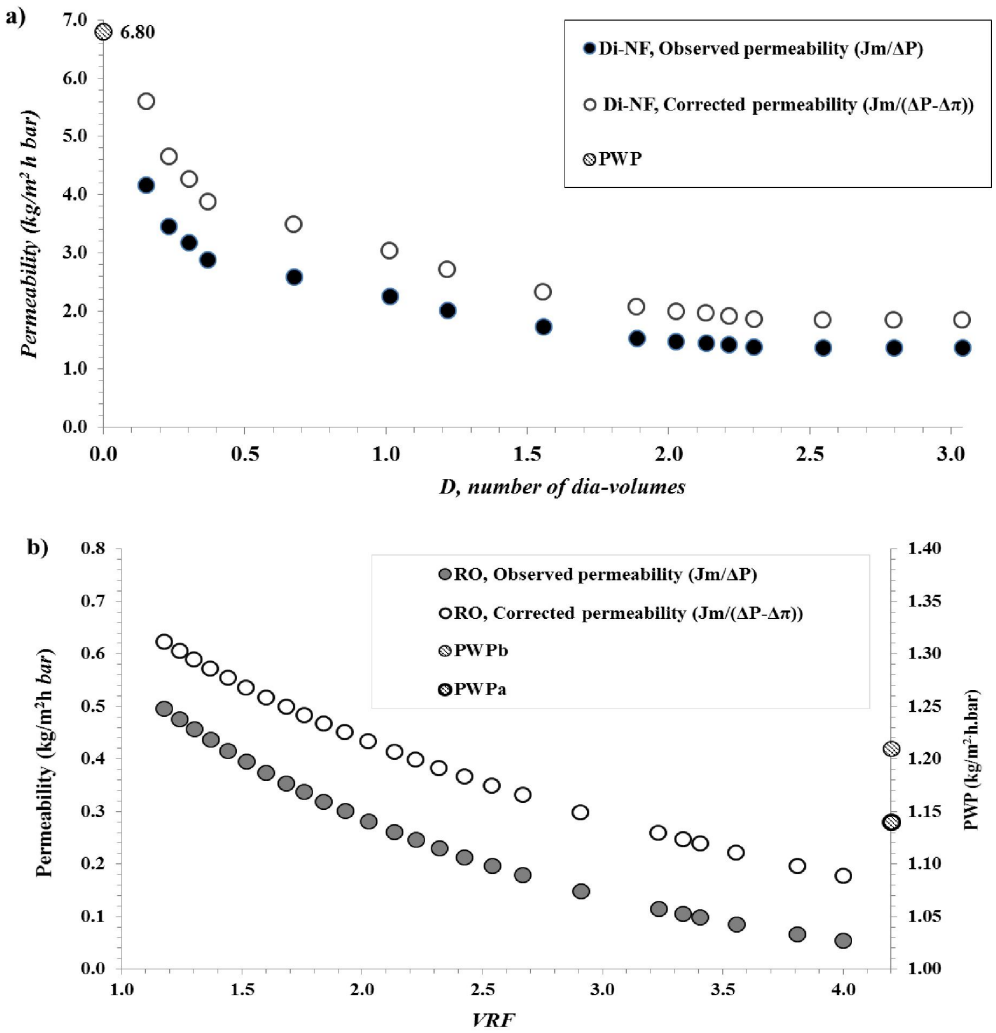


Figure 5.2.4 Observed and corrected permeability of carob extracts produced from two-step extraction during pilot scale Di-NF and RO.

a) Permeability of **CAE$_2$** during Di-NF versus the number of dia-volumes, D (Desal 5-DK membrane, 50 °C and 8 bar). The osmotic pressure -corrected permeability (J_m/ (ΔP-$\Delta\pi$)) values were calculated by considering the effect of osmotic pressure (2.2 bar) **b)** Permeability of **CAE$_1$** during RO versus the volume reduction factor, VRF (SW30 membrane, 25 °C and 30 bar). The osmotic pressure-corrected permeability was calculated by considering the effect of osmotic pressure (increase from 6.1 to 20.7 bar) during concentration filtration.

5.2.2.2 Fractionation and concentration of carob extracts

As a general trend, a slightly higher retention of phenolic compounds compared to sugars was observed during Di-NF of the carob extract (**CAE**, from one-step extraction) with the Desal 5-DK membrane (**Fig. 5.2.5)**. The same trend was reported by Díaz-Reinoso et al. (2009) when they filtered a grape pomace aqueous extract by a NF membrane with similar characteristics (Desal 5-DL). The retention of the most abundant low molar mass phenolic compounds in the carob extract, i.e. gallic acid, was ~ 48% (±0.3), which means that it passed strongly to the permeate side. The higher molar mass phenolic compounds (catechin and its derivatives), which have more market value than gallic acids, seemed to be recovered almost completely, due to the fact that the average retention of the total phenolic compounds (including gallic acids) was ~97.5%. Because gallic acid permeated through the membrane, the retention of the total phenolic compounds increased slightly along Di-NF. The proportion of gallic acid in the total reduction of phenolic compounds (5%) was about 80%. Thus, almost complete separation of catechin and its derivatives from gallic acid could indeed be realized. According to the empirical model **(Paper IV)**, ~80% gallic acid was removed at the dia-volume of three, and almost complete removal of gallic acid occurred at the dia-volume of six.

As **Fig. 5.2.5** shows, the retentions of sugars (glucose, fructose and sucrose) along Di-NF were in the range of 88-94%. Based on these retentions, the empirical simulation of Di-NF (**Paper IV**) showed that about 30 dia-volumes would be needed in order to remove about 97%, 94% and 90% of glucose, fructose and sucrose, respectively. Therefore, complete fractionation between catechin and its derivatives and sugars would be possible only at very high dia-volumes. However, high dia-volumes may also affect the yield of high value phenolic compounds. The contents of the phenolic compounds and sugars in the concentrates after Di-NF and NF of **CAE** are shown in **Fig. 5.2.6**. With one dia-volume, the content of different sugars in the **CAE** solution decreased by 4-8%. The removal of gallic acid (75%) seemed to be the main reason for the reduction in the total phenolic compounds content with Di-NF.

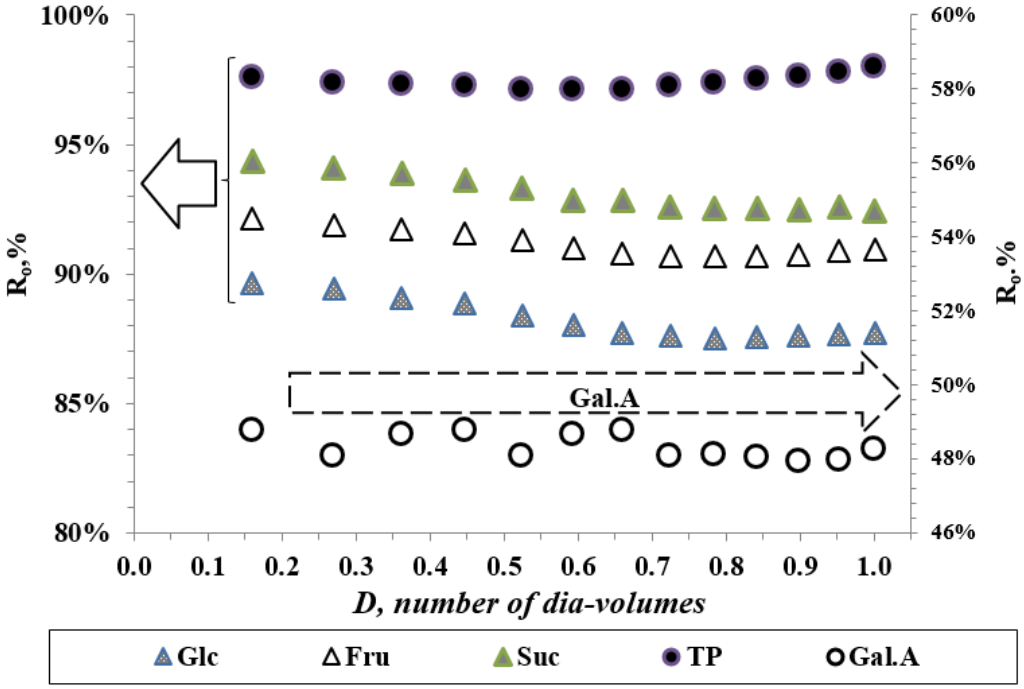


Figure 5.2.5 Variation in the observed retention (R_o,%) of glucose (Glc), fructose (Fru), sucrose (Suc), total phenolic compounds (TP), and gallic acid (Gal.A) during laboratory scale Di-NF of **CAE** with Desal 5-DK membrane versus the number of dia-volumes, D(-)

The diafiltered **CAE** (**R_{Di}**, **Fig. 5.2.6)** was concentrated to a VRF of 2.8. At this VRF, the concentration factors for sugars and phenolic compounds were 2.5 and 2.8, respectively. Therefore, almost all phenolic compounds and most of the sugars were concentrated in the NF concentrate fraction, (**R**, **Fig. 5.2.6).** Fractionation of the one-step carob extract (**CAE**) by Di-NF followed by the concentration step with NF improved the purity of the total phenolic compounds. When the ratio of TP (g)/sugars (g) was used as the indicator of purification efficiency, its value in the final NF concentrate was 12% higher compared to the original extract (feed) i.e. **CAE**.

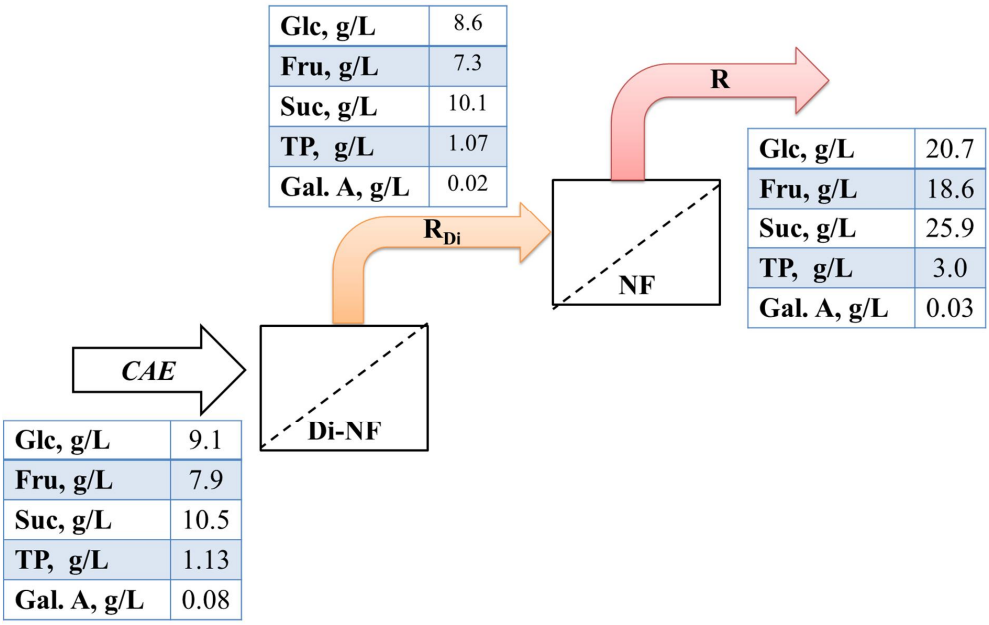


Figure 5.2.6 Laboratory-scale dia-nanofiltration (1 dia-volume) and nanofiltration (VRF of 2.8) of **CAE**: content of sugars and total phenols in the Di-NF and NF concentrate streams ($\mathbf{R_{Di}}$ and **R**, respectively).

In the pilot-scale trial, a spiral wound module was used for Di-NF→NF of the second step extract (**CAE_2**). The Di-NF of **CAE_2** had advantages over the Di-NF of the one-step extract (**CAE,** dilution factor 3.9**),** as no dilution was required. This was due to the lower viscosity of **CAE_2** (7 times lower sugar content). Therefore, the lower amount of filtered volume needed less membrane area in the NF step. In **CAE_2** only a small amount of gallic acid (low-value phenolic compound) was quantified (less than 0.05 g/L). Thus, most gallic acids were extracted efficiently together with most of the sugars in the first extraction step. Independently of the dia-volume, the retention of valuable phenolic compounds was almost 100%, so they were efficiently recovered. The retentions of glucose and fructose were 94% and 96% during Di-NF, so their removal would need a high number of dia-volumes. The retention of sucrose was > 99%. Therefore, the hydrolysis of sucrose to glucose and fructose is needed to improve its removal by Di-NF.

Due to the similar molar masses of sugars and phenolic compounds, their efficient fractionation by Di-NF was not possible. Díaz-Reinoso et al. (2009), who nano-filtered a grape seed aqueous extract, have reported a similar conclusion. Therefore, the diafiltered **CAE_2** was subjected to NF to concentrate the valuable phenolic compounds, i.e. high molar mass ones. **Fig. 5.2.7** presents the contents of **total** phenolic compounds and sugars after NF (final VRF value of ~ 6) in the concentrate stream **R_2**. At this VRF, almost all of the total phenolic compounds were concentrated (CF 5.9), whereas the concentration factors (CF) of glucose, fructose and sucrose were 4.1, 4.7 and 5.6, respectively. The purity indictor, i.e. (TP (g)/sugars (g) ratio) in the final concentrate, **R_2**, was 18% higher than in the original extract, i.e. **CAE_2**. The final concentrate contained almost 11 g/L of phenolic compounds. To our knowledge, there are no studies on the concentration of carob extracts by NF. The reported concentration values vary when the phenolic compounds have been concentrated with NF from biomass-based extracts (VRF 6, 150-300 Da membranes). Prudêncio et al. (2012) obtained similar concentration of total phenolic compounds (11 g/L) when a mate tree aqueous extract (1.6 g/L phenolic compounds) was concentrated with NF. The concentration of phenolic compounds increased from 0.2 g/L to 1 g/L when Díaz-Reinoso et al. (2009) subjected grape pomace aqueous extracts to NF. In both these studies, the antioxidant activity in the concentrates increased with NF.

Although NF did not improve the purity of the phenolic compounds significantly, it can be used to take the water out and produce a fraction suitable for further separation processes. This fraction could be processed e.g. by adsorption to recover the phenolic compounds. In related studies, Díaz-Reinoso et al. (2009; 2010) employed NF prior to polymeric resin adsorption and/or solvent extraction with ethyl acetate for the recovery of phenolic compounds with high antioxidant activity from a pressed and distilled grape pomace aqueous extract. In their studies, at a VRF of 5.5–6.5, the phenolic compounds with antioxidant activity were concentrated by factors in the range of 3–6. Other options, such as enzymatic hydrolysis of sucrose to glucose and fructose using commercial enzymes like invertase (Petit and Pinilla, 1994) could be used before Di-NF↔NF to enable more efficient removal of sugars from phenolic compounds (Pinelo et al. 2009). As NF is operated at mild temperature conditions, it can be assumed that the biological activity of the separated phenolic compounds would be preserved. Kumazawa et al. (2002) have found high antioxidant activity of phenolic contents in the aqueous extract of carob residues.

The NF permeate was like a dilute sugar solution with a low content of phenolic compounds. Therefore, in order to operate the process with minimum discharge and to benefit from all streams it could be combined with $\mathbf{CAE_1}$ and subjected to RO. In this study, $\mathbf{CAE_1}$ (the first extract of two-step extraction) was only used to concentrate the sugars with RO. The compositions of various RO streams (VRF 4) are shown in **Fig. 5.2.7**. As could be assumed, RO retained all the sugars present in the $\mathbf{CAE_1}$ liquor. The RO concentrate had a high sugar content (~ 160 g/L) and low phenolic content (1.5 g/L, mainly gallic acid). So, it could be utilized as fermentation feedstock for medical and food industry, where products such as xanthan (Roseiro et al. 1991 a) and mannitol (Carvalheiro et al. 2011), as well as the special chemical pinitol (Macias Camero and Sanjuan Merino, 2003) could be produced. Moreover, the RO permeate had fresh-water quality, and it could be reused in the aqueous extraction of carob residues. In a similar study, Scordino et al. (2007) produced a purified and concentrated sugar fraction (~ 250 g/L) by RO (VRF of 4) from pigmented orange pulp wash water, where the presence of phenolic compounds was not reported. The solution was pretreated with resin adsorption and ultrafiltration before RO to remove phenolic compounds and proteins. Scordino et al. (2007) suggest employing this fraction as a natural sweetener in food and beverage industries.

It can be concluded that by combining two-step aqueous extraction with membrane filtration, two distinct natural fractions from carob kibbles can be produced (**Paper IV**). The fraction where catechin and its derivatives are enriched could be suitable for the nutraceuticals market, and the other fraction enriched in sugars has promising qualities as feedstock for medical and functional food applications. In this process, all the dilute side streams can be recirculated.

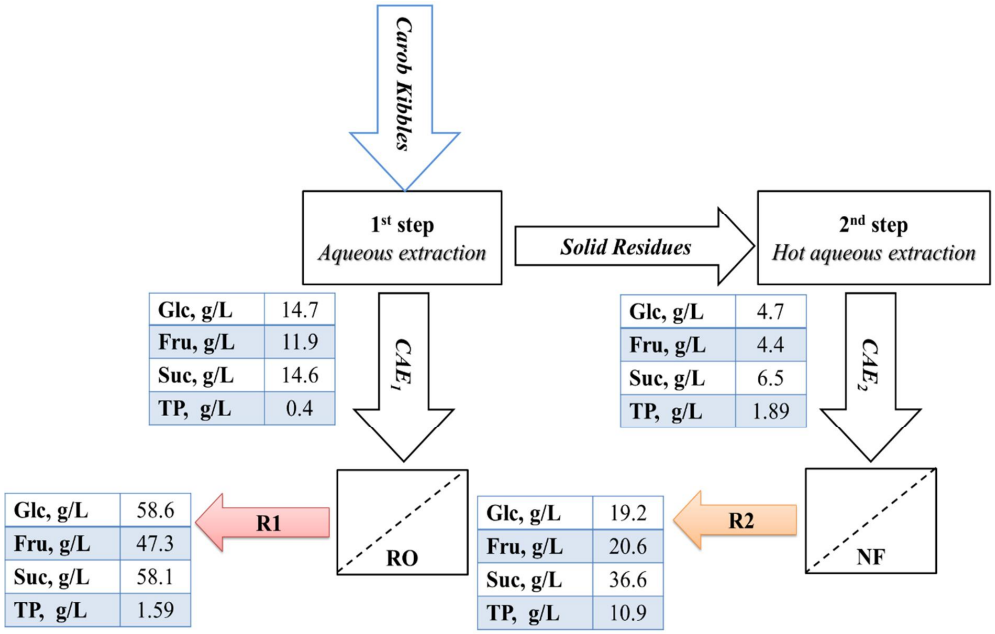


Figure 5.2.7 Pilot-scale reverse osmosis of **CAE$_1$** (VRF of 4) and nanofiltration of **CAE$_2$** (VRF of 6): content of sugars and total phenolic compounds in the RO and NF concentrate streams (**R$_1$** and **R$_2$**, respectively).

6 Conclusions

This study focused on the exploitation of biomass residues by recovering their value-added compounds. The main aim was to recover spruce hemicelluloses, i.e. GGMs, and phenolic compounds of carob kibbles. To recover these compounds, sustainable separation approaches based on aqueous extraction and membrane-based separation were developed.

Galactoglucomannans (GGMs) can be utilized as a renewable raw material supplies for a wide range of bio-based products like food additives, hydrogels and sustainable barrier packaging films. In this study, GGMs were separated from a pressurised hot water extract (wood autohydrolysates) made from spruce saw dust. Regenerated cellulose (RC) ultrafiltration membranes (cut-off values 10 and 30 kDa) with a high shear rate technique (CR-filter) fractionated the spruce autohydrolysates successfully into concentrated fractions of GGMs with specific molar mass distributions. Compared to the original wood autohydrolysates, the concentrated fractions had a higher concentration of high molar mass GGMs with higher purity and low lignin content (< 10%).

The highest concentration achieved was ~400 g/L with 80% GGM purity (average molar mass 5.3 kDa). The quality of the concentrated fractions of the GGMs was comparable to that of the wood autohydrolysates reported in the literature to be suitable for making sustainable barrier films and hydrogels. In addition, from the industrial perspective, the concentrated fractions of GGMs obtained in this study had the advantage of producing high-grade GGMs with precipitation. This could offer a cost-efficient process, reducing the consumption of ethanol significantly.

The permeability of the wood autohydrolysates during UF was affected by the content of the feed, as well as the possibility of different feed components to form a gel layer on the membrane surface. The decline in the permeability of autohydrolysates with RC membranes was mainly due to the increase of viscosity, which was associated with the increased concentration of high molar mass components during UF. The fouling of the RC membranes was generally low. From the economic point of view, the higher flux and low fouling tendency of the tested membranes means longer life time operation.

To enhance the performance of GGM recovery, DF and PCD oxidation were investigated. With DF, high molar mass GGMs were purified from low molar mass ones. Moreover, impurities like lignin, xylans and monosaccharides were removed with different portions. The results proved that only a partial removal of lignin could be achieved by DF. Therefore, in order to achieve a higher purity of the hemicellulose, another purification method is needed prior to diafiltration, such as adsorption or degradation (oxidation) of lignin molecules. PCD oxidation was found effective in enhancing the permeability of the original wood autohydrolysates and the reduction of autohydrolysate turbidity. Removal of wood lipophilic extractives and lignans was also achieved by oxidation. However, it did not decrease the amount of lignin, the main impurity in hemicellulose concentrates, significantly.

Carob kibbles are an important renewable source of valuable compounds, such as fermentable sugars and phenolic compounds. The results showed that an aqueous extraction at mild operating conditions offers a sustainable, selective process for the recovery of sugars and phenolic compounds from carob residues. One-step extraction recovered only about 20% of the phenolic compounds, corresponding to an extraction yield of 0.6 g $_{GAE}$ /100g dry mass of carob kibbles. The extract contained a significant amount of carbohydrates (110 g/L), and the amount of high molar mass valuable compounds, i.e. catechin and its derivatives, was rather low. The membrane-based

separation of phenolic compounds and sugars from this extract was inadequate. Therefore, two-step aqueous extraction at different temperatures (30 and 100 °C) was developed. It gave a superior yield of phenolic compounds, i.e. about 70%, corresponding to 2 g $_{GAE}$/100g of carob dry matter. It also upgraded the quality of the extracts obtained from carob residues by improving the separation of the sugars from the phenolic compounds in the extraction stages. In this approach, it seemed that the removal of sugars in the first extraction step facilitated the isolation of more high molar mass phenolic compounds from the carob solids. The two-step extraction was flexible to scale up, and it was found to be an effective method to obtain sugars and polyphenol-rich streams that can be further processed e.g. in biorefineries or food industries. The carob extracts obtained by aqueous extraction were treated by NF and reverse osmosis. Based on the experimental work, an integrated separation process with the advantage of the zero-discharge principle was proposed to separate sugars from valuable phenolic compounds. This separation was beneficial for obtaining two distinct natural streams from carob kibbles. One of the extracts was enriched in sugars (glucose, fructose and sucrose) with a minimal content in phenolic compounds and suitable for fermentation processes in e.g. medical, food and beverage industries. The other extract contained high value phenolic compounds (catechin and its derivatives) with a reduced concentration of gallic acids (lower market value than catechins) and monomeric sugars that can be used as a nutraceutical dietary ingredient.

As a conclusion, membrane filtration processes demonstrate high potential as separation techniques in the valorisation of biomass residues by the production of valuable concentrated fractions of value-added compounds, in particular mono- polysaccharides and phenolic compounds. The integration of an aqueous extraction with membrane processes could offer a sustainable and green fractionation process applicable for biorefinery. However, depending on the demands of product quality, further purification of the concentrated fractions, for instance with adsorption, might be needed. This approach could be applied in the industrial scale and extended to other biomass residues. The environmental advantages and cost efficiency of this approach are worthwhile to consider for investigation in the future work.

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Publication I

Al Manasrah, M., Kallioinen, M., Ilvesniemi, H., Mänttäri, M.

Recovery of galactoglucomannan from wood hydrolysate using regenerated cellulose ultrafiltration membranes

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Recovery of galactoglucomannan from wood hydrolysate using regenerated cellulose ultrafiltration membranes

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ABSTRACT

Hemicelluloses show promise as a renewable source of raw material for various industrial processes. In this study, galactoglucomannan was recovered from pressurized hot water extract of spruce-sawdust in two steps using hydrophilic regenerated cellulose ultrafiltration membranes having different molecular weight cut-off values. The first step was concentration of galactoglucomannan (GGM) by ultrafiltration using a flat sheet unit and the second step was purification of the retained galactoglucomannan by diafiltration using reverse osmosis filtered water. The highest GGM retention (88%), purity (63%) and recovery (70%) were achieved with the UC005 membrane (cut-off value 5-kDa) at a volume reduction (VR%) of 86%. The UC010 and UC030 membranes (cut-off values 10- and 30-kDa, respectively) partly separated xylan from GGM. Generally, diafiltration did not improve the purity of the GGM due to overlapping of the GGM and lignin molar mass distributions and the fact that most of free low molar mass lignin had already been removed in the concentration filtration step. However, by diafiltration, partial removal of xylan and complete removal of monosaccharides from the GGM rich concentrate was achieved.

1. Introduction

Hemicelluloses are a major natural source of polysaccharides and represent approximately 20–30% of the dry weight of wood based biomass. Hemicelluloses are branched polymers formed by various types of sugar units arranged in different proportions and with different structures. The polymerisation degree of hemicelluloses ranges from 100–200 so hemicelluloses have relatively low molar mass compared with other wood polymers (Timell and Syracuse, 1967).

Hemicelluloses have received considerable attention as a promising source of renewable raw material for several value-added products (Willför et al., 2008). Organic acids such as acetic acid, methane, monosaccharides, sugar alcohols, and solvent alternatives to petroleum-derived chemicals are some of the potential products which have been refined from hemicelluloses (Werpy et al., 2004). Hemicelluloses are now used as a raw material for food additives, thickeners, emulsifiers (Mikkonen et al., 2009), adhesives, binders, adsorbents, and anti-tumor agents (Werpy et al., 2004; Ebringerova, 2006; Willför et al., 2008). Recently, other novel products have been developed, such as cationic biopolymers, packaging films (Hartman et al., 2006; Hansen and Plackett, 2008), hydrogels (Söderqvist Lindblad et al., 2001) and long-chain alkyl ester derivatives. In the forest-based biorefinery concept pulp and paper production is combined with conversion of hemicelluloses to fuels (Kenealy et al., 2007; Hu et al., 2008).

Hemicelluloses can be isolated from wood or biomass by extraction. Bobleter et al. (1976) pioneered a hot water pre-treatment of biomass and Mok and Antal (1992) achieved complete removal of hemicelluloses from biomass and herbaceous materials without high degradation using around 15 min liquid water extraction at 200–230 °C. Hot water has been used to extract hemicelluloses from a number of wood species (Amidon and Liu, 2009). Recently, liquid water at high temperature and pressure has been used to fractionate biomass into its components in a method called pressurized hot water extraction (PHWE) (Ando et al., 2000; Liu and Wayman, 2005; Tunc and Van Heiningen, 2008; Yu et al., 2008; Leppänen et al., 2010). PHWE is used as a pre-treatment method for solid materials to recover flavonoids and other phenolic compounds from aspen knotwood (Hartonen et al., 2007), and polysaccharides from mushrooms (Chien et al., 2007). The key advantages of PHWE over other extraction techniques are its low cost, non-toxicity and the environmental benefits of using water as a solvent (Hartonen, 1999).

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PHWE of biomass generates dilute brown liquor, called hydrolysate. In addition to hemicelluloses, the hydrolysate contains other dissolved compounds originating from the biomass. If the goal is separation of hemicelluloses from other compounds in the hydrolysate, ultrafiltration (UF) can be considered one of the most promising methods since it has low energy requirements, high selectivity, and requires no additional chemicals. UF separation mechanism is selective exclusion of particles in which solute components with molar mass higher than the membrane cut-off are retained while the solvent and solute components with molar mass lower than the membrane cut-off pass through the membrane.

In the pulp and paper industry, membrane processes are used to recycle valuable materials and to purify process water for reuse (Mänttäri et al., 2002; Huang et al., 2008; Persson et al., 2010). Large scale UF has been deemed an industrially attractive method to recover dissolved polysaccharides, mainly hemicelluloses, from the process water of mechanical and thermo-mechanical pulping (TMP) of spruce wood (Willför et al., 2008; Persson et al., 2010).

Persson et al. (2006, 2007) have concluded that isolation of hemicelluloses from TMP process streams using appropriate membrane processes can be beneficial. In their research, they pre-treated TMP process waters containing hemicelluloses (1–2 g/L) by microfiltration. The hemicelluloses were then concentrated using UF and further purified using diafiltration. They found that fouling of hydrophobic polyethersulphone (PES) membranes was significant. Conversely, the hydrophilic UF membranes offered high permeate flux and low fouling tendency. With hydrophilic UF membranes, a hemicelluloses fraction with about 60 g/L concentration at almost 80% purity was obtained for TMP process water.

Desired components in UF concentrates can be further purified by diafiltration. Andersson et al. (2007) found that diafiltration is probably the most cost-efficient method to separate hemicelluloses from smaller molar mass compounds such as small oligosaccharides, monosaccharides, and salts. Increasing the amount of water in diafiltration usually increases the purity of the retained hemicelluloses.

In the present study, the performance of hydrophilic regenerated cellulose ultrafiltration (RC-UF) membranes with different molecular weight cut-off values was evaluated in the separation of the dominant hemicellulose in spruce, i.e., galactoglucomannan (GGM), from sawdust extract. In addition, the efficiency of diafiltration in purification of the retained GGM was evaluated. The criteria used for evaluation of the membrane performance were permeate flux and membrane fouling, in addition to separation efficiency parameters (retention, recovery (yield) and purity of GGM). The aim of this study was to ascertain the most suitable hydrophilic membrane to recover valuable GGM extracted by pressurized hot water from low value biomass materials such as sawdust.

2. Methods

2.1. Pressurized hot water extraction

The dry solid sample of the extracted compounds was prepared by the Finnish Forest Research Institute (Metla). The pressurized hot water extraction (PHWE) solution was prepared in a 3 L extraction unit filled with around 600 g of dried spruce sawdust. The sawdust was soaked in hot water then heated water was pumped through the reactor in a way that ensured flow-through conditions within the extraction vessel. The extraction temperature and the water solution flow rate were 180 °C and150 ml/min, respectively. At the end of the 53 min of extraction, 8 L of the PHWE liquors (with about 1.8 wt.% total solids content) were collected. After evaporation of water using a rotary evaporator and drying the solids using vacuum drier, the dry weight of the extract was 24% of the initial dry weight of the saw dust placed in the extractor.

2.2. Raw materials

The feed solution was prepared by dissolving 10 g of the solid extract in 1 L of water (concentration 10 g/L). The solution could be described as a light brown liquid containing mostly dissolved hemicelluloses, monosaccharides, lignin and its degradation compounds, besides minor amounts of wood extractives. The dissolved solids in the solution contained about 82% (i.e., 20% out of dry wood) oligo- and polysaccharides (mainly galactoglucomannan (GGM)) and 9% monosaccharides). Fig. 1 shows the structure of the hemicelluloses and branched sugar/acid groups released during the PHWE process. Table 1 presents the composition of the PHWE liquor (wood hydrolysate). GGM, the dominant hemicellulose in spruce, is mostly present in a polymeric (oligomeric) configuration. About 20% of the galactose units were partially hydrolysed during PHWE at mild acidic conditions. The arabinoglucuronoxylan (xylan) content in the extraction liquor was significantly lower than that of GGM. About 70% of the xylan was in a polymeric (including oligmeric) configuration. Most of the arabinose side groups were hydrolysed during the PHWE. The ratio of GGM to xylan (oligomeric and polymeric) in the extraction liquor was around 5, which is significantly higher than that measured for raw spruce wood (approximately 2) (Alén, 2000). The corresponding ratio calculated for total carbohydrates was about 3, showing that a higher proportion of xylan had degraded to monosaccharides. The ratio was still somewhat higher than that typically measured in spruce, indicating slightly better release of GGM from the spruce sawdust during the PHWE, despite the fact that GGM retained its polymeric configuration better than xylan.

2.3. Membranes

Hydrophilic RC-UF membranes were used to concentrate hemicelluloses from PHWE liquors. Table 2 presents the main characteristics of the tested membranes. Measured pure water permeabilities at 65 °C were 35, 48 and 350 (kg/(m^2h bar)) for the UC005, UC010 and UC030 membranes, respectively.

2.4. Filtration experiments

Membrane filtration experiments were carried out using a cross-flow flat sheet membrane module with an active membrane surface area of 0.01 m^2. The wood hydrolysate solution was pumped from a feed tank (volume 3 L) to the membrane module using a hydra-cell pump (Wanner Engineering Inc.) that applies trans-membrane pressure in the system. A frequency converter was used to control the pump velocity. During the filtration experiments, the cross-flow velocity was maintained in the turbulent regime (cross flow velocity = 2 m/s) to minimise the concentration polarization effect.

Before use, the membranes were washed with water in an ultrasonic bath three times for 15 min. Pure water flux (PWF) measurements were carried out before and after each filtration of the PHWE liquor to detect fouling of the membranes.

Filtrations of the PHWE liquors were carried out in two steps, as shown in Fig. 2. In the first step, RC-UF membranes with different cut-offs were used to concentrate and fractionate the hemicelluloses. The concentrate was recycled to the feed tank and permeate was collected in a separate vessel and measured gravimetrically. The concentration mode filtration continued until the desired volume reduction (VR) was achieved. (VR is defined as the ratio of the volume of the permeate to the volume of the initial feed).

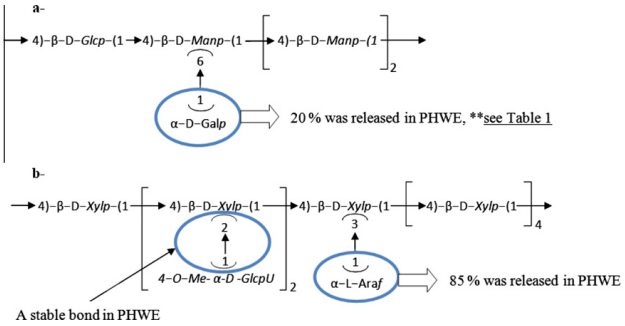

Fig. 1. Simplified structural scheme of the main softwood hemicelluloses. (a – Galactoglucomannan (GGM), b – Arabinoglucuronoxylan (xylan)) and changes during the PHWE, where Glcp is the D-glucopyranose unit; Manp is the D-mannopyranose unit; Galp is the D-galactopyranose unit; Xylp is the xylopyranose unit; 4-O-Me-α-DGlcpU is the methyl-α-D-glucuronic acid unit; and Araf is the arabinofuranose unit.

Table 1
Composition of the feed solution made from the extract of spruce sawdust.

Analysed parameters		
TOC, mg/L	4030	*Calculated based on the assumption that 40% of organic compounds consist of carbon
Total amount of organics, mg/L*	10070	**GGM content is based on the asumption that all analysed mannose, glucose and galactose units are from GGM. Part of galactose might also originate from galactan (compression wood of spruce). Acetyl groups are released during PHWE extraction (pH decreased due forming of acetic acids) and they are not included in calculation of GGM content.
Lignin, mg/L	1010	
Turbidity, NTU	1350	
pH	4	
Conductivity, μS/cm	380	
Not analysed	2020	

Carbohydrates composition

	Total	Man	Glc	Gal	**GGM** **	Ara	Xyl	4-O-Me-GlcA	Xylan	Rha	GlcA	GalA
Hemicellulose and oligomers**, mg/L	6050	3560	900	400	**4860**	70	890	30	**990**	40	20	190
Monosaccharides, mg/L	990	120	50	110	**280**	290	340	20	**650**	20	0	10
Total carbohydrates, mg/L	7040	3680	950	510	**5140**	360	1230	50	**1640**	60	20	200

The bold values mean hemicelluloses (GGM and Xylan) and the non-bold values are monosaccharides that formed the GGM and Xylan polymers.
Man: mannose, Glc: glucose, Gal: galactose, Ara: arabinose, Xyl: xylose, 4-O-Me-GlcA: 4-O-methyl-glucuronic acid, Rha: rhamnose, GlcA: glucuronic acid, GalA: galacturonic acid.

Table 2
Regenerated cellulose ultrafiltration (RC-UF) membranes and their properties (Manufacturer: Microdyn-Nadir, Germany).

Membrane	Cut-off (kDa)		Pure water flux[d] (L/(m^{2}h))	pH Range	Max. temperature (°C)
	Manufacturer[a]	Experimental			
UC030	30	10[b]	>300		
UC010	10	2.5[c]	>40	1–11	55
UC005	5	2.5[c]	>25		

[a] MICRODYN-NADIR (2007).
[b] Kallioinen (2008).
[c] Platt et al. (2002).
[d] Test conditions: 3 bars, 20 °C, stirred cell 700 RPM (MICRODYN-NADIR, 2007).

In the second step, a diafiltration technique was used to purify the retained hemicelluloses. During diafiltration, a specific volume of RO filtered water (κ = 0.054 μS/cm, T = 25 °C) was added at intervals to the concentrate in the feed tank, and a similar volume of permeate was removed. In this study, two diafiltration steps were performed with a diavolume coefficient (the ratio of the diafiltration water volume to the concentrate volume) of approximately 2 for each step. The UF and diafiltration steps were carried out at 65 °C. The relatively high temperature was chosen to minimize the need for cooling after water extraction of wood chips/sawdust in real practices at mill sites. It should be noted that the temperature was higher than that recommended by the manufacturer (Table 2), but the tested RC membranes have been shown to be able to withstand such high temperatures (Kallioinen et al., 2007).

2.5. Analysis methods

Before analysis, feed and concentrate samples were centrifuged at 500*g* for 30 min to remove suspended solids and fibers. To esti-

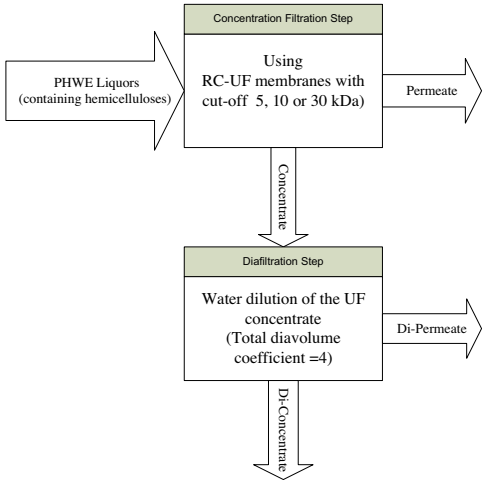

Fig. 2. Schematic illustration of the filtration experiments (PHWE: Pressurized hot water extraction, RC-UF: Regenerated cellulose ultrafiltration, and Di: Diafiltration).

mate the retention of various compounds and to evaluate the separation and purification efficiency parameters, all liquid streams were analyzed for their total organic carbon using a TOC-5050 analyzer to determine the organic solute content (i.e., carbohydrates, lignin, organic acids and wood extractives).

The amount of total hemicelluloses in the filtration samples (includes polysaccharides, oligosaccharides and monosaccharides) was estimated from freeze-dried samples using acid methanolysis followed by gas chromatography (GC) analysis (Sundberg et al., 1996). Free monosaccharides were determined by GC after direct silylation of the original sample. Analysis of lignin content was based on correlation of the lignin concentration with absorbance of ultraviolet light at 280 nm. In this study, a correlation coefficient of 17.8 L/(g cm) was used for estimation (Örså et al., 1997). To ensure accurate values, the samples were first pre-treated by methyl-tertbutylether (MTBE) extraction to remove wood extractives having also an absorbance band at 280 nm and thus interfere with the analysis of lignin.

Conductivity and pH were measured to determine the ionic content of the samples. Conductivity was measured with a digital conductivity meter (Knick Konduktometer 703) calibrated with 0.01 M KCl. The presence of suspended solids and possible extractives was detected by measuring turbidity using a Hach 2100AN IS turbidimeter.

2.6. Calculations

Mass flux is the rate of permeate mass flow across a membrane surface area. In this study, it was calculated by Eq. (1):

$$J_m = \frac{m_p}{A_m} \quad (1)$$

where J_m is the mass flux (kg/(m^{2}h)), m_p is the mass flow rate (kg/h) and A_m is the membrane surface area (m^2).

Selectivity of a membrane for a solute is described by retention. The observed membrane retention of the solute was calculated by Eq. (2) (Strathmann et al., 2006):

$$R_o = 1 - \frac{\ln(1 - \frac{C_p \Delta}{C_f})}{\ln(1 - \Delta)} \quad (2)$$

where R_o is the observed retention; C_p is the concentration of accumulated solute in the permeate at a specific recovery rate, C_f is the feed concentration, and Δ is the recovery rate (accumulated volume of permeate divided by initial feed volume which is corresponding to VR definition and value in this study).

Membrane fouling was calculated by comparing the difference between the pure water flux before and after the filtration, Eq. (3).

$$\text{Fouling}\,(\%) = \frac{PWF_b - PWF_a}{PWF_b} \cdot 100\% \quad (3)$$

where PWF_b is the pure water flux before filtration (kg/(m^{2}h)) and PWF_a is the pure water flux after filtration (kg/(m^{2}h)).

The recovery of hemicelluloses during filtration was calculated by Eq. (4):

$$\text{Recovery}\,(\%) = \frac{m_{hemi(C)}}{m_{hemi(F)}}.100\% \quad (4)$$

where $m_{hemi(C)}$ is the mass of hemicelluloses in the concentrate and $m_{hemi(F)}$ is the mass of hemicelluloses in the feed.

The purity of the hemicelluloses was defined as the ratio between the carbon content in the hemicelluloses (about 40%) and the total organic carbon (TOC) in the final concentrate and was calculated by Eq. (5):

$$\text{Purity}\,(\%) = \frac{0.4 \bullet C_{hemi(C)}}{TOC_{(C)}} \cdot 100\% \quad (5)$$

where $C_{hemi(C)}$ is the concentration of the hemicelluloses (e.g. GGM) and $TOC_{(C)}$ is the total organic carbon in the final concentrate.

3. Results and discussion

3.1. Permeate flux and fouling

Permeate flux is one of the most important parameters to evaluate the performance of pressure-driven membrane filtration processes. If the retention of the solute by the membrane is satisfactory, the permeate flux becomes a fundamental factor to optimize the membrane filtration process.

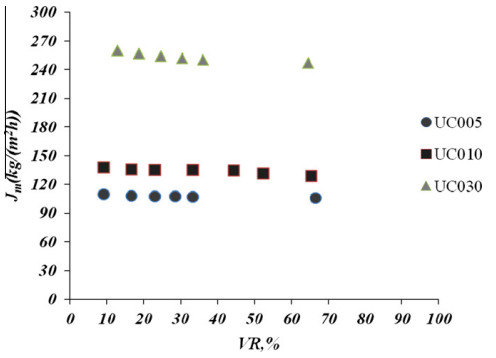

Fig. 3. Permeate flux during concentration of the PHWE liquor with the UC030 (filtration pressure was 1 bar), UC010 (3 bar) and UC005 (3.5 bar) membranes at 65 °C.

Table 3
Permeate fluxes at the end of the concentration filtration and two diafiltration steps, and fouling (%) of the RC-UF membranes.

Membrane	Pressure (bar)	J_m (kg/m^{2}h))				Decrease/change by two diafiltrations			
		1st step		2nd step		TOC	Hemicelluloses	Lignin	Fouling (%)
		PWF_b	UF	Dia_1	Dia_2				
UC030	1	350	245	131	114	8.7–>3.7 g/L	12.2–>4.7 g/L	1.5–>1.2 g/L	26
UC010	3	165	135	125	123	7.6–>4.7 g/L	11.7–>8.6 g/L	1.5–>1.3 g/L	16
UC005	3.5	130	107	86	86	13.4–>9.5 g/L	24.6–>15.1 g/L	3.6–>3.1 g/L	18

Dia_1, Dia_2 are diafiltrations with a diavolume coefficient of 2.

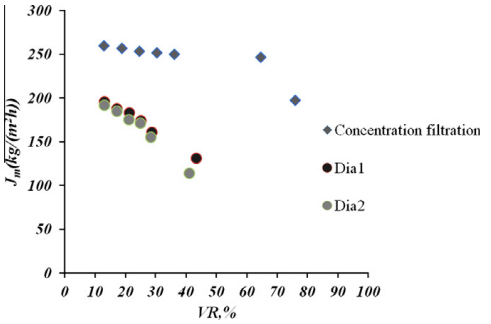

Fig. 4. Permeate flux of the UC030 membrane (1 bar, 65 °C, cross-flow velocity ~2 m/s) during concentration filtration (VR = 75%) and two diafiltrations (diavolume coefficient was 2 for each one).

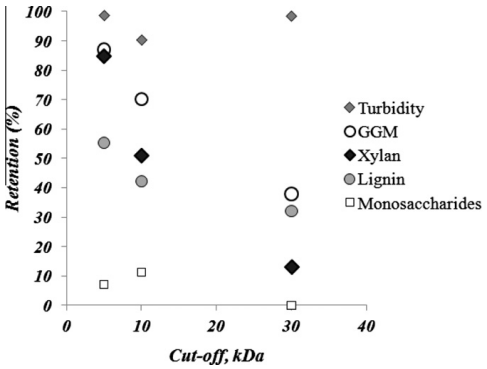

Fig. 5. Effect of RC membrane cut-off on the retention of hemicelluloses (GGM and xylan), lignin, monosaccharides and turbidity.

Fig. 3 shows the permeate fluxes through the UC005, UC010 and UC030 membranes. Permeate flux through the RC membranes was generally high. The membrane with the highest cut-off had the highest permeate flux, as could be expected. Fouling tendency (Table 3) of the membranes was low. The high permeate flux and more open structure of the UC030 membrane compared to the other RC membranes studied could explain its slightly higher fouling (26%).The experiments were conducted at 65 °C and therefore, membrane compaction might also have caused a reduction in pure water flux. The fouling could hence be lower than calculated based on the changes in pure water fluxes (Eq. (3)).

Table 3 shows that the permeate fluxes of the UC030 membrane were significantly lower at the end of the diafiltration steps than at the end of the UF concentration step. The flux decreased substantially even though the TOC, hemicelluloses and lignin content of the concentrate decreased from 8.7 to 3.7 g/L, from 12.2 to 4.7 g/L and from 1.5 to 1.2 g/L, respectively during the diafiltrations. Furthermore, as Fig. 4 shows, the slope for flux decrease was significantly higher in the diafiltration stage. Permeate flux of the UC010 and UC005 membranes dropped only slightly as the purification by diafiltration proceeded.

It seems that the flux decreased more when the relative amount of high molar mass substances in the retentate side increased. With the tighter UC010 and UC005 membranes the flux decrease in the diafiltration steps was lower (compared with that of the UC030 membrane) because they retained more medium size molar mass and low molar mass solutes compounds. This probably diminished the capability of the polymers to form a gel-like structure on the membrane surface.

3.2. Recovery of hemicelluloses

The results in Fig. 5 reveal clear differences in the ability of the membranes to separate hemicelluloses, mainly GGM, from lignin. The best choice for purification of GGM from PHWE liquor was the UC005 membrane, which had the greatest difference between the GGM and lignin retentions (88% and 55%, respectively). Moreover, the highest GGM recovery (almost 70%) was achieved by this membrane, since the GGM concentration in the final concentrate was 22 g/L at a VR% value of 86%.

Retentions of monosaccharides were low with the tested membranes. Lignin retention of the UC030 membrane was clearly lower (about 30%) compared to that of the other membranes but hemicelluloses also passed through the membrane (GGM retention was only 38%). The turbidity measurements indicate that most of the suspended solids and possible extractives were retained also with the largest pore size tested UC030 membrane. For the main hemicelluloses in spruce wood, retention of GGM was higher than xylan retention when the 10- and 30-kDa membranes were used. According to the literature, xylan in spruce wood has a slightly higher molar mass than GGM (Othmer, 1995). In this study, xylan retention was low, suggesting that the backbone of xylan was partially degraded in the PHWE process. The carbohydrate analysis presented in Table 1 confirms this assumption. Thus, partial separation of xylan from GGM could be possible with the UC010 or UC030 membrane.

The UC005 and UC010 membranes retained both hemicelluloses better than they retained lignin. With the UC030 membrane, lignin retention was higher than xylan retention, which indicates that the molar mass distributions of lignin and hemicelluloses are significantly different. It can be assumed that lignin (UV absorbing material) mostly consists of low and high molar mass compounds and smaller amounts of medium size compounds. Overlapping of the hemicellulose and lignin molar masses and the presence of lignin-hemicellulose complexes make complete separation of lignin from hemicelluloses directly by UF unrealistic.

With the UC005 membrane, total hemicellulose purity increased in concentration filtration from 60% to 73% and GGM purity from 48% to 63%. The recovered and concentrated hemicelluloses were further purified using diafiltration. Purification of hemicelluloses by diafiltration reduced the total recovery of hemicelluloses in different portions depending on the membrane cut-off. Analysis of hemicellulose measured both the polymeric and oligomeric compounds. Therefore, it is not surprising that during diafiltration a significant part of the hemicelluloses was lost and that the lowest reduction in recovery was achieved with the smallest pore size tested UC005 membrane. Diafiltration might therefore improve the purity of hemicelluloses having the highest molar mass, even though the overall hemicellulose purity decreases.

In diafiltration, lignin retention was significantly higher (80%) than that in the preceding concentration filtration. The increased lignin retention explains why GGM purity decreased during diafiltration. It seems that during concentration filtration most of the permeable lignin (low molar mass UV absorbing materials) passed through the membrane. The residual lignin might have high molar mass or still be bounded to the backbone of the hemicellulose, thus resulting in high lignin retention. Although diafiltration was not able to improve GGM purity, it partially facilitated separation of xylan from GGM. Furthermore, two stage diafiltration efficiently removed monosaccharides from the hemicellulose concentrate; monosaccharide content in the concentrate after diafiltration was from 20 to 50 mg/L depending on the membrane.

3.3. Separation efficiency of monosaccharides

GGM retained its polymeric configuration in the PHWE process and only minor amounts of glucose and mannose existed as monosaccharides in the extract. However, about 20% of the galactose side groups were released as monosaccharides. Xylan was partially degraded to monosaccharides in the PHWE process. About 30% of the xylose and 80% of the arabinose were present as monosaccharides. These compounds permeated the used UF membranes easily and after diafiltration (total diavolume coefficient = 4) more than 95% of the xylose and arabinose was recovered into the permeate. Monosaccharides recovery can be readily increased by concentrating the solution further with tighter membranes. The monosaccharides concentration was, however, relatively low (about 1 g/L) which might restrict the economic viability of monosaccharide recovery.

4. Conclusions

The aim of this study was to evaluate the performance of three RC-UF membranes in recovery of GGM from spruce sawdust extract. The smallest pore size membrane tested (UC005) retained almost 90% of the GGM, increasing the GGM purity from 48% to 63%. Partial separation of xylan from GGM was achieved with the UC010 and UC030 membranes. Diafiltration was able to purify hemicelluloses from monosaccharides. Purification of GGM from lignin fragments was only partial. To improve the hemicellulose purity, another purification method is therefore needed prior to diafiltration, such as adsorption or degradation of lignin molecules.

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Publication II

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Improvement of ultrafiltration performance by oxidation treatment in the recovery of galactoglucomannan from wood autohydrolyzate

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Improvement of ultrafiltration performance by oxidation treatment in the recovery of galactoglucomannan from wood autohydrolyzate

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ABSTRACT

The possibility to enhance ultrafiltration in the recovery of galactoglukomannan (GGM) from wood autohydrolysate with the gas-phase pulsed corona discharge (PCD) oxidation was studied. The filtration capacity, membrane fouling, and purity of hemicelluloses in the membrane concentrates were used as criteria to evaluate the benefits of the hybrid separation process. The results showed that the PCD oxidation significantly improved the filterability of the wood autohydrolyzate, although its effect on the fouling of the very hydrophilic cellulose-based UF membranes was low. The positive influence on filterability can be at least partly explained by the decreased viscosity of the oxidized autohydrolyzates. Oxidation modified the structure of the lignin but its effect on the lignin molar mass was small. As a result of oxidation, the average molar mass of hemicelluloses was also slightly decreased. Therefore, the influence of oxidation on the purity of the concentrated hemicellulose fractions was not as high as expected. However, oxidation removed lignans and lipophilic wood extractives, which have some influence on the purity of the produced hemicellulose fractions.

1. Introduction

Hemicelluloses are the second most common group of polysaccharides in nature. Globally, pulp mills treat approximately 70 Mt of hemicelluloses annually. Typically, hemicelluloses are not utilized in the most efficient way in the mill and they are usually incinerated in the recovery boiler although their heating value is relatively low. Therefore, hemicelluloses are a huge, existent raw material source for future oil-free economy.

Galactoglucomannans (GGMs) are the predominant hemicelluloses in the soft woods and their recovery and refining to value added products have recently attracted the attention of researchers [1–3]. One of the promising methods to extract GGMs from wood material is pressurized hot water extraction [4–8]. During the extraction other wood compounds are also dissolved into the wood autohydrolyzate and, therefore, further fractionation and purification is needed before the hemicelluloses can be further processed to compounds substituting oil-based compounds. Typically, wood autohydrolyzates contain phenolic compounds such as lignin and its degradation products, and lignans. In addition, small amounts of organic acids and wood lipophilic extractives are present. Lipophilic extractives and lignans have low molar masses, which makes application of membrane technology, in particular ultrafiltration (UF), theoretically feasible in recovery and purification of dissolved high molar mass hemicelluloses [9–14]. The approach, however, often suffers from a noticeable decrease in filtration capacity making the process uneconomical [13,15]. In addition, UF is not selective as regards hemicelluloses, as other high molar mass compounds, such as lignin, are retained simultaneously.

The feasibility of the UF process may be improved by using a proper hydrophilic membrane [16,17], controlling filtration conditions (e.g. high shear rate on the membrane surface to reduce concentration polarization, and thus, usually also membrane fouling), and by applying suitable pre-treatment to the feed solution [13–15]. Lignin and wood extractives have shown to be potential foulants when wood originating solutions are treated. Various pre-treatment processes are able to degrade, inactivate, or remove these foulants prior to UF. Oxidation of the foulants is an attractive fouling management alternative, because it might lead to small molar mass lignin compounds permeating the membrane and offers the possibility to valorize lignin recovered from the

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permeate fraction. Moreover, the waste amount produced in the hybrid process combining oxidation and ultrafiltration is easier to minimize compared to a process combining adsorbents and ultrafiltration. Oxidation might also separate lignin from hemicellulose–lignin complexes and thus enable improvement of purity of the concentrated high molar mass hemicellulose fraction.

Different oxidation methods, for instance wet oxidation [18,19] and ozonation [20,21] have been studied in the degradation of lignin present in different biomass based solutions. Recently, oxidation with the pulsed corona discharge (PCD) method has also proven to decompose lignin in aqueous solutions [22] at an energy efficiency exceeding the one of traditional ozonation; this is due to utilizing short-living oxidants [23]. The degradation of lignin to vanillin and syringaldehyde as oxidation products has been presented as a possible perspective to valorize lignin using the PCD treatment [22]. In the PCD oxidation, only a small energy input is needed to generate ozone and hydroxyl radicals from oxygen and water and, therefore, less energy is needed compared to ozonation. Koivula et al. [13] showed earlier that pretreatment of spruce autohydrolyzates with PCD oxidation significantly improved the flux through a hydrophobic polysulphone membrane and reduced membrane fouling. However, the effect of PCD oxidation on the composition and properties of the wood autohydrolyzate was not examined in details. Furthermore, the effect of oxidation on the filtration performance of hydrophilic membranes, which generally have a lower fouling tendency in the treatment of wood originating solutions, was not studied.

Therefore, the aim of this study was to discover the influence of PCD oxidation pretreatment on the performance of a membrane-based recovery and purification process for hemicellulose (galactoglucomannan), when hydrophilic membranes are applied. The filtration capacity, membrane fouling, and purity of hemicelluloses in membrane concentrates were used as criteria to evaluate the benefits of the hybrid separation process. The effect of oxidative treatment on the autohydrolyzate composition and properties were analyzed and the filtration results explained based on the analysis results.

2. Materials and methods

2.1. Wood autohydrolyzate

Wood extract (autohydrolyzate) was prepared by the treatment of 29.4 kg of dry spruce saw dust with water in a 300 L flow-through extraction vessel at the volumetric flow rate of 14 L/min. The extraction temperature and time were 170 °C and 52 min. The total amount of wood autohydrolyzate was 728 kg, making the water/wood ratio approx. 25. The extraction equipment is described in detail by Kilpeläinen et al. [24].

The extracted liquor contained 2.6 g/L of organic carbon and about 3.8 g/L of carbohydrates. The proportion of monomeric sugars was less than 10% of the total carbohydrates. The average molar mass of hemicellulose was approximately 7 kDa. Due to autohydrolyses and cleavage of acetic acid during the extraction in hot water the pH of wood autohydrolyzate was 4.1. Lignin was expectedly the main impurity in the wood autohydrolyzate. Its content was evaluated by the UV absorbance at 280 nm comprising 0.6 g/L. The liquor also contained about 28 mg/L of lipophilic extractives, 44 mg/L of lignans, 440 mg/L of organic acids and less than 10 mg/L of furan compounds.

2.2. Membrane filter and membranes

In order to minimize membrane fouling, hydrophilic cellulose based UF membranes (water–air interface contact angles below 15°) in a high shear rate membrane filter were used in this study [16,17]. According to the manufacturers, the cut-off values of the membranes used were 30 and 10 kDa for the UC030 (Microdyn-Nadir) and the RC70PP (Alfa Laval) membranes, respectively. The membranes were selected based on earlier experience of the filterability of membranes with similar types of solutions [12,13,16]. A high shear rate cross-rotational (CR) filter was used, because it enables a high turbulence on the membrane surface, thus reducing the effect of concentration polarization [25].

2.3. Filtration experiments

All filtrations were made in the concentration mode to volume reduction factors (VRF) numerically presented in Fig. 1. During the filtration the permeate was collected in a separate vessel, and the concentrate was recirculated back into the feed tank. The pressure, temperature and rotor velocity were kept constant during filtrations. Filtrations were made at a pressure of 1 bar and 2 bar with the 30 kDa and the 10 kDa membrane, respectively. The rotor velocity in the CR-filter was approximately 9 m/s and the temperature 65 °C.

Sequential filtrations were carried out by filtering the previous stage concentrate or permeate using the same or a lower cut-off membrane as shown in Fig. 1. Based on the cut-off values 10 and 30 kDa, two kinds of hemicellulose fractions were recovered by the membranes used. Oxidation treatment was applied to three solutions (Fig. 1):

1. The original wood autohydrolyzate prior to the filtration with the 30 kDa membrane (UC030 III PCD).
2. The 30 kDa membrane concentrate prior to further concentration filtration with the 30 kDa membrane (filtration UC030 II PCD).
3. The 30 kDa membrane permeate (UC030 I) which was at first pre-concentrated by the 10 kDa membrane (RC70PP I) prior to further concentration filtration with the 10 kDa membrane (Filtration RC70PP II PCD).

Membrane performance was evaluated by measuring the permeate flux, pure water permeabilities before (PWP_b), and after (PWP_a) the filtration of wood autohydrolysate and by analyzing the collected samples. Fouling was calculated from the pure water permeabilities and presented as a per cent difference in pure water fluxes before and after the wood autohydrolyzate filtration (Eq. (1)).

2.4. Oxidative treatment

Oxidative treatment was studied with the aim of improving the filtration efficiency and the hemicellulose fraction purity. A schematic diagram of the experimental set-up is shown in Fig. 2. The system consists of a pulse generator (power supply) and a simple reactor described earlier [23]. The power supply generates the discharge pulses of voltage pulse amplitude 20 kV, the current 380–400 A, and 100 ns in duration at pulse repetition frequency of 840 pulses per second (pps). The energy delivered to the reactor was calculated as an integral product of voltage and current peak areas. The maximum delivered energy was 250 W at the maximum pulse repetition frequency (840 pps). The energy consumption efficiency of the pulse generator was 67%.

An autohydrolyzate solution in the amount of 40 L was circulated from the reservoir tank through the reactor by a pump. The autohydrolyzate passed from the top of the reactor through a perforated plate and spread between the electrodes. The autohydrolyzate passed through the PCD zone where the target compounds reacted with hydroxyl radicals, ozone, and other

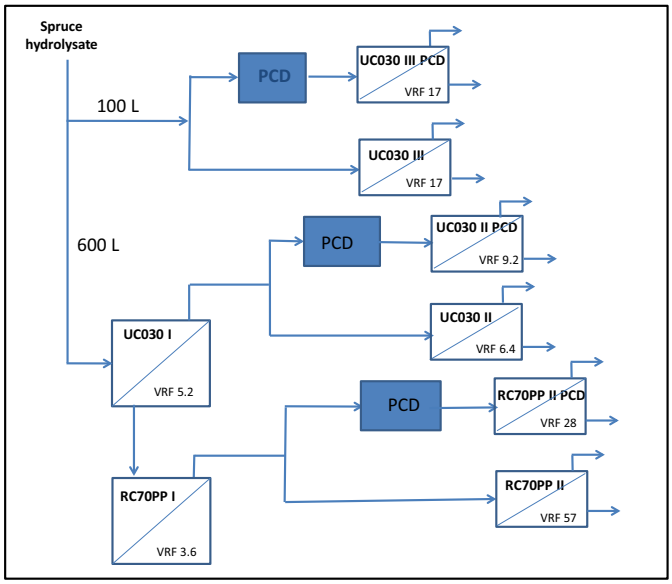

Fig. 1. Sequences of filtration and oxidation treatments.

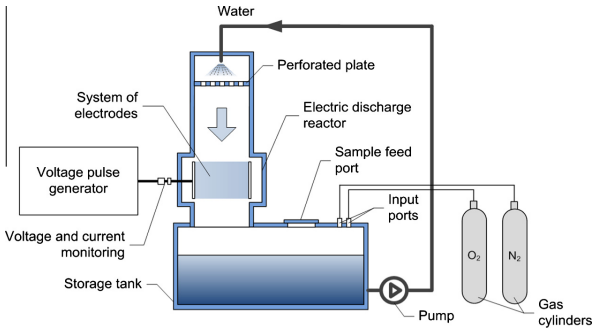

Fig. 2. Schematic illustration of the experimental setup.

short-living oxidants before returning back to the tank. To achieve an initial concentration of about 90% of oxygen in the reactor, pure oxygen gas was introduced to the reactor prior to the experiment.

The effect of the treatment intensity on the composition of the wood autohydrolyzate was studied by collecting samples from the reactor with a 15 min increment from 0 to 45 min and at 120 mins of treatment. The energy delivered to the reactor was calculated as a product of the pulsed power and treatment time relative to the treated autohydrolyzate sample, and comprised about 5400 kJ/m^3 for a 15 min of treatment. The samples of wood autohydrolyzates oxidized for 45 min were filtered as described in Fig. 1.

2.5. Analytical methods

Before analysis, feed and concentrate samples were centrifuged to remove suspended solids and fibers. Conductivity and pH were measured at 25 °C to determine the ionic content of the samples. Conductivity was measured with a digital conductivity meter (Knick Konduktometer 703) calibrated with 0.01 M KCl. The presence of suspended solids and possible extractives was detected by measuring turbidity using a Hach 2100AN IS turbidimeter.

Total organic carbon (TOC) was analyzed using a Shimadzu TOC-5000A analyzer (Shimadzu TOC-5050A analyzer with ASI 5000A autosampler, 680 °C, high purity synthetic air 4.0 as the carrier gas at 150 mL/min, two parallel samplings, deviation < 2%). The purity of hemicelluloses is related to the total organic carbon content of the samples.

Detailed sugar analysis was carried out to estimate the concentrations of hemicelluloses and monosaccharides. The carbohydrates were analyzed according to Sundberg et al. [26]. The carbohydrate content was analyzed by a gas chromatography (GC, HP6890 with injector HP7683, Agilent, USA). The column

was a 25 m/0.20 mm i.d. wide-bore capillary column with a nonpolar phase (HP-1, Agilent Technologies) having a film thickness of 0.11 μm. Carbohydrates were analyzed as monosaccharides derivatives, thus, before analysis hemicelluloses/polymeric carbohydrates were transformed to monosaccharides by acid methanolysis followed by silylation. Monomeric carbohydrates were analyzed in the same way but without acid methanolysis.

Wood lipophilic extractives and lignans were extracted from the samples by methyl *tert*-butyl ether (MTBE) extraction and, after silylation, analyzed by GC [27].

Lignin was determined by UV spectrophotometry (as UV absorbance at 280 nm) after removing lignans and other extractives by MTBE extraction of the samples [28]. The absorbance value was converted to a lignin concentration (g/L) by dividing the absorbance with the absorptive coefficient 17.8 L/(cm g). The UV absorbance was also measured in the original samples before the MTBE extraction. The UV absorbance after the MTBE extraction mostly measures polymeric lignin compounds.

Furfural and hydroxymethylfurfural (HMF) were analyzed by high performance liquid chromatography (HPLC) with an UV detector at 280 nm. The column Agilent Poroshell 2.7 μm, EC-C18 120 Å (4.6 × 50 mm) was used, at an eluent flow rate of 1.2 mL/min. The injection volume was 3 μL and the used eluents were 95% water +0.5% acetic acid and 5% methanol +0.5% acetic acid. The total analysis time was 15 min.

The analysis of carboxylic/hydroxylic acids was made according to Rovio et al. [29]. Beckmann-Coulter P/ACE MDQ capillary electrophoresis (CE) equipment with a photodiode array UV/Vis detector was used in the analysis. Uncoated fused silica capillaries of 50 μm I.D. and with a length of 50/60 cm (effective length/total length) were employed in the experiments. Acids were monitored by indirect UV detection at 281 nm. The applied voltage was 30 kV. The used electrolyte solution consisted of 20 mM of 2,3-pyrazinedicarboxylic acid, 65 mM of tricine, 2 mM of $BaCl_2$, 0.5 mM of cetyl trimethylammonium bromide and 2 mM of urea.

To evaluate changes in the structure of the polymeric compounds, molar mass distributions as well as the viscosity of the wood autohydrolyzates were analyzed. The average molar mass (Mw) was determined by size-exclusion chromatography (SEC) in on-line combination with a multi-angle-laser-light-scattering (MALLS) instrument (miniDAWN, Wyatt Technology, Santa Barbara, USA, λ_0 = 690 nm) with three scattering angles of 41.5°, 90.0°, and 138.5° and a refractive index (RI) and UV detectors (Shimadzu Corporation, Japan). A two column system 2× Ultrahydrogel TM linear 7.8 mm × 300 mm + guard column was used. An 0.1 M $NaNO_3$ solution, after being filtered through a 0.1 μm Anodisc 47 membrane filter, was used as the eluent at a flow rate of 0.5 mL/min. The samples were filtered through a 0.22 μm nylon syringe filter before injection. The dn/dc value 0.15 was used [30]. The injection volume was 200 μL. Astra software (Wyatt Technology, Santa Barbara, USA) was used to interpret the data. Kinematic viscosity was measured by viscometer (COMECTA I, no. 4020, size 1, constant 0.0091131).

2.6. Calculations

Membrane fouling was calculated by comparing the difference between the pure water permeability before and after the filtration, as the following Eq. (1) shows.

$$\text{Fouling } (\%) = \frac{PWP_b - PWP_a}{PWP_b} \cdot 100\% \quad (1)$$

where PWP_b is the pure water permeability before filtration and PWP_a is the pure water permeability after filtration (kg/(m^2 h bar)).

The yield of hemicelluloses in the membrane concentrate was calculated by:

$$\text{Yield } (\%) = \frac{m_{hemi(C)}}{m_{hemi(F)}} \cdot 100\% \quad (2)$$

where $m_{hemi(C)}$ is the mass of hemicelluloses in the concentrate and $m_{hemi(F)}$ is the mass of hemicelluloses in the feed.

The purity of the hemicelluloses was defined as the ratio between the carbon content in the hemicelluloses, comprising about 40% of hemicellulose molecular mass, and the total organic carbon (TOC) in the final concentrate and was calculated by:

$$\text{Purity } (\%) = \frac{0.4 \cdot C_{hemi(C)}}{TOC_{(C)}} \cdot 100 \quad (3)$$

where $C_{hemi(C)}$ is the concentration of the hemicelluloses (e.g. GGM) and $TOC_{(C)}$ is the total organic carbon concentration in the final concentrate.

3. Results and discussions

3.1. Effect of oxidative treatment on filtration capacity and fouling

The effect of oxidative treatment on the filtration capacity is illustrated in Fig. 3. The oxidative treatment had a significant effect on the flux when the original wood autohydrolyzate was concentrated (filtration UC030 III PCD, in Fig. 1). The increase of flux was even more remarkable when the concentrate of the 30 kDa membrane (filtration UC030 I) was concentrated further after the PCD treatment (filtration UC030 II PCD). When the permeate of the 30 kDa membrane was further filtered with the 10 kDa membrane, and the concentrate of this 10 kDa filtration was treated with oxidation, the filterability of oxidized concentrate did not differ from the non-oxidized concentrate (Fig. 3b). Therefore, it can be concluded that the 30 kDa membrane sufficiently removed foulants, causing a decrease of flux through the 10 kDa hydrophilic cellulose membrane. As a result, the effect of oxidation on the flux of the hydrophilic 10 kDa cellulose membrane was negligible (Fig. 3b) if the solution was pre-filtered prior to oxidation.

As Table 1 shows the measured fouling values were low which indicates that the decrease in flux was mostly caused by other phenomena such as concentration polarization, the increase of osmotic pressure, the increase of viscosity or the formation of a gel layer on the membrane surface when the polymeric compounds were concentrated. Removal of the high molar mass compounds by pre-filtration or their partial degradation by oxidation presumably decreases the solution viscosity and the potential of the compounds to form a gel layer during concentration filtration.

The viscosity measurements proved that the oxidation significantly reduced the viscosity of the UF concentrate. When the original wood autohydrolyzate and the pre-oxidized extract were concentrated by the 30 kDa membrane to VRF 17 the kinematic viscosity coefficient in the concentrates comprised 1.71 mm^2/s and 1.36 mm^2/s, respectively. The viscosity of oxidized liquor was about 20% lower, and the flux (Fig. 3a) was about 30% higher than that of the original liquor. Therefore, the change in viscosity explained a significant part of the flux increase observed after pre-oxidation of the autohydrolyzate.

3.2. Effect of oxidative treatment on the chemical composition of autohydrolyzate

Fig. 4(a) and (b) show the effect of PCD treatment time on the quantity of parameters of the wood autohydrolyzate. Since the largest changes in the parameters were observed at 45 min of oxidation with little progress after that, the detailed analysis of autohydrolyzate was performed in the samples collected at this treatment time.

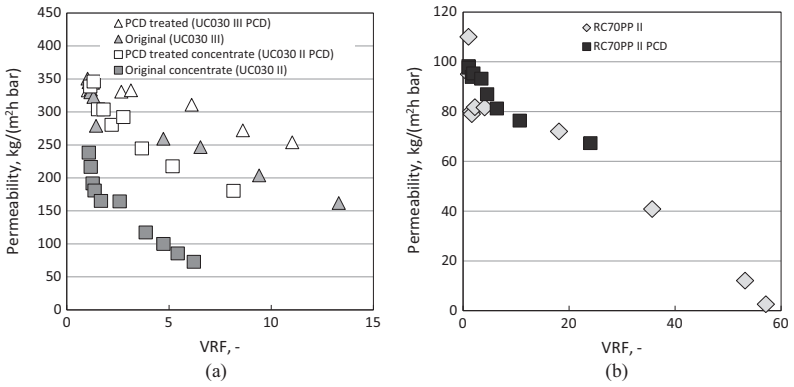

Fig. 3. Effect of PCD treatment on the permeability of (a) the UC030 (30 kDa) and (b) the RC70PP (10 kDa) membrane with the following feed solutions: UC030 membrane – original autohydrolyzate and pre-concentrated autohydrolyzate with and without the PCD treatment; RC70PP membrane – primary permeate of UC030 pre-concentrated at the RC70PP membrane with and without the PCD treatment (see Fig. 1).

Table 1
Pure water permeabilities and membrane fouling with and without oxidative pre-treatment of the autohydrolyzate.

	Feed solution	Filtration code in Fig. 1	PWP_b, g/(m² h bar)	PWP_a, kg/(m² h bar)	Fouling, %
UC030	Original extract	UC030 I	305	270	11%
	Original extract	UC030 III	314	310	1%
	Oxidized original extract	UC030 III PCD	296	298	−1%
	Concentrate from filtration I	UC030 II	308	270	12%
	Oxidized concentrate from filtration I	UC030 II PCD	307	275	10%
RC70 PP	UC030 permeate	RC70PP I	104	102	2%
	Concentrate from the filtration RC70PP I	RC70PP II	102	107	−5%
	Oxidized concentrate from the filtration RC70PP I	RC70PP II PCD	108	104	4%

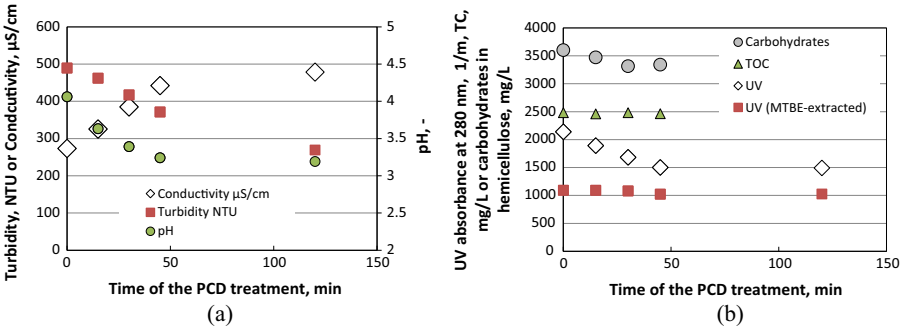

Fig. 4. Effect of the time of oxidation treatment on the composition of the wood autohydrolyzate.

The growth in electric conductivity and acidity of PCD-treated wood autohydrolyzate indicate the formation of acids common for oxidation processes [31]. The CE analysis showed the presence of acetic, formic, glycolic, oxalic and glucoisosaccharinic (GISA) acids. Table 2 shows the concentration of acids increasing with oxidation time except for the acetic acid. The GGM is partly acetylated and the acetyl groups are already liberated from hemicellulose during the hot water treatment and they form acetic acid, which decreases the pH and accelerate the hydrolysis. Therefore, the amount of acetic acid no longer increased in the oxidation. The results showed that the main organic acid formed in oxidation was formic acid, the concentration of which more than doubled from 50 to 119 mg/L. Concentrations of other identified organic acids, i.e. lactic, succinic, malic, 2,5-dihydroxypentanoic, 2-hydroxybutanoic acid and xyloisosaccharinic (XISA) acids were around 10 mg/L. The total increase in acids concentration did not exceed 140 mg/L, making the total sum after oxidation 570 mg/L, which comprised about 10% of the total organic carbon.

Table 2
Concentration of organic acids (mg/L) in the original autohydrolyzate and after different periods of the oxidation treatment.

Treatment time, min	Concentration, mg/L					
	Acetic acid	Formic acid	Glycolic acid	Oxalic acid	Gisa	Other acids
0	270	50	27	12	24	49
15	293	87	43	23	23	48
30	293	105	45	29	33	54
45	284	119	46	33	31	63

The analysis of carbohydrate composition showed the predominant character of GGMs comprising over 75% of hemicelluloses in the autohydrolyzate. Pre-oxidation caused about a 7% loss of hemicellulosic carbohydrates (Table 3). The amount of monomeric hexoses slightly increased, although their total concentration did not exceed 2% of hexoses. About 28% of the pentoses existed in monomers and their concentration slightly decreased during oxidation. The main monomeric pentose was arabinose, comprising about 70% of all the monomeric pentoses' content.

The results indicate that oxidation did not increase the amount of furfural and 4-hydroxymethylfurfural, but their concentrations remained at a very low level being less than 10 mg/L also after the treatment. Thus, it can be assumed that the minor loss in carbohydrates is mostly due their degradation to organic acids.

An interesting observation was made concerning the UV absorbance behavior in autohydrolyzate solutions and those samples treated with the MTBE extraction (Fig. 4). The PCD oxidation reduced the UV absorbance of wood autohydrolyzate samples, e.g. from an initial 2200–1500 m^{-1} in the 45 min of treatment (Fig. 4b). However, when the samples were compared after the MTBE extraction, only a minor effect of oxidation on the UV absorbance could be seen. Methyl *tert*-butyl ether is known as a solvent for lipophilic wood extractives and also for extracting phenolic compounds such as lignans, small molar mass phenols, and furan type compounds optically active at 280 nm but not polymeric lignin. Therefore, the degrading UV absorbance of the original samples, compared to the constant one of MTBE-extracted autohydrolyzate, indicate selective degradation of lignans and other small molar mass phenolic compounds in PCD-oxidation.

It seems that the oxidation intensity was not sufficient to degrade a significant amount of polymeric lignin from the autohydrolyzate. However, based on the SEC/MALLS analysis oxidation modified the lignin structure. The oxidation treatment had a noticeable influence on the signal of the UV detector by spreading the signal over time (i.e. lignin concentration at different retention times) although only a minor effect on the average molar mass was seen. The retention time of most of the UV absorbing compounds in the original wood autohydrolyzate was from 40 to 42 min – spreading from 38 to 44 min as a result of oxidation. This indicates the formation of new UV absorbing compounds the molar masses of which remained predominantly unchanged being for instance 2910 g/mol for the UV absorbing compounds in the untreated, and 2760 g/mol for the UV absorbing compounds in the pre-oxidized autohydrolyzate.

The decreased turbidity (Fig. 4a) in the PCD treatment indicates the removal of lipophilic wood extractives, which is confirmed in their detailed analysis. For instance, the lipophilic extractives and lignans in the original feed solution in the filtration UC030 III (Fig. 1) were reduced as a result of 45-min PCD-treatment from 26 and 42 mg/L to 6 and 2 mg/L, respectively. The efficient removal of wood lipophilic extractives is shown in Fig. 5. Lipophilic wood extractives are reported to foul the membranes in many studies, this fouling mostly concerns hydrophobic membranes [32]. Fouling of hydrophilic membranes in this study was not significant.

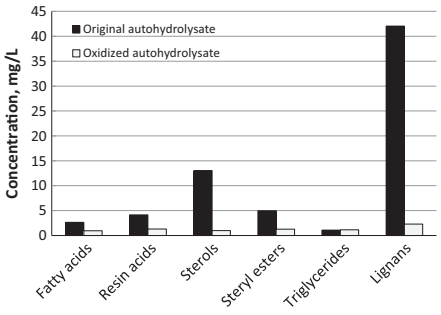

Fig. 5. Concentration of wood lipophilic extractives and lignans in the original wood autohydrolyzate and after 45 min of oxidation treatment.

Table 3
Polymeric and monomeric sugar compounds in the wood autohydrolyzate after the oxidation treatment.

Treatment time, min	Hemicelluloses, mg/L				Monomeric carbohydrates, mg/L				Sugars loss, %	
	0	15	30	45	0	15	30	45	Hemicellulose	Monomers
Arabinose	99	105	88	99	198	185	184	179	~0	10
Rhamnose	66	64	63	67	7	7	7	7	~0	~0
Xylose	559	540	519	520	73	68	67	66	7	9
∑Pentoses	724	709	670	687	277	260	258	252	5	9
Mannose	1820	1744	1672	1671	14	19	20	20	8	−39
Galactose	393	381	361	369	19	36	36	35	6	−80
Glucose	502	483	464	467	3	5	4	5	7	−87
∑Hexoses	2714	2608	2497	2507	36	59	60	60	8	−65
Glucuronic acid	15	18	17	20	1	0	0	0	−36	
Galacturonic acid	146	134	128	125	2	1	2	2	14	
Total, mg/L	3599	3470	3313	3340	316	321	320	314	7	1

Although evident changes in the composition of organic compounds were seen after oxidation, the amount of TOC remained constant (Fig. 3b). The energy delivered to oxidation was not high enough to cause the mineralization of organic compounds. This was most probably due to the high concentration of the organic compounds in the treated stream.

3.3. Purity of the high molecular mass galactoglucomannan

The possibility of degrading the impurities existing in the wood autohydrolyzate to the extent that they would not be retained together with hemicellulose in the downstream UF process and would not disturb filtration performance, made the oxidation step an attractive pretreatment option. The main impurity in the wood autohydrolyzate is lignin, which might disturb the film-forming process and cause unwanted odor and color in the hemicellulose based products. Other impurity groups are the lipophilic wood extractives consisting of different resin and fatty acids, waxes, steryl esters, and triglycerides. Their molecular masses are low compared to the recovered hemicelluloses and to the cut-off value of the membranes used. However, they also exist in colloidal form and are, therefore, partly retained by the UF membrane. The lipophilic wood extractives can form sticky deposits on process equipment, or they may create spots e.g. in films formed from hemicellulose.

As presented above, under the experimental conditions used in this study, oxidation treatment for 45 min did not degrade lignin significantly; however the oxidation modified the lignin structure. This, together with the other changes originating from the oxidation of the autohydrolyzate, lead to a situation in which the concentrated fraction produced from the oxidized wood autohydrolyzate contained less lignin compared to the

Table 4
Filtration results for original (UC030 III) and pre-oxidized (UC030 III PCD) wood autohydrolyzates at VRF = 17.

	pH –	Conductivity µS/cm	Turbidity NTU	TOC mg/L	Lignin mg/L	Lipophilic extractives, mg/L	Lignans mg/L	Pentoses mg/L	Hexoses mg/L	Sugar acids mg/L
Original feed	4.1	278	551	2555	624	26	42	623	2339	142
UC030 III permeate	4.1	277	40	1958	545	25		560	1364	116
UC030 III concentrate	4.0	325	8528	13,010	2404	38	52	1611	16,216	647
Yield at VRF 17, %		7%	93%	30%	23%	9%	7%	15%	42%	27%
Concentration factor at VRF 17		1.2	15.5	5.1	3.9	1.5	1.2	2.6	6.9	4.6
Pre-oxised feed	3.2	478	269	2548	579	6	2	595	2060	121
UC030 III PCD permeate	3.2	460	11	1990	500	5		407	1084	78
UC030 III PCD concentrate	3.2	481	3226	10,084	1888	18	2	1228	12,470	441
Yield at VRF 17, %		6%	72%	24%	20%	19%	6%	12%	36%	22%
Concentration factor at VRF 17		1.0	12.0	4.0	3.3	3.2	1.1	2.1	6.1	3.7

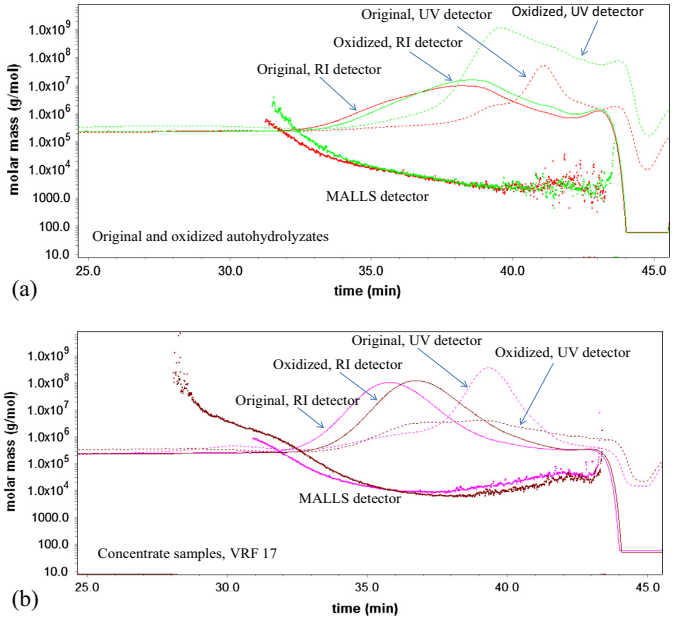

Fig. 6. SEC chromatograms (MALLS, UV and RI detectors) of (a) the original and oxidized wood autohydrolyzates and (b) their concentrates of 30 kDa membrane (number average molar mass).

concentrated fraction produced without pre-oxidation. For example, at VRF = 17 the lignin concentrations were 2.4 and 1.9 g/L in untreated and oxidized autohydrolyzates respectively. However, purity of GGM in the concentrated fractions was only slightly improved due to the pre-oxidation used. The reason for this were the minor changes the oxidation caused in the hemicellulose structures leading a to decrease in GGM retention. As Table 4 shows, the concentration of hemicelluloses (hexoses) increased 6-fold and lignin about 3-fold when a VRF of 17 was achieved. The ultrafiltration with the 30 kDa membrane (VRF 17) only slightly reduced the lignin proportion of the total carbon from 16%, in the original feed, to 11% in the final concentrate when the non-oxidized autohydrolyzate was treated. The results were rather similar to the pre-oxidized autohydrolyzate.

As already mentioned, oxidation caused a decrease in the average molecular mass of hemicelluloses and decreased the high molecular mass hemicellulose yield achieved in the ultrafiltration. For instance, the calculated weight average molar masses of hemicelluloses (RI detector in Fig. 6) were 7000 and 6300 g/mol for the original and pre-oxidized autohydrolyzates, and after the concentration to the VRF-value of 17 19,700 g/mol and 14,800 g/mol, respectively. The yield of hexose sugars in the UF was 42% and 36% with the original and pre-oxidized autohydrolyzates, respectively. UF naturally decreased the concentrate polydispersity by concentrating high molar mass compounds and permeating low molar mass compounds. The polydispersity (2.0) of the original autohydrolysate decreased to about 1.6 in the UF concentrate. The oxidation also probably had minor effect on the polydispersity of polymeric compounds, although further experiments are needed to confirm the effect.

The oxidation significantly reduced the turbidity of the autohydrolyzate and almost completely removed the lipophilic wood extractives (Figs. 4 and 5 and Table 4). Turbidity was well retained by UF membrane (concentration factor 15 in Table 4) and, therefore, its removal by oxidation before UF significantly improved the concentrate purity.

Lignans, which are hydrophilic, low molecular mass compounds, were efficiently degraded in the oxidation. Moreover, their retention in the ultrafiltration was negligible. Thus, their degradation did not improve the purity of the produced hemicellulose fraction. The oxidation treatment also lead to the formation of minor amounts of organic acids thus causing some loss of carbohydrates. The organic acids have low molar masses, and they permeate 30 and 10 kDa membranes completely. Therefore, they will not be accumulated in the membrane concentrate, and the diafiltration of concentrates can be applied if further purification is needed.

4. Conclusion

This study focused on the examination of the possibility of enhancing ultrafiltration efficiency in the recovery of GGM from spruce autohydrolyzate by applying a pre-oxidation step implemented with a pulsed corona discharge (PCD)-method. Moreover, the possibility to improve the purity of the produced high molecular mass GGM fraction with the PCD pre-oxidation was evaluated.

It was shown that although the mild oxidation used in the study was not seen to mineralize the organic compounds, oxidation still significantly improved the filtration capacity of the wood autohydrolyzate. This occurred despite the fouling of the hydrophilic cellulose membranes being very low, even without the pre-oxidation. The improvement of the capacity was partly due to the reduced viscosity of the oxidized wood autohydrolyzate. The PCD oxidation treatment removed the majority of wood lipophilic extractives and lignans from the wood autohydrolyzate, and low molar mass organic acids, mainly formic acid, were formed. The oxidation did not significantly degrade the lignin but did modify the lignin structure. Applying pre-oxidation led to a slightly lower lignin content in the produced UF concentrate, and the oxidation also resulted in some decrease in the hemicellulose molar mass and lower retention. Approximately a 10% hemicellulose loss was also measured as occurring in the pre-oxidation step. Therefore, it can be concluded that the oxidation prior to the UF significantly improved the filtration capacity, but its effect on the hemicellulose–lignin ratio was small.

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Publication III

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Selective recovery of phenolic compounds and carbohydrates from carob kibbles using water-based extraction

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Selective recovery of phenolic compounds and carbohydrates from carob kibbles using water-based extraction

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ABSTRACT

Carob kibbles are an important renewable source of valuable compounds, such as fermentable sugars and phenolic compounds. However, the selective recovery of these compounds is not a trivial task. In this work, a strategy was developed to enable the recovery of both classes of compounds by means of a water-based extraction.

One-step extraction recovered only approximately 20% of the phenolic compounds, corresponding to an extraction yield of 0.6 g Gallic acid equivalents (GAE)/100 g dry mass of carob kibbles. The obtained extract contained a significant amount of carbohydrates (110 g/L). The alternative two-step extraction developed enabled higher compound selectivity together with an increase in the yield of the phenolic compounds to about 70%, corresponding to 1.9 g_{GAE}/100 g carob dry matter.

The two-step extraction was easily scaled-up and is an effective method to obtain significantly separated carbohydrates and polyphenol-rich streams that can be further processed, e.g., in biorefineries or food industries, respectively.

1. Introduction

Agro-food industry wastes, such as olive, grape and carob residues, can be exploited as raw materials for various valuable products, and these agro-food residues have received considerable attention as an abundant and inexpensive renewable resource for chemical, biotechnological and pharmaceutical applications. Carob kibbles, in particular, are recognized as having great potential, not only due to its high content of easily fermentable sugars, but also due to its phenolic content. Carob kibbles are the result of mechanical treatment of carob pods, using a kibbler, after the high-value seeds (~10% of the carob pod weight) have been extracted for the production of locust bean gum (Albergaria et al., 1999). Currently, carob kibbles are mainly used as animal feed, despite its valuable composition. The soluble sugars in carob kibbles can reach up to 50% on a dry basis (Roseiro et al., 1991a; Petit and Pinilla, 1995; Avallone et al., 1997) and the kibbles can be used in a wide range of applications, including the production of ethanol, citric acid (Roukas, 1998), xanthan (Roseiro et al., 1991b) and mannitol (Carvalheiro et al., 2011), as well as the specialty chemical pinitol (Macias Camero and Sanjuan Merino, 2003). Several studies have drawn attention to this residue as a good source of valuable phenolic compounds (Owen et al., 2003; Makris and Kefalas, 2004).

The phenolic compounds are a group of very diverse chemicals that include e.g., phenolic acids and aldehydes, hydroxycinnamic acids and its derivatives, flavonoids, lignans, or tannins (Manach et al., 2004). Many of these compounds have been shown to present useful traits that support their use as bioactive compounds for human health. As such, the study of their selective recovery is a significant scientific and industrially applied relevant topic. Moure et al. (2001) reviewed various extraction and recovery methods of antioxidant compounds (mainly phenolic compounds) from agricultural and industrial residues, and this has been complemented by other studies, e.g., considering residues such as olive seeds and olive mill waste water (Paraskeva and Diamadopoulos, 2006; Marco et al., 2007), grape seeds (Casazza et al., 2011), potato peels (Singh and Saldaña, 2011), carob (Turhan et al., 2006), and many other biomass residues (Junior et al., 2010).

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In carob kibbles, the major phenolic compounds present are condensed tannins (Marakis, 1996), but many other phenolic compounds can be recovered by water extraction. For instances, different forms of gallic acid, including the free form and its derivatives (gallotannins and methyl gallate); catechin, flavonol glycosides, myricetin rhamnoside, eriodictyol glycoside, quercetin glycoside and quercetin rhamnoside have been described (Avallone et al., 1997; Corsi et al., 2002; Papagiannopoulos et al., 2004). As such, relevant bioactivities have been described in carob, namely antioxidant activity (Kumazawa et al., 2002; Makris and Kefalas, 2004), together with others e.g., anti-proliferative activity for special cancer lines (Roseiro et al., 2011).

Several methodologies have been used for the extraction of phenolic compounds from carob residues. Papagiannopoulos et al. (2004) found that acetone-water extraction produces a higher yield of phenolic compounds than water extraction. Owen et al. (2003) claimed that Soxhlet extraction using methanol gives higher yield and greater variety in phenolic compounds content than water batch extraction at room temperature. Aqueous acetone extraction was found to be highly efficient in the recovery of phenolic compounds from carob pods. The extraction was performed as two room-temperature water extractions to remove carbohydrates from the carob pods before subjecting the solid residues to boiling water extraction (Kumazawa et al., 2002). Recently, supercritical CO_2 extraction has also been tested for the recovery of phenolic compounds from de-sugared carob kibbles (Bernardo-Gil et al., 2011). Some of these methods have demonstrated good performance at lab-scale; however, their conversion to a larger scale is not without challenges. Water-extraction based processes can overcome some of these problems and performs well at a larger scale, in both economic and environmental terms.

The present work aims to develop a sustainable, efficient and selective water-based extraction procedure for the recovery of phenolic compounds and sugars from carob kibbles that is suitable for scale-up from lab-scale to industrial scale. Two different strategies are tested, a single-step and a two-step procedure. The fractionation should support further application of the carbohydrates and phenolic compounds independently, for example, as a biorefinery substrate or as raw materials for the food and pharmaceutical industries.

2. Materials and methods

2.1. Carob kibbles

Carob (*Ceratonia siliqua* L.) kibbles (chopped and deseeded carob pods) were obtained from a local de-seeding factory in Algarve, Portugal, and then stored in plastic containers at room temperature in a dark and dry place prior to use. The moisture content of the carob kibbles was 10–13%. Screening of the kibbles using selected sieves (Retsch, Germany) with different pore sizes and an appropriate sieve shaker (EVS1, Endecotts, England) showed that 27% was larger than 8 mm, 40% of the material was between 8 and 4 mm, 21% between 2 and 4 mm, and only 11% of the kibbles was smaller than 2 mm.

2.2. One-step extraction

Carob kibbles were subject to aqueous extraction by mixing with water at liquid-to-solid ratios (LSR) between 2 and 50, w/w. To enable optimization of the LSR, five extractions were performed in duplicate using an appropriate amount of carob kibbles (100 g, 50 g and 25 g, for higher ratios) and purified water based on the tested LSR. The extractions were carried out for 5 h in an orbital incubator (Infors Unitron HT, Switzerland) set at 50 °C.

To enhance the extraction yield, the liquid extract was separated from the remaining solids using a manual hydraulic press (Sotel, Portugal) up to 200 bar. Suspended solids in the extract were removed by centrifugation using a Heraeus Sepatech centrifuge (12,000 rpm, 10 min at 4 °C) and the liquid extract was stored in a freezer until further use.

The extraction conditions were further optimized by studying the effects of temperature (30–100 °C) and time (20–300 min), based on previous knowledge and the scale-up potential/practicality. The assays were carried out either in the orbital incubator described above or in a water bath (for temperatures >80 °C).

A Doehlert experimental design (Doehlert, 1970) consisting of 7 sets of experiments A–G (Table 1) was applied to establish the experimental conditions. All assays were done at least in duplicate to provide a measure of the inherent experimental error.

The model used to express the responses was a second-order polynomial model:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \epsilon \quad (1)$$

where Y is the response, X the independent variables, and the subscripts 1 and 2 refer to extraction temperature and time, respectively. β_0 is the regression coefficient at the center point; β_1 and β_2 are the coefficients of the variables 1 and 2 (main effects), respectively; β_{12} is the two-factor interaction coefficient between variables 1 and 2; and β_{11} and β_{22} are the quadratic coefficients for variables 1 and 2; ϵ is independent random errors, assumed to be normally and independently distributed. The linear multiple regressions to Eq. (1) and its analysis of variance (ANOVA) were carried out using the Microsoft® Excel 2010 regression tool pack. All replicates were used. The best water extraction conditions were determined using the Microsoft Excel® 2010 Solver tool based on the best-fit equation using a constrained model. Coded representation of the variables was used for all calculation purposes (X_1 = temperature; X_2 = time).

2.3. Two-step extraction

In the first step, the extraction temperature was set at 30 °C. Two different LSR (10 and 20) and extraction times (210 min and 300 min) were tested in order to achieve the highest carbohydrates yield together with the lowest total phenolics removal.

After definition of the operating conditions for the first water extraction step, the two-step water extraction was tested using 100 g and 1.2 kg of carob kibbles. After the first step, the liquid fraction was separated from the remaining solids by pressing using the hydraulic press described above. The pressed solids were recovered and sampled (1–2 g) to rapidly quantify moisture content in an automatic AMB 50 moisture balance (Adam Equipment, CT), in order to determine the amount of water needed to achieve the proper LSR for second step extraction. The optimized operation conditions from the one-step extraction experiments were used in

Table 1
Codified matrix for the Doehlert experimental design for two variables and the corresponding experimental matrix. Each row represents an experimental trial.

Variables Coded			Real	
Trial	X_1	X_2	Temperature (°C)	Time (min)
A	−1.00	0.00	30	150
B	0.00	0.00	65	150
C	1.00	0.00	100	150
D	−0.5	−0.866	47.5	20
E	−0.5	0.866	47.5	280
F	0.5	−0.866	82.5	20
G	0.5	0.866	82.5	280

the second step extraction to achieve a high phenolic compounds yield.

A pilot-scale trial was conducted using 4 kg of carob kibbles and the first step was performed using 4 batches (1 kg of kibbles for each batch). The solids from all batches were pooled as one batch and placed in the autoclave for the second extraction, i.e., the hot water extraction, as described above.

2.4. Soxhlet extraction

In order to estimate the content of total extractable carbohydrates and phenolic compounds in the carob kibbles, water Soxhlet extraction was carried out. The extraction was performed in duplicate using 4 g of carob kibbles in an initial water volume of 200 mL. The extraction lasted for 16 h with 5–6 cycles/h. The total yield obtained by the Soxhlet extraction was used to calculate the percentage of the extracted components for each assay.

2.5. Extract analysis

2.5.1. Sugar composition

Sucrose, glucose and fructose were analyzed by HPLC using a Sugar Pak-1 column (Waters, Milford, USA). The temperature of the column was maintained at 80 °C, and the carbohydrates were eluted with calcium-EDTA (50 mg/L) at a flow rate of 0.5 mL/min. The HPLC system was an Agilent 1100 Series (Waldbronn, Germany) system equipped with a RI detector (G1362A) for sugar quantification. Aliphatic acids and gallic acid were quantified using an Aminex HPX-87H column (Bio-Rad Hercules, CA, USA) operating at 50 °C. The mobile phase was 5 mM H_2SO_4 and the flow rate 0.6 mL min^{-1}. Gallic acid was quantified with UV detector based on 280 nm data. Before HPLC analysis, all samples were filtered through 0.45 μm membrane filters.

2.5.2. Total phenolics evaluation

The amount of total phenolics in the extracts was determined with the Folin–Ciocalteu method, using gallic acid as the standard (Singleton et al., 1999), and with a JASCO spectrophotometer (model V-530, Japan). Absorbance was measured at 765 nm after 15 min. incubation at 45 °C. Total phenolic compounds were expressed as g_{GAE} (gallic acid equivalents)/L by comparison to the gallic acid standard curve and converted to g_{GAE}/100 g carob kibbles on dry weight basis.

2.5.2. Phenolic profile

Capillary zone electrophoresis (CZE) was carried out in an Agilent Technologies CE system (Waldbronn, Germany) equipped with a diode array detector (DAD), which was used to obtain the phenolic profile in the extract. The CZE analysis was done based on the procedure of Roseiro et al. (2013). The separation buffer was 15 mM sodium tetraborate decahydrate in 10% MeOH (used as an organic modifier for higher separation performance and selectivity of CZE), adjusted to pH 9.3. The separation voltage and current were 30 kV (0.5 min ramp up) and 120 μA, respectively. The capillary temperature was kept at 30 °C during separations. Before analysis, the samples were filtered through 0.45 μm membrane filters. Electropherograms were taken at 220, 280, 320 and 375 nm. Between-run preconditioning of the capillary was performed by flushing with 0.1 M NaOH (3 min) followed by the buffer (3 min). To enable identification of the phenolic compounds, solutions of standard phenolic compounds were analyzed and their UV spectra stored. The identification of the phenolic compounds in the extract sample was determined by electrophoretic comparisons (migration times and UV spectra) with the authentic standards.

3. Results and discussion

3.1. Carob kibbles characterization

In carob characterization, the content of carbohydrates and phenolic compounds in carob pod extract and fiber presented in the literature varies greatly. This variation is probably a result of the different extraction procedures and methods of analysis used, although plant quality, geographical origin, climatic conditions, harvesting and storage also play a role. Examples of the reported values for total phenolic compounds in carob pods vary between 1.9 mg/g (Avallone et al., 1997) and 9.3 mg/g (Makris and Kefalas, 2004). The high content of carbohydrates (48–56%) in carob pods is well reported (Roseiro et al., 1991a; Petit and Pinilla, 1995; Avallone et al., 1997; Batlle and Tous, 1997). In the present study, the measured concentration of total phenolics was 0.7 g/L (2.8 g_{GAE}/100 g dry carob kibbles ~28 mg/g), which is significantly higher than previously reported values for carob pods in related literature. The concentration of carbohydrates (glucose, fructose and sucrose) was 13.8 g/L (54.6 g carbohydrates/100 g dry carob kibbles). The total dissolved solids concentration was 14.9 g/L, which indicates that most of the solids extracted from the kibbles were only carbohydrates and phenolic compounds, and no significant extraction of structural compounds, protein or fat occurs, as expected. As such, the residual solids will be mainly composed of structural components that can be further upgraded within the biorefinery framework, where special attention should be given to the structural phenolic compounds present.

3.2. Selection of the liquid-to-solid ratio for one-step water extraction

The liquid-to-solid ratio (LSR) is a key operating parameter in aqueous extraction. LSR ranging from 2 to 50 were tested at constant temperature and time (50 °C and 5 h, respectively). Table 2 and Fig. 1 show the yield of glucose, fructose and sucrose, and total phenolics, respectively. Generally, the yield of carbohydrates and also phenolic compounds increased as the LSR increased. This increase was more pronounced for sucrose, which is the most abundant component in carob kibbles. These results are in agreement with those of Petit and Pinilla (1995), who found that the efficiency of carbohydrates (mainly sucrose) recovery (% of sugar extracted from the total sugar content) from carob pulps increased with increasing LSR.

Although a rather high LSR was used in our study, it was not sufficient to release all the carbohydrate content. For example, at LSR of 4, ~84% of the carbohydrates and only 11% of total phenolics

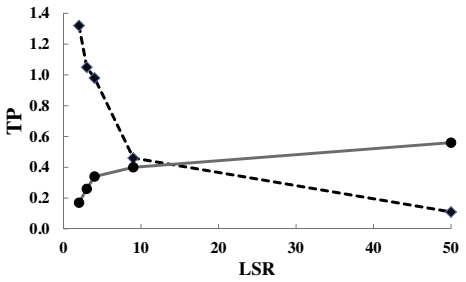

Fig. 1. Effect of LSR on the concentration of total phenolics (TP) in the liquid extract (g/L) (♦) and on extraction yield (●) (g_{GAE}/100 g carob dry weight). Extraction temperature and time were 50 °C and 5 h, respectively.

Table 2
Yields (Y) and concentrations of glucose (Glc), fructose (Fru) and sucrose (Suc) at different LSR. Extraction temperature and time were 50 °C and 5 h, respectively.

LSR	[Glc] g/L	Y_{Glc} (g_{Glc}/100 g dry mass)	[Fru] g/L	Y_{Fru} (g_{Fru}/100 g dry mass)	[Suc] g/L	Y_{Suc} (g_{Suc}/100 g dry mass)
2	65.5	8.6	40.3	5.3	93.6	12.3
3	51.8	12.7	32.2	7.9	72.2	17.7
4	38.4	13.6	24.0	8.5	66.7	23.6
9	16.4	14.5	10.3	9.1	28.7	25.4
50	2.9	14.7	1.9	9.6	5.5	27.6

[*] Coefficient of variation is below 0.5.

were recovered from the carob kibbles. At extremely high LSR of 50, the amount of carbohydrates and total phenolics extracted was 95% and 20%, respectively.

Pinelo et al. (2004) found that a higher amount of water extractable phenolic compounds was obtained from almond hulls and pine sawdust when higher LSR ~10 was applied. Casazza et al. (2011) also concluded that increase in LSR generally has a positive effect on the yield of phenolic compounds recovered from grape seeds by ethanol. In the present study, the relatively low yield of total phenolics suggests that extremely high LSR is not the key to obtain high phenolic compounds recovery. It was observed that in order to recover phenolic compounds from carob kibbles efficiently, LSR = 4 seems to be adequate (Fig. 1), as the total phenolics yield linearly increased with LSR up to value of 4. This result is broadly in line with Casazza et al. (2011), who found that LSR over 5 does not greatly improve extraction yield of total phenolics from grape seed. In our study, only a slight nonlinear increase in total phenolics yield was observed at LSR over 4. Furthermore, extremely high LSR produces very dilute liquors containing significant amounts of water that needs to be processed in further purification operations, thus hindering process economics.

3.3. Optimization of the temperature and time of one-step water extraction

Based on the LSR optimisation, an LSR value of 4 was used in the optimization of the other operating conditions of the extraction, specifically, temperature and time. A statistical experimental design was used to define the operational conditions within the studied ranges, chosen based on previous knowledge (Roseiro et al., 1991a, 2011) and the envisage viability for operation at large-scale.

The yield of glucose, fructose, sucrose and total phenolics obtained for each experimental trial is presented in Table 3. In general, the extraction temperature and time have a significant effect on the recovery of phenolic compounds. In this study, the yield of the phenolic compounds increased as the temperature (studied range: 30–100 °C) increased, as expected. Several studies on carob residues have shown the same trend (e.g., Turhan et al., 2006). At higher extraction temperatures, the plant tissue is "softened" more than at lower temperatures and, therefore, the interaction between polyphenols and protein or polysaccharides diminishes, leading to improvement in their solubility and diffusivity (Roseiro et al., 2013). The highest yield of phenolic compounds was 0.55 g_{GAE}/100 g dry carob kibbles, obtained at 100 °C for 150 min. This value was lower than the one obtained by Makris et al. (2007). They succeeded in obtaining about 1.4 g_{GAE}/100 g carob dry weight, but by using a methanol/acetone/water mixture as the extraction solvent. An 80% aqueous acetone extraction, which provided 0.93 g_{GAE}/100 g of carob pods (Makris and Kefalas, 2004), was also more efficient than the one-step water extraction used in this study. But the highest yield value obtained in this study was much higher than the 0.045 g/100 g extractable polyphenols recovered from milled carob using pressurized hot water extraction in an accelerated solvent extractor (Papagiannopoulos et al., 2004). Nevertheless, the results for phenolic compounds shown in Table 3 compare favorably to the 0.3 g_{GAE}/100 g of carob pulp obtained by supercritical extraction of carob kibbles (Bernardo-Gil et al., 2011).

The carbohydrates (glucose, fructose and sucrose) were released in different proportions depending on the operating temperature and time (Table 3). It was observed that the yield of carbohydrates increased with time, as previously reported by Roseiro et al. (1991a), where the sugar concentration reached a steady state value (200 g/L) after 6 h of aqueous extraction at 20 °C (LSR = 2). While some literature, e.g., Manso et al. (2010), has claimed no significant difference in the extraction efficiency of carbohydrates with time, our results revealed a clear relationship, albeit mainly at lower temperatures. This finding is probably due to the high portion of carbohydrates (62% of their content in kibbles) which can be already recovered at shorter time i.e., 20 min (at the same extraction temperature of 47.5 °C). However, at 30 °C a high portion of carbohydrates (76%) can already be released from the carob kibbles and only a slight increase in the yield of carbohydrates was observed above this temperature (maximum 13%). This observation was in agreement with Manso et al. (2010), who claimed that temperature increase (25–75 °C) does not greatly improve the yield of sugar extraction. The effect of extraction time and temperature on the sugar recovery has been studied by several authors with different results. Turhan et al. (2006) found that the highest sugar yield was obtained at 85 °C. Conversely, Petit and Pinilla (1995) claimed that the efficiency of sugar extraction decreases at temperatures higher than 30 °C.

3.4. Statistical analysis of experimental data of one-step water extraction

Empirical modeling of the extraction process can be a suitable tool to quantify the impact that operating parameters have on the total phenolics recovery. Furthermore, it also enables better fine-tuning of the extraction strategy. The response surface model approach was effective for finding the relation between the relevant operating conditions (namely, extraction temperature and time) and the composition of the extract. Table 4 presents the regression coefficients estimated for the polynomial model based on the experimental design data as well as the coefficient of determination (R^2) for the different responses. As an example, the 3D response surface for the total phenolics yield is given in Fig. 2.

The correlation of the composition of the carob kibbles extract with the studied variables by the proposed equation was able to give statistically significant regressions at ANOVA analysis confidence level, p value <0.05.

The linear coefficient of the temperature is statistically significant for all compounds, clearly demonstrating its relevance for recovery performance. This coefficient is significant for carbohydrates, especially for the most abundant (sucrose), and reveals that the yield of sucrose increased significantly with temperature. The temperature quadratic effect is statistically significant for total phenolics, showing that higher temperatures will not continuously increase the yield of phenolics. The coefficient for this effect was positive for glucose, meaning that the recovery of glucose slightly increased at high temperature. There was no effect for the temperature quadratic coefficient on the yields of fructose and sucrose.

Table 3
Yield (*Y*) of glucose (Glc), fructose (Fru), sucrose (Suc) and total phenolics (TP) obtained at the LSR of 4 and at different temperature and time conditions based on the Doehlert experimental design. (Coefficient of variation is 0.5 for sugars and 0.04 for total phenolics).

Trial	Temperature (°C)	Time (min)	Y_{Glc} (g_{Glc}/100 g dry mass)	Y_{Fru} (g_{Fru}/100 g dry mass)	Y_{Suc} (g_{Suc}/100 g dry mass)	Y_{TP} (g_{GAE}/100 g dry mass)
A	30	150	12.2	6.9	22.3	0.25
B	65	150	12.7	7.4	25.5	0.45
C	100	150	13.8	8.1	26.4	0.55
D	47.5	20	9.7	6.7	17.3	0.22
E	47.5	280	12.9	7.3	22.5	0.33
F	82.5	20	11.0	7.0	23.6	0.47
G	82.5	280	13.2	7.8	25.9	0.52

Table 4
Estimated regression coefficients for the diverse responses analyzed.

	b_0	b_1	b_2	b_{12}	b_{11}	b_{22}	R^2
Total phenolics	**1.25 ± 0.04 (0.00)**	**0.35 ± 0.02 (0.00)**	**0.24 ± 0.02 (0.00)**	**0.11 ± 0.05 (0.05)**	**−0.17 ± 0.05 (0.01)**	**−0.37 ± 0.05 (0.00)**	0.98
Sucrose	**68.79 ± 2.30 (0.00)**	**7.06 ± 1.30 (0.00)**	**5.54 ± 1.30 (0.00)**	**−6.17 ± 2.70 (0.05)**	-3.86 ± 2.80 (0.21)	**−7.19 ± 2.80 (0.03)**	0.88
Glucose	**34.21 ± 0.01 (0.00)**	**1.57 ± 0.00 (0.00)**	**4.08 ± 0.00 (0.00)**	**−2.30 ± 0.01 (0.00)**	**0.46 ± 0.01 (0.00)**	**−2.11 ± 0.01 (0.00)**	0.99
Fructose	**19.93 ± 0.16 (0.00)**	**1.09 ± 0.10 (0.00)**	**0.95 ± 0.01 (0.00)**	−0.05 ± 0.19 (0.75)	0.05 ± 0.20 (0.82)	0.28 ± 0.20 (0.82)	0.97

Data is presented as "Coefficient ± standard error (*p*-value)". Coefficients statistically significant at *p* value = < 0.05 are in bold.

This trend might be due to their high solubility in water even at room temperature.

Extraction time is statistically significant and positively influenced the recovery of total phenolics and all carbohydrates. The value of the time effect coefficient was much higher for carbohydrates, which might be due to the ease of sugar extraction and their high content in carob kibbles compared to the phenolic compounds content. The extraction time quadratic coefficient was statistically significant for phenolic compounds, glucose and sucrose recovery, which shows that for the studied range, longer extraction times will not be beneficial. The interaction coefficient for extraction temperature and time is negative and statistically significant for glucose and especially sucrose, indicating that high extraction temperatures and long operating times negatively affects their recovery. In the studied range, combining high temperature and time had a positive effect on the extraction of phenolic compounds, but not on fructose. The relative values of β_1 and β_2 showed that the effect of extraction temperature on recovery of all components is higher than the effect of time, except for glucose. The extraction temperature was considered the key parameter for phenolic compounds extraction from carob kibbles also in a previous study (Roseiro et al., 2013).

Based on the above discussion, it can be seen that the use of the response surface model approach was effective for identifying and quantifying the effect of the main water extraction operating parameters. The proposed model and its estimated coefficients were thus applied to determine the optimal operational conditions for the recovery of phenolic compounds that were found to be 100 °C and 220 min (at LSR = 4). In order to further validate the model an experimental assay was carried out at the prescribed conditions, and the total phenolic yield was 0.6 g, TP/100 g kibbles, and the corresponding carbohydrates yield was 49.1 g, sugar/100 g kibbles, both in close agreement with the predicted by the model.

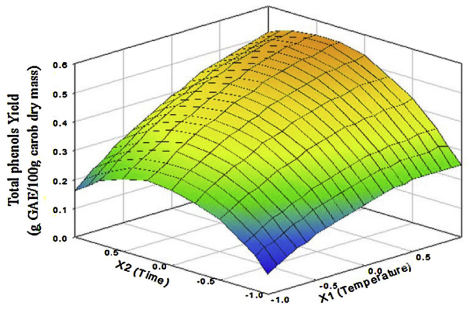

Fig. 2. Response surface for the yield of total phenolics as a function of coded extraction temperature (X_1, real studied range: 30–100 °C) and time(X_2, real studied range: 20–300 min).

3.5. Identification of phenolic compounds in one-step water extracts

The identification of individual phenolics by capillary electrophoresis (Fig. 3) showed that gallic acid is the main component present in the extracts obtained at 30 °C with a matching of over 99.9% with authentic standard. Gallic acid was also detected in extracts obtained at temperatures lower than 82.5 °C, even for longer extraction time. At 100 °C, the temperature at which the highest total phenolics content was obtained, catechin derivatives were also identified: namely, (−)-epigallo catechin gallate (EGCG), (−)-gallocatechin gallate (GCG) and (−)-epicatechin (EC), with a matching of 97% for catechins derivatives and 99.6% for gallic acid. The same components have been previously identified by Corsi et al. (2002), who analyzed an infusion of carob pods with boiling water, and more recently, in the aqueous decoction of carob kibbles (Roseiro et al., 2013). Electropherograms of the other operating conditions (data not shown) showed that gallic acid could be detected in all conditions, regardless of the extraction time; while catechin derivatives could be identified only at higher temperatures, i.e., 82.5 °C and 100 °C. The amount and type of the identified components clearly vary with the extraction temperature and extraction time. It can be concluded that gallic acid and catechin derivatives (which are also the major catechins found in green tea, recognised as one of the most effective antioxidants (Ananingsih et al., 2011)) are the major phenolic compounds found in the aqueous extracts of carob kibbles.

3.6. Two-step water extraction

Based on the results described above, a two-step extraction strategy was developed, aiming to obtain most of the carbohydrate fraction in the first step and recover phenolic compounds in the second. In order to avoid phenolic compounds losses during the separation of the carbohydrates, the temperature was set to 30 °C in the first extraction. The use of an extraction

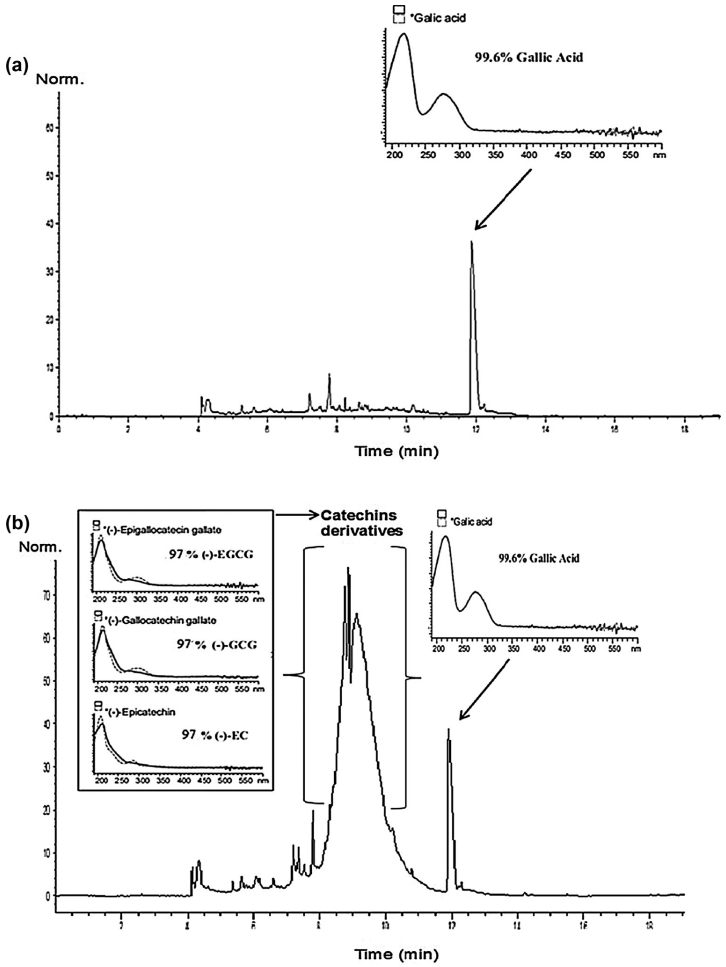

Fig. 3. Electropherogram at 280 nm showing the phenolic compounds profile of the carob kibbles extracts obtained at (a) 30 °C and (b) 100 °C for 150 min and LSR = 4. Matching % was obtained by comparison with authentic standards run at the same conditions as the sample.

temperature close to room temperature for carbohydrates extraction helps to avoid significant solubilization of phenolic compounds, which can cause undesirable effects when these carbohydrates are used for fermentation processes (Petit and Pinilla, 1995). Water extraction at 30 °C for 150 min (as shown in Table 3) was able to release a high amount of carbohydrates (76% of extractable sugars) with a relatively low amount of phenolic compounds corresponding to the low molar mass

Table 5
Yield (*Y*) of glucose (Glc), fructose (Fru), sucrose (Suc) and total phenolics (TP) at different conditions of the first extraction step. (Coefficient of variation is 0.5 for sugar and 0.04 for total phenolics).

Trial conditions (Temperature, LSR, time)	Y_{Glc} (g_{Glc}/100 g dry mass)	Y_{Fru} (g_{Fru}/100 g dry mass)	Y_{Suc} (g_{Suc}/100 g dry mass)	Y_{TP} (g_{GAE}/100 g dry mass)
30 °C, 10:1, 210 min	11.4	7.6	19.1	0.3
30 °C, 10:1, 300 min	13.3	9.0	21.7	0.4
30 °C, 20:1, 210 min	10.5	7.2	20.5	0.3
30 °C, 20:1, 300 min	10.5	7.2	21.9	0.4

Table 6
Yield of glucose (Glc), fructose (Fru), sucrose (suc) and total phenolics (TP) produced by two steps extraction. (Coefficient of variation is 0.5 for sugars and 0.04 for total phenolics).

Step #	Trial conditions (Temperature, LSR, time)	Y_{Glc} (g_{Glc}/100 g dry mass)	Y_{Fru} (g_{Fru}/100 g dry mass)	Y_{Suc} (g_{Suc}/100 g dry mass)	Y_{TP} (g_{GAE}/100 g dry mass)
1[a]	30 °C, 10:1, 300 min	10.7	7.1	22.8	0.4
2	100 °C, 4:1, 220 min	1.6	1.1	3.7	1.9
1[b]	30 °C, 10:1, 300 min	11.5	8.1	23.8	0.3
2	100 °C, 4:1, 220 min	1.6	1.1	3.8	1.9

[a] Carob kibbles were 100 g and 1.2 kg.
[b] Carob kibbles were 1.2 kg.

of Gallic acid (0.3 g/100 g dry mass). Water extraction around room temperature has been suggested by Roseiro et al. (1991a) for recovery of carbohydrates without high loss of phenolic compounds. Evaluation of the trial conditions (Table 5) showed that the highest effective separation of carbohydrates was obtained at LSR = 10 and 300 min extraction time, but there was no statistically significant difference in the carbohydrate yield when the LSR was doubled from 10 to 20 (p-value = 0.062358). A longer extraction time seems to allow a higher carbohydrate extraction, but this is also not statistically significant. A LSR of 10 was proposed by Manso et al. (2010) for isolation of sugar (at 25 °C for 1 h) from milled carob, releasing about 94% of the sugar (corresponding to 47 g/L). Manso et al. (2010) found that at LSR of 20 no beneficial yield effect was observed. In this study, water extraction at 30 °C for various extraction time and LSR gave low yields of total phenolics in all trials (Table 5), which further suggests that extraction temperature might have the predominant effect on the phenolic extraction.

The two-step extraction was performed with different solid loads. The results (Table 6) show that most of the carbohydrates and a low amount of total phenolics were extracted in the first extraction step. In contrast, the second extraction yielded a much lower amount of carbohydrates and a higher amount of phenolic compounds. Multistage sugar extraction from carob pods was tested by Roseiro et al. (1991a), who produced liquors containing 200 g/L sugar, corresponding to about 60% yield. Roseiro et al. (1991a) also presented that a steady sugar concentration with a fully diffusion-limited system at various LSR (from 0.67 to 8) was reached after 6 h of water extraction processing of carob kibbles. Control of the solid particle size, for example in the grinding of the carob kibbles, was suggested as a way to increase the total phenolics yield by weakening the carob fibers and increasing the contact area with water (Petit and Pinilla, 1995). Results in Table 6 indicate that with the two-step extraction, most of the carbohydrates (81%) can be removed in the first step with quite low yield of phenolic compounds (~0.4 g_{GAE}/100 g dry mass).

The second extraction step produced a stream enriched in phenolic compounds with 1.9 g_{GAE}/100 g dry mass (~70% of the total phenolics in the tested carob kibbles) and lower sugar presence in the extract. This phenolics extraction yield is comparable with the results of a three-step solvent extraction using 70% acetone, which gave as high as 1.95 g_{GAE}/100 g (Avallone et al., 1997) but is higher than the results of Makris and Kefalas (2004) and Makris et al. (2007). It can be concluded that the two-step extraction using water as an environmental friendly solvent that is proposed in this study offers an effective alternative to organic solvents for recovery of phenolic compounds from carob kibbles. The capillary zone electrophoresis identification (Fig. 4) of the phenolic compounds in the second extraction, i.e., the hot water extraction, indicated extraction of catechin derivatives; namely, (−)-epigallo catechin gallate (EGCG), (−)-gallocatechin gallate (GCG) and (−)-epicatechin (EC)) and gallic acid, as expected.

3.7. Pilot-scale two-step water extraction

The pilot-scale extraction was performed using 4 kg of carob kibbles. The yield of the phenolic compound in the first extraction step

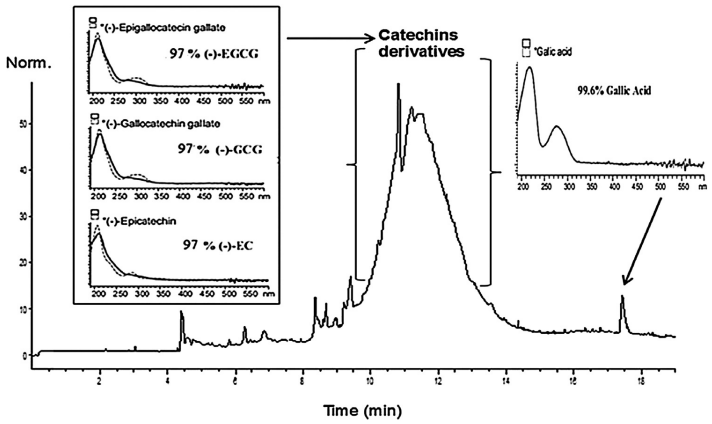

Fig. 4. Electropherogram at 280 nm showing the phenolic compounds profile of the carob kibbles extracts produced from the second, hot water extraction (100 °C, LSR = 4, 220 min). Matching % was obtained by comparison with authentic standards run at the same conditions as the sample.

was 0.5 g_{GAE}/100 g dry mass. Lower yield of phenolic compounds (0.9 g_{GAE}/100 g dry mass, corresponding to 32%) was observed in the second extraction step at pilot-scale compared to the lab-scale trial, probably due to a lack of mixing in the autoclave, which might have led to a decrease in the mass transfer of phenolic compounds to liquid phase with time (saturation concentration limit). In this trial, most of the carbohydrates (88%) could be removed in the first step, where the yield of glucose, fructose and sucrose was 12.8 g Glc/100 g dry mass, 11.1 g Fru/100 g dry mass and 24 g Suc/100 g dry mass, respectively. In the second step, the yield of glucose, fructose and sucrose was 2.2 g Glc/100 g dry mass, 1.8 g Fru/100 g dry mass and 2.5 g Suc/100 g dry mass, respectively. As a result, almost total removal of carbohydrates was achieved with the two steps in this pilot-scale trial.

4. Conclusions

The selective recovery of phenolic compounds and sugars from carob kibbles is not trivial. In this study, the highest yield of phenolic compounds in one-step extraction at optimised conditions (LSR = 4, 100 °C for 220 min) was 0.6 g_{GAE}/100 g dry carob kibbles, i.e., 20% of the total phenolics content. Two-step extraction showed improved phenolic content yield and improved separation of the carbohydrates from the phenolic compounds. Furthermore, it was easily scaled-up.

The present study shows that water extraction at mild operating conditions offers a sustainable, selective process for the recovery of sugars and phenolic compounds from carob residues.

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Publication IV

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Nanofiltration and reverse osmosis as a platform for production of natural botanic extracts: The case study of carob by-products

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ABSTRACT

Carob kibbles are a low-cost and renewable source of economically relevant phenolic compounds (high value catechin and its derivatives and gallic acid) and abundant in small sugars. This work aims at producing two distinct natural extracts from carob kibbles, one extract enriched in catechin and its derivatives for the nutraceuticals market and an extract enriched in sugars for the food industry. This valorisation strategy involves an integrated process based on membrane technology that fulfils the zero discharge principle and may be applied to other agro-industrial by-products. Different aqueous extraction schemes were considered (a one-step process and a two-steps approach). The aqueous extracts obtained were fractionated by diananofiltration and the fractions obtained were evaluated in terms of their content in target products. An integrated scheme for production of fractionated extracts is proposed based on the experimental work developed assuring, simultaneously, a minimal use of resources and emission of waste.

1. Introduction

Carob by-products from the carob seed gum industry (carob kibbles), have a high content in marketable sugars and phenolic compounds, but are currently only used in low value applications such as animal feed and regional confectionary. The development of a valorisation strategy for carob kibbles should take into consideration these two classes of compounds. Besides the high concentration of mono- and disaccharides (glucose, fructose and sucrose), several studies reported that carob kibbles have a high variety of antioxidant phenolic compounds comparable to olive residues [1]. According to Avallone et al. [2] and Corsi et al. [3], water extractable carob phenolic compounds are mainly gallic acid and also (−)-catechin and its derivatives: (−)-epigallocatechin, (−)-epicatechin gallate, and (−)-epigallocatechin gallate, also present in green tea. Furthermore, carob derived phenolic compounds have also been shown to present anti-tumural activities [4,5].

The valorisation strategy of carob kibbles should involve an effective separation between (more valuable) phenolic compounds and sugars, increasing the economic value of their crude extracts. The production of an extract from carob kibbles enriched in catechins and its derivatives is very attractive for the profitable nutraceuticals market. Additionally, the production of a natural extract enriched in mono- and disaccharides has also economical interest to the food industry in general. Nevertheless, this fractionation is a demanding task because sugars are much more abundant than phenolic compounds in carob kibbles extracts and the target compounds have similar molecular mass.

The release and recovery of phenolic compounds from carob kibbles was carried out by extraction with water, a common biocompatible solvent [3,6]. In our previous work [7], a one-step aqueous extraction from carob kibbles was optimised aiming the recovery of phenolic compounds. However, the extract obtained presented a high concentration in mono- and disaccharides and gallic acid. Therefore, a sequential two-step extraction process was developed in order to obtain two different fractions: a first extract enriched in sugars and gallic acid with a reduced concentration in phenolic

Abbreviations: c_i, concentration of compound i; D, number of diafiltration volumes; GAE, gallic acid equivalents; J_v, volumetric flux; L_p, permeability; MWCO, molecular weight cut-off; NF, nanofiltration; p, pressure; R_i, apparent rejection of compound i; RO, reverse osmosis; VRF, volume reduction factor; V_w, volume of solvent added to the retentate; subscript perm, permeate.

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compounds, and a second extract enriched in higher molecular mass phenolics, almost free of sugars and gallic acid. Actually, this process made possible the production of a first step extract enriched in sugars, free of valuable phenolic compounds, despite a minor contamination in gallic acid. A second step extract, enriched in valuable phenolic compounds was also obtained, with a minor contamination in gallic acid, but still with a significant presence of sugars (although less contaminated in sugars than the extract produced with the one-step extraction protocol).

This work discusses the process developed for further improving the separation between valuable phenolic compounds from sugars and gallic acid. In order to separate the target phenolic compounds from sugars and gallic acid (which have lower molecular mass) it was decided to select a highly retentive membrane for the phenolic compounds. The strategy was to retain as much as possible the high molecular mass species. This approach allows for obtaining permeates essentially free of phenolic compounds and assure the quantitative retention of these valuable target compounds. Additionally, if economically relevant, the permeating species may be further processed in order to concentrate them, using reverse osmosis.

Once the highly retentive membrane was selected, two different operating approaches could be followed: fractionation by nanofiltration, namely using a cascade approach suggested by Refs. [8,9]; or a diananofiltration using water as a washing solvent, where sugars and gallic acid are soluble, being removed through the permeate. Both approaches could be considered, but in this work it was decided to use a diananofiltration approach because the feed stream is highly viscous due to the elevated concentration of sugars. Diananofiltration with addition of fresh washing solvent allows for operation under relatively mild and controlled viscosity conditions, enabling for operation under suitable external mass transfer conditions, with advantage over nanofiltration. When this restriction imposed by the viscosity of the feed stream is not relevant, the use of nanofiltration cascades with recycle of the retentates should be taken into consideration [8,9].

Diananofiltration is commonly used for the removal of genotoxins from active pharmaceutical ingredients [10]. Several examples of diananofiltration in agro-industrial applications are also reported in literature, namely in the partial removal of the monovalent salts from oligosaccharides in a model solution of soybean [11] and from high value protein and lactose in a cottage cheese whey [12], or to remove mainly aliphatic acids and furfural derivatives from sugars (xylose, arabinose and glucose) in olive pomace aqueous extracts [13]. Nevertheless, the use of diananofiltration for the recovery/purification of valuable phenolic compounds has only been reported, to our knowledge, in the removal of pesticides and recovery of bioactive steryl esters from deodorizer distillates [14,15].

This work aims for recovering, fractionating and purifying valuable phenolic compounds (catechin and its derivatives) from small sugars (glucose, fructose and sucrose) present in carob by-products. The valorisation strategy was defined with the objective of producing one purified extract rich in catechin and its derivatives for the profitable nutraceuticals market and one purified extract rich in small sugars for the food industry, increasing the overall value of carob extracts. This strategy involved the development of an integrated process based on membrane technology that fulfils the zero discharge principle. The approach followed may be easily extended to other agro-industrial by-products.

2. Experimental

2.1. Materials

Carob (*Ceratonia siliqua* L.) kibbles (chopped and deseeded carob pods) were obtained from a local de-seeding factory (Algarve, Portugal), and then stored in plastic containers at room temperature in a dark and dry place prior to use. Screening of the kibbles using selected sieves (Retsch, Germany) with different pore sizes and an appropriate sieve shaker (EVS1, Endecotts, England) showed that 27% was larger than 8 mm, 40% of the material was between 8 and 4 mm, 21% between 2 and 4 mm, and only 11% of the kibbles was smaller than 2 mm. Aqueous extracts from carob kibbles were produced as described previously [7]. The membranes used in this work are described in Table 1, with specifications obtained from the producers. Desal 5 DK, which is a hydrophilic nanofiltration membrane, was chosen in order to enable the permeation of the hydrophilic sugars and gallic acid. A membrane with a higher molecular mass cut-off was not chosen in order to assure a total rejection of hydrophobic catechin (molecular mass of 290.3 Da) and its derivatives with molecular mass between 400 and 500 Da. The membrane SW30, a reverse osmosis membrane, was chosen in order to assure a total rejection of mono- and disaccharides (glucose and fructose with a molecular mass of 180 Da and sucrose 342 Da).

2.2. Membrane processing

The membrane experiments performed are summarised in Table 2. The one-step extract was centrifuged (12,000 rpm, 10 min at 4 °C) and the 2nd extract of the two-steps extraction process was pre-filtered with a 20 μm fabric filter for 15 min. In order to exhibit a viscosity value similar to water at 20 °C (1 mPa s) and hence enabling membrane processing, the one-step extract was diluted with water at a mass dilution ratio of 3.9 and the extracts used in both diafiltration processing (see Table 2), were heated at 50 °C. High temperature and relatively low transmembrane pressure values were chosen during diananofiltration experiments, in order to assure the lowest possible apparent rejection values of gallic acid and sugars and, consequently, enhance their separation from catechin and its derivatives.

The one-step experiment was carried out with the membrane unit operated in a dead-end mode with a gas control unit (METCell, Membrane Extraction Technology, UK) at a rotor speed of 300 rpm, using a Desal 5DK flat sheet membrane. The temperature of the retentate was maintained constant by a controlled temperature bath. The 2nd extract of a two-steps extraction process was operated in a cross-flow mode. The membrane modules used had a spiral wound configuration with dimensions 2.5″ diameter and 40″ length: a Desal 5 DK 2540 C1076 nanofiltration module and a SW30 2540 reverse osmosis module. The temperature of the retentate was maintained constant using a heat exchanger.

In each membrane experiment (diananofiltration, nanofiltration and reverse osmosis experiments), the initial and the final feed solution, cumulative permeate samples (permeate accumulated up to a defined time, t) and "instantaneous" permeate samples collected along time (instant permeate samples collected at time "t") were characterised in terms of content in sugars (glucose, fructose, sucrose), in total phenolic compounds and in gallic acid (see below). The apparent rejections of total phenolic compounds TP, glucose, fructose, sucrose, and gallic acid, R_i (%), were calculated during the diananofiltration experiments through Eq. (1):

$$R_i = 1 - \frac{c_{i,perm}}{c_{i,feed}} \quad (1)$$

where $c_{i,perm}$ (g/L) and $c_{i,feed}$ (g/L) are respectively the (instant not accumulated) concentration of compound i under study in the permeate and the feed (retentate) compartments.

During diafiltration, the volume of water added to the retentate, V_w (L), is the volume required to maintain the retentate volume at a constant value since the beginning of the experiment, V_{feed} (L). The number of diafiltration volumes, D (–), is the ratio between the

Table 1
Characteristics of the membranes studied in this work.

Membrane	Type of membrane	Producer	MWCO (Da)	Membrane material	Maximum operating temperature (°C)	Maximum operating pressure (bar)
Desal 5 DK	Nanofiltration	GE Osmonics	150–300[a]	PA-TFC	50	30[c]
SW30	Reverse osmosis	Dow Chemicals	[b]	PA-TFC	45	69

PA-TFC, polyamide thin-film composite membrane.
[a] For uncharged organic molecules.
[b] 99.4% salt rejection.
[c] At a temperature higher than 35 °C.

Table 2
Scheme of the membrane experiments performed in this work.

Extract	Mode of operation	T (°C)	Δp (bar)	Membrane model	Membrane area (m^2)
Extract from one-step process	Nanofiltration operated in diafiltration mode	50	12	Desal 5 DK	51.4×10^{-4}
2nd extract of two-steps process	Nanofiltration (NF) operated in diafiltration mode followed by NF concentration	50	8	Desal 5 DK 2540 C1076	2.6
1st extract of two-steps process	Reverse osmosis in a concentration mode	25	30	SW30 2540	2.8

volume of washing solvent added (water in this study) and the initial volume of feed solution to be processed. Samples of the retentate and of the cumulative permeate were taken whenever the permeation of one volume V_{feed} was reached, in order to establish mass balances. The dianafiltration experiment performed with the one-step extract was completed when 1 diafiltration volume was reached. The dianafiltration experiment performed with the two-step extract was completed when 3 diafiltration volumes were reached.

When the system is operated in a diafiltration mode, the concentration of compound i under study in the feed (retentate) compartment, may be obtained from the following mass balance on the compound i in the feed vessel, as in [16,17]:

$$\begin{aligned} &\frac{d(V_{feed}\cdot c_{i,feed})}{dt} = -J_v \cdot A_{memb} \cdot c_{i,perm} \\ &c_{i,feed} \cdot \frac{d\,V_{feed}}{dt} + V_{feed} \cdot \frac{d\,c_{i,feed}}{dt} = -J_v \cdot A_{memb} \cdot c_{i,perm} \end{aligned} \quad (2)$$

Since the feed volume V_{feed} (L) and the apparent rejection of compound i, R_i (%), (calculated through Eq. (1)) remain constant, Eq. (2) can be expressed as:

$$\begin{aligned} &\int_{t_0}^{t} dt = -\int_{c_{i,feed}(t=0)}^{c_{i,feed}(t=t)} \frac{V_{feed}}{J_v \cdot A_{memb} \cdot c_{i,feed} \cdot (1-R_i)} dc_{feed,i} \\ &\int_{t_0}^{t} dt = -\frac{V_{feed}}{J_v \cdot A_{memb} \cdot (1-R_i)} \int_{c_{i,feed}(t=0)}^{c_{i,feed}(t=t)} \frac{1}{c_{i,feed}} dc_{feed,i} \end{aligned} \quad (3)$$

After integration, Eq. (3) can be expressed as:

$$t - t_0 = -\frac{V_{feed}}{J_v \cdot A_{memb} \cdot (1-R_i)} \cdot \ln\left(\frac{c_{feed}(t)}{c_{feed}(t=0)}\right) \quad (4)$$

The volume of the permeate produced up to time t, V_P (L), equals to the volume of solvent (water) added to the retentate, V_w (L), may be calculated through Eq. (5):

$$V_P = J_v \cdot A_{memb} \cdot (t - t_0) \quad (5)$$

Combining Eqs. (4) and (5), the concentration values of each compound i in the feed compartment (retentate), $c_{i,feed}$ (g/L), are then estimated through Eq. (6):

$$\begin{aligned} &c_{i,feed}(t) = c_{i,feed}(t=0) \cdot \exp\left[\frac{-V_w \cdot (1-R_i)}{V_{feed}}\right] \\ &c_{i,feed}(t) = c_{i,feed}(t=0) \cdot \exp\left[-D \cdot (1-R_i)\right] \end{aligned} \quad (6)$$

2.3. Analysis methods

2.3.1. HPLC analysis

Sucrose, glucose and fructose were analysed by HPLC (Agilent Technologies Liquid Chromatographer 1100 Series System, Waldbronn, Germany) using a Sugar Pak-1 column (Waters, Milford, USA). The column was maintained at 80 °C, and the sugars were eluted with calcium-EDTA 50 mg/L at a flow rate of 0.5 ml/min. The HPLC system was equipped with a RI detector (G1362A) for sugar quantification. Organic acids, mainly gallic acid, were quantified using an Aminex HPX-87H column (Bio-Rad, Hercules, CA, USA) operated at 50 °C. The mobile phase was 5 mM H_2SO_4 and the flow rate 0.6 ml/min. Gallic acid was quantified with UV detector based on the absorbance at 280 nm. Before HPLC analysis all feed, permeate and concentrate samples were filtered through 0.45 μm filters.

2.3.2. Quantification of total phenolic compounds

The amount of total phenolic compounds in all collected filtration samples was determined based on the Folin–Ciocalteu method using gallic acid as standard and a Jasco spectrophotometer (Jasco V-530, Japan). 100 μL of each sample was mixed with 5 ml of the 1/10 (v/v) diluted Folin–Ciocalteu reagent and 4 ml of 7.5% Na2CO3. Absorbance was measured at 765 nm after 15 min of incubation at 45 °C. Total phenolic compounds were expressed as g GAE (gallic acid equivalents)/L by comparison with a calibration curve for gallic acid.

2.3.3. Analysis by capillary zone electrophoresis

Capillary zone electrophoresis (CZE) (Agilent Technologies CE system, Waldbronn, Germany) equipped with a diode array detector (DAD) was used to obtain the phenolic profile and identify various types of phenolic compounds in the feed, permeate and concentrate samples. The CZE analysis was done based on the procedure of Roseiro et al. [4]. The separation buffer was 15 mM sodium tetraborate decahydrate in 10% methanol (used as organic modifier for higher separation performance and selectivity of CZE), adjusted to a pH of 9.3. The separation voltage and current were 30 kV (0.5 min ramp up) and 120 μA, respectively. The capillary temperature was kept at 30 °C during separations. ChemStation data software and a fused-silica uncoated internal diameter 50 μm and 62/56 cm effective length, extended light path capillary also from Agilent was used. Before analysis the samples were filtered through a 0.45 μm membrane. Electropherograms measurements were taken at 220, 280, 320 and 375 nm. The pre-conditioning of capillary between runs was performed using 3 min flushing firstly with 0.1 M NaOH then with buffer. For identification, solutions containing known phenolic compounds were also analysed as standard where their UV spectra were stored to establish a library of UV spectra of the standard compounds. The identification of phenolic compounds was determined by

electrophoretic comparisons (migration times) and UV spectra with the authentic standards.

3. Results and discussion

3.1. Non-fractionated extracts

The one-step water extract from carob kibbles, optimised by Almanasrah et al. [7] for the recovery of phenolic compounds (at a liquid to solid mass ratio of 4, 100 °C and during 220 min), had a dark appearance. Due to the presence of a high concentration of sugars, the extract presented a high viscosity. In order to enable membrane processing and avoid low volumetric fluxes, the extract was diluted with water at a mass dilution ratio of 3.9 and heated at 50 °C to lower viscosity (the value of viscosity chosen was 1 mPa s, the water viscosity value at 20 °C). The characterisation of the extract after dilution is shown in Table 3. The phenolic compounds present, identified through capillary zone electrophoresis (CZE) analysis by Almanasrah et al. [7], were the high value catechin (molecular mass of 290.3 Da) and catechin derivatives, namely epi-catechin gallate (molecular mass of 442.37 Da), (−)-epigallo catechin gallate EGCG (molecular mass of 458.4 Da), (−)-gallocatechin gallate GCG (molecular mass of 458.4 Da), (−)-epicatechin EC (molecular mass of 290.3 Da) and quercetin (molecular mass of 302.2 Da), also present in green tea extracts.

The two-steps aqueous extractions from carob kibbles were also optimised in a previous work [7]. The first step extract, optimised for the recovery of sugars (at a liquid to solid ratio of 10, 30 °C and during 300 min) had a light brown syrup appearance and was concentrated by reverse osmosis (RO). The second step extract, produced from first step solid residues, was optimised for the recovery of phenolic compounds with the same operating conditions used for the one-step extraction, and then was processed by diananofiltration. The final retentate of diananofiltration process was further concentrated by nanofiltration, using the same membrane and set-up. The characterisation of both extracts is presented in Table 4. Their viscosities under the operating conditions selected were close to 1 mPa s, the water viscosity at 20 °C. Therefore, no dilution of extracts was necessary before membrane processing.

Table 3
Characterisation of the one-step water extract of carob kibbles after dilution with water (mass dilution ratio of 3.9).

Parameter	
Temperature of extraction, °C	100
Total phenolic compounds, g/L	1.13
Gallic acid, g/L	0.08
Total sugars, g/L	27.5
Glucose, g/L	9.1
Fructose, g/L	7.9
Sucrose, g/L	10.5
Viscosity at 50 °C, mPa s	1.01
pH	4.8

3.2. Fractionation of extracts by diananofiltration

Diananofiltration was used to enable the removal of sugars and gallic acid from higher molecular catechin and its derivatives. Firstly, the one step extract optimised by Almanasrah et al. [7] for the recovery of phenolic compounds was processed by diananofiltration. Secondly, due to the high concentration of sugars in the one step extract, the second extract of the two-steps extraction process, enriched in phenolic compounds, was also processed by diananofiltration.

3.2.1. Fractionation of the one step carob extract by diananofiltration

The diluted one step extract (dilution ratio of 3.9) enriched in phenolic compounds (1.13 g/L, see Table 3), was processed by diananofiltration at 50 °C. The most abundant individual phenolic compound present and the only one to be quantified individually was gallic acid (molecular mass of 170.1 Da) [7]. Moreover, the extract was highly "contaminated" with small sugar molecules (glucose, fructose and sucrose), much more concentrated than the phenolic compounds, which represents a challenge for the purification of the high value phenolic compounds.

The use of diananofiltration was assessed in order to remove the contamination of sugars and gallic acid from the high value phenolic compounds. Particularly, diananofiltration aimed for obtaining a fraction of extract retained by the membrane (retentate) with maximised concentrations of high value larger phenolics (corresponding to high rejection of these compounds) and a fraction of extract permeating the membrane, enriched in sugars and gallic acid (corresponding to low rejection of these compounds).

Fig. 1 shows the ratio of the volumetric flux, J_v, to transmembrane pressure, $J_v/\Delta p$ (L/m^2 h)/(bar), and the apparent membrane permeability corrected for the osmotic pressure effect, L_p (L/m^2 h)/(bar), as a function of the number of diafiltration volumes, D (–). The corrected permeability values were obtained from Darcy's law:

$$J_v = L_p \cdot (\Delta p - \Delta\pi) \quad (7)$$

where $\Delta\pi$ (bar) is the osmotic pressure difference between the retentate and the permeate streams, which was estimated in this work through the van't Hoff equation.

Fig. 1 shows the parameter $J_v/\Delta p$ (L/m^2 h)/(bar), besides the apparent corrected permeability, L_p (L/h m^2 bar), in order to highlight that the effect of the osmotic pressure on the observed permeabilities was not negligible. The osmotic pressure difference

Table 4
Characterisation of the first and second steps extracts from a two-steps extraction before processing, respectively, by reverse osmosis and diananofiltration.

Parameter	1st step extract (feed solution for reverse osmosis concentration)	2nd step extract (feed solution for diananofiltration)
Temperature of extraction, °C	30	100
Total phenolic compounds, g/L	0.4	1.9
Gallic acid, g/L	0.14	0.05
Total sugars, g/L	41.2	15.6
Glucose, g/L	14.7	4.7
Fructose, g/L	11.9	4.4
Sucrose, g/L	14.6	6.5
Viscosity, mPa s	1.01[a]	1.10[b]
pH	4.95	4.75

[a] At 30 °C.
[b] At 50 °C.

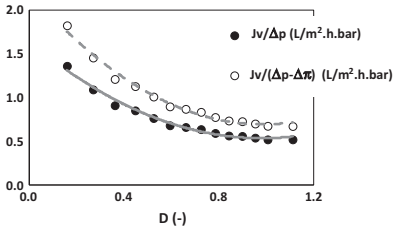


Fig. 1. Diananofiltration experiment at 12 bar and 50 °C using Desal 5DK membrane with the diluted one step carob extract as feed solution. $J_v/\Delta p$ and the apparent corrected permeability, $L_p = J_v/(\Delta p - \Delta\pi)$ (L/m^2 h)/(bar), plotted against the diafiltration volume, D (–).

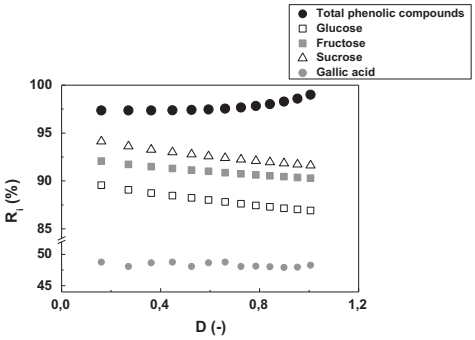


Fig. 2. Diananofiltration experiment at lab scale at 12 bar and 50 °C using a Desal 5DK membrane with the diluted one step carob extract as feed solution. Apparent rejections of total phenolic compounds (calculated in equivalents of gallic acid, GAE), glucose, fructose, sucrose and gallic acid, R_i (%), plotted against the diafiltration volume, D (–).

between the retentate and the permeate streams, $\Delta\pi$, decreased only around 10%, following the decrease of total concentration of solutes in the retentate compartment (contrarily to what happens when membrane processing is accomplished in the concentration mode). Therefore, the effect of the osmotic pressure difference remained almost unchanged during the experiment. The decrease of the apparent corrected permeabilities, which is not explained by the effect of the osmotic pressure, may be explained by an effect of mass resistance in the feed boundary layer at the membrane surface. Namely, the transport of solutes in the feed boundary layer towards the membrane was most probably not efficient enough to compensate the transport of solutes across the membrane, probably due to fouling effects. This mass transfer resistance effect stabilised along the experiment, evidenced by the stabilised values reached in Fig. 1.

The apparent rejections of total phenolic compounds TP, glucose, fructose, sucrose, and gallic acid, R_i (%), were plotted as function of the diafiltration volume, D (–), as shown in Fig. 2.

The mass balance of each compound under study closed within less than 5%.

The increase of the rejection of total phenolic compounds along the experiment may be partly explained by the permeation of gallic acid, which is more relevant in the beginning of the experiment due to its low value of apparent rejections, see Fig. 2. Gallic acid was the only individual phenolic compound present being able to be quantified individually.

The experimental and estimated concentration values of each compound i in the feed compartment (retentate), $c_{i,feed}$ (g/L), during the diananofiltration experiment were plotted as function of the diafiltration volume, D (–), as shown in Fig. 3.

In this work, the apparent rejection of each compound, considered for the estimation of the retentate concentrations, was the average value of the experimental rejections obtained for each compound (shown in Fig. 3), since these values did not varied more than 2% along the experiment.

Figs. 2 and 3 show that the high value, larger molecular mass phenolic compounds (catechin and its derivatives) may be recovered almost completely, due to the fact the total phenolic compounds were almost totally rejected (with an apparent rejection value of 97.5%) and the low value gallic acid permeated the membrane (with an apparent rejection factor of 48.3%). As such, an effective separation of catechin and its derivatives from gallic acid may indeed be accomplished by diananofiltration. According to the model, nearly 80% of the gallic acid is removed at a diafiltration volume of 3 and near 100% of the gallic acid is removed at a diafiltration volume of 6. However, the apparent rejections of the sugars were still too high. An effective separation of catechin and its derivatives from sugars would only be possible at very high values of diafiltration volumes which, combined with the dilution of the extract for enabling the diafiltration processing, would turn the process very expensive for the high quantity of water involved. Moreover, very high values of diafiltration volumes would lead to a significant loss of the high value phenolic compounds. According to the model, at a diafiltration volume of 30, 97%, 94% and 90% of respectively glucose, fructose and sucrose are removed but with a loss of total phenolic compounds of 53%, as shown in Fig. 3.

3.2.2. Fractionation of the second step extract by diananofiltration

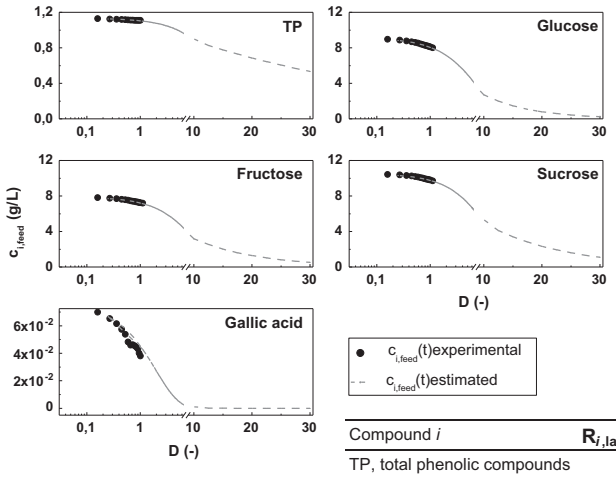
Diananofiltration was used to process the second step extract enriched in phenolic compounds (1.9 g/L) at 50 °C (see Table 4). This extract presents a minor contamination in gallic acid (0.05 g/L) but still an important concentration in sugars (15.6 g/L), although lower than obtained with the one step extract procedure (27.5 g/L in sugars after dilution). The objective of diananofiltration was the same as previously discussed in Section 3.2.1.

Fig. 4 shows the ratio of the volumetric flux to transmembranar pressure, $J_v/\Delta p$ (L/m^2 h)/(bar), and the apparent corrected permeability, L_p (L/h m^2 bar), as a function of the diafiltration volume, D (–).

Similarly to the diafiltration of the one step extract, the effect of the osmotic pressure on the observed permeabilities (Fig. 4) is not negligible but during all the experiment this effect remained almost constant ($\Delta\pi$ decreased around 6% during this diafiltration process, less than previously observed for the one step extract). The trend of the apparent corrected permeabilities along the experiment was also similar to the behaviour previously observed and may be explained by fouling effects.

The apparent rejections of total phenolic compounds, glucose, fructose and sucrose, R_i (%), were plotted as function of the diafiltration volume, D (–), as shown in Fig. 5. The mass balance for each compound under study closes with differences lower than 7%. Gallic acid was very much diluted in the feed solution (0.05 g/L, see Table 4) and could not be quantified neither in the retentate nor in the permeate, representing a minor contamination.

The experimental and estimated concentration values of each compound i in the feed compartment (retentate), $c_{i,feed}$ (g/L), during the diananofiltration experiment are plotted as function of the diafiltration volume, D (–), as shown in Fig. 6. For estimation of concentrations in the retentate, the apparent rejections for the total phenolic compounds and sucrose were considered 100%, the



Compound *i*	$R_{i,lab}$ (%)
TP, total phenolic compounds	97.5
Glucose	87.8
Fructose	90.9
Sucrose	92.5
Gallic acid	48.3

Fig. 3. Diananofiltration experiment at lab scale at 12 bar and 50 °C using a Desal 5DK membrane with the diluted one step carob extract as feed solution. Retentate concentrations of total phenolic compounds TP (calculated in equivalents of gallic acid, GAE), glucose, fructose, sucrose and gallic acid, $c_{i,feed}$ (t) (g/L), plotted against the diafiltration volume, D (–). Dashed lines are independently estimated values (not fittings) obtained from Eq. (6).

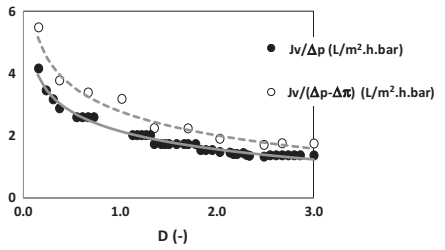


Fig. 4. Diananofiltration experiment at 8 bar and 50 °C using Desal 5DK membrane with the second step carob extract (of a two-steps extraction process) as feed solution. $J_v/\Delta p$ and the apparent corrected permeability, $L_p = J_v/(\Delta p - \Delta\pi)$ (L/h m^2)/(bar), plotted against the diafiltration volume, D (–).

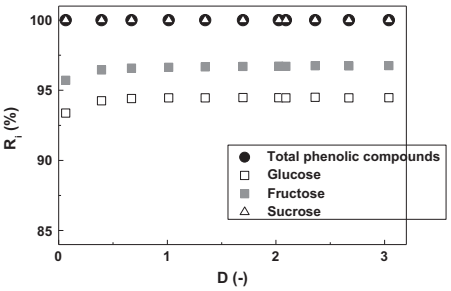
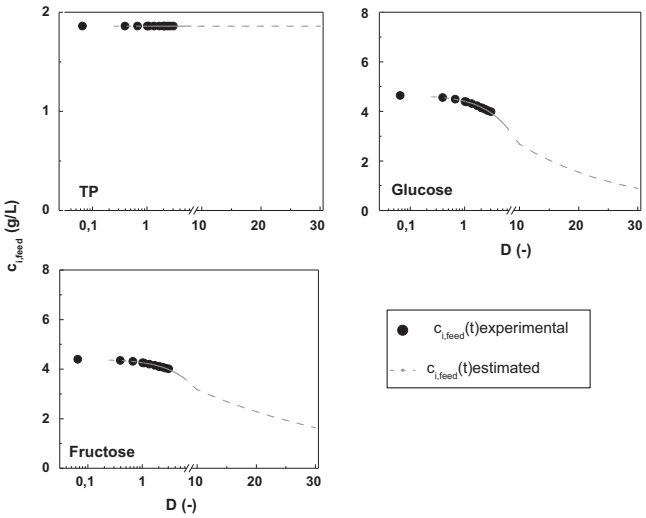


Fig. 5. Diananofiltration experiment at 8 bar and 50 °C using a Desal 5DK membrane with the second step carob extract as feed solution. Apparent rejections for total phenolic compounds (calculated in equivalents of gallic acid, GAE), glucose, fructose and sucrose, R_i (%), are plotted against the diafiltration volume, D (–).

experimental constant value for these compounds, and the apparent rejections for glucose and fructose were considered as the average values of the experimental rejections for each compound, since these values varied less than 0.5% along the experiment, see Fig. 6.

In terms of the quality of the extract enriched in valuable phenolic compounds, the second step extract presents advantages over the one step extract. The second step extract was less contaminated in gallic acid (0.05 g/L) than the one step extract (0.08 g/L), becoming a minor contamination after processing by diananofiltration. Additionally, it was less contaminated by sugars (15.6 g/L) than the one step extract (27.5 g/L after dilution). The diafiltration of the second step extract has also advantages over the diafiltration of the one step extract. Firstly, the one step extract required a 3.9 dilution before membrane processing due to its high viscosity, so diafiltration had to process 3.9× more extract and consequently required 3.9× more membrane area, which represents an important increase of cost (the second step extract did not require any dilution). Regarding the valuable phenolic compounds, their yield of recovery in the second step extract diafiltration was higher (see Almanasrah et al. [7]) and they were totally recovered (retained by the membrane), with an apparent rejection of 100% independently of the diafiltration volume used (the apparent rejection of phenolic compounds with the one step extract was 97.5%). Glucose and fructose may be completely removed if a sufficiently high number of diafiltration volumes is used. Sucrose was not possible to remove (permeate) during diafiltration of the second step



Compound *i*	$R_{i(pilot)}$ (%)
TP, total phenolic compounds	100
Glucose	94.5
Fructose	96.7
Sucrose	100

Fig. 6. Diananofiltration experiments at 8 bar and 50 °C using a Desal 5DK membrane with the second step carob extract as feed solution. Retentate concentrations of total phenolic compounds (calculated in equivalents of gallic acid, GAE), glucose, fructose and sucrose, $c_{i,feed}$ (t) (g/L), plotted against the diafiltration volume, D (–).

extract but it may easily be hydrolysed to monomeric sugars and removed by diafiltration.

In terms of the quality of the extract enriched in sugars (first extract of the two steps-process), the two-steps extraction presents significant advantages over diafiltration of the one step extract. The first step extract of the two-steps extraction process was optimised for the recovery of sugars, achieving an interesting yield of recovery (total sugars concentration of 41.2 g/L), free of valuable catechin and its derivatives, although with a contamination of gallic acid (0.14 g/L). Diafiltration of the one step extract does not allow for separating sugars from gallic acid, catechin and its derivatives, as shown in Fig. 3.

3.3. Proposed integrated process for the production of extracts

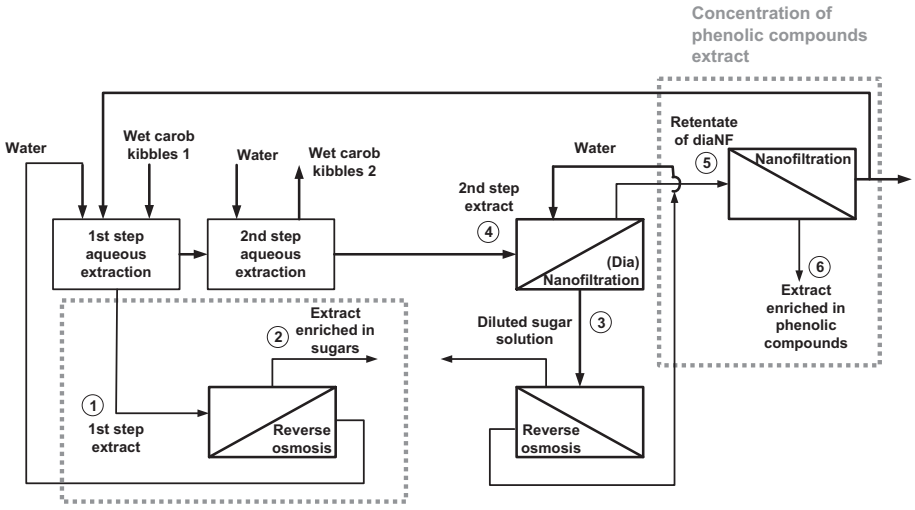
The scheme to produce extracts from carob kibbles, obtaining one extract enriched in high value phenolic compounds (catechin and its derivatives), separated from one extract enriched in sugars, is proposed in Fig. 7. Additionally to the production of extracts and diananofiltration of the extract enriched in high value phenolic compounds, a final step for concentration of the extracts is also proposed. The chemical characterisation of the streams of the process is also shown in Fig. 7.

As the permeate of the diananofiltration is very diluted (stream 3), this stream may be processed by reverse osmosis in order to fulfil the zero discharge principle as shown in Fig. 7. The first step extract was concentrated by reverse osmosis and the final retentate of the diananofiltration was processed by nanofiltration in the concentration mode, resulting respectively to streams 2 and 6 of Fig. 7, which volumetric fluxes are shown in Fig. 8.

In the concentration of the first step extract, all compounds under studied were concentrated by a factor of 4 (corresponding to apparent rejections of 100%), as it was experimentally confirmed. The concentration of the (high value) phenolic compounds (retentate of diananofiltration) was carried out by nanofiltration with advantages over the use of a reverse osmosis membrane. In particular, the contaminants glucose and fructose were partially removed and the fluxes were higher, see Fig. 8.

4. Conclusions

This work proposes an integrated process for obtaining two distinct natural extracts from carob kibbles, one concentrated in sugars (glucose, fructose and sucrose) with a minimal content in phenolic compounds, to be used in the food industry, and another purified in high value phenolic compounds (catechin and its derivatives) with a reduced concentration of gallic acid and monomeric sugars, to be used as a nutraceutical ingredient. The separation of sugars from phenolic compounds was quite challenging because sugars are present in much higher concentration than



Streams	1	2	3	4	5	6
[TP] (g/L)	0.40	**1.59**	0.00	1.89	1.86	**10.90**
[Glucose] (g/L)	14.7	**58.6**	0.8	4.7	3.9	**19.2**
[Fructose] (g/L)	11.9	**47.3**	0.5	4.4	3.9	**20.6**
[Sucrose] (g/L)	14.6	**58.1**	0.0(2)	6.5	6.5	**36.6**
[Gallic acid] (g/L)	0.14	**0.56**	(*)	0.05	(*)	(*)

Fig. 7. Flow-chart of the production of extract enriched in high value phenolic compounds (stream 6) and of extract enriched in sugars (stream 2) from carob kibbles and their chemical characterisation. (*) Below detection.

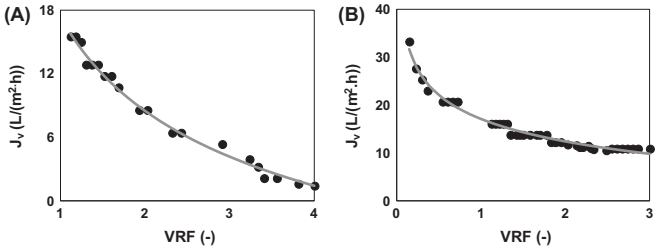


Fig. 8. Concentration of the carob extracts. (A) Reverse osmosis experiment at 30 bar and 30 °C using a SW30 membrane with the first step extract as feed solution (stream 1 from Fig. 7). Volumetric fluxes J_v (L/h m^2) plotted against the volume reduction factor, VRF (–). (B) Nanofiltration experiment at 8 bar and 50 °C using a Desal 5DK membrane with the final retentate of the dianafiltration experiment extract as feed solution (stream 5 from Fig. 7). Volumetric fluxes J_v (L/h m^2) are plotted against the volume reduction factor, VRF (–).

phenolic compounds and all compounds under study have similar molecular mass. The overall process also proposes the use of water as a biocompatible extraction solvent and it fulfils the zero discharge principle.

The obtained extract enriched in sugars was free of catechin and its derivatives, which is advantageous, and had a minor gallic acid contamination. This extract represents a concentrate of the extract enriched in sugars obtained in a previous work [7]. In the obtained extract enriched in high value phenolic compounds, all the target compounds were fully recovered, as desired. The contamination by sugars may be significantly removed by dianafiltration, as it was proven in this work, and the contamination by gallic acid was eliminated.

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