Separation and Extraction of Proteins and Polysaccharides from the Seaweed *Palmaria Palmata* using Enzyme Digestion

by

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PREFACE

This master thesis was possible thanks to Matís, a food and biotechnology R&D institute located in Reykjavik, Iceland. It commenced 1st of February 2016 and ended with a presentation held the 27/4 2017. The purpose of the project was to find a method of enzymatic extraction of polysaccharides and proteins in *Palmaria palmata* (*P. palmata)* a red seaweed. The project focuses mainly on extraction using proteases, which catalyse the hydrolysis of proteins, and xylanase, that catalyse the hydrolysis of xylan. Xylan is the main polysaccharide in *P. palmata.* The findings intend to be beneficial and useful within the human food and animal feed industry. These new methods and ways to utilize this large ocean resource may lead to more sustainable and environmental solutions compared to already existing terrestrial options such as soy and other grains.

This project would not have been possible without my supervisors at Matís, I would like to thank my supervisor Björn Viðar Aðalbjörnsson for support, help and guidance through the project. Moreover, I would like to show gratitude to Rósa Jónsdóttir for her support and encourage in the project. In addition, a special thanks to my new friend and coworker Málfríður Bjarnadóttir. Furthermore, I would like to thank my colleagues, friends and new acquaintances on Matís for your valuable knowledge, helpful guidance in the lab and around the facilities.

I would also like to show gratitude to my supervisor Johan Svensson Bonde and my examiner Leif Bülow at Lunds Tekniska Högskola for being supportive and helping me out in the initial and final phase of the project.

Finally, I would also like to thank my family and friends for always being there for me and support me in what I do, including my work with this project. Your presence and support are invaluable!

Anna Nilsson

ABSTRACT

Seaweed has a great potential within human food and animal feed industry. *Palmaria palmata* (*P. palmata*) or more commonly dulse, is a type of red seaweed which has a high protein content (8-35%), rich in minerals such as iodine and iron and contain high levels of dietary fibers. The main polysaccharide in dulse is xylan. It has been suggested that the xylans are linked to the proteins in the seaweed. This may decrease the accessibility and the digestion of the proteins present in dulse. This paper intends to find and optimize methods for extraction of the proteins and separation of the proteins and polysaccharides in dulse. The methods used to treat dulse, includes protease hydrolysis, hydrolyse of xylan with xylanase. The analytical methods to analyze the nutritional content includes SDS-PAGE, Bradford assay, phenol-sulfuric acid method, TLC and HPEAC-PAD. Hydrolysis with proteases showed limited success, only a small increase in protein content (total 46.6%) was found when hydrolysing with Umamizyme. Hydrolysis with xylanase showed greater success with a protein concentration of 53.4%. Hydrolyse with xylanase showed best potential when separating polysaccharides from proteins and extracting proteins in dulse. Further optimization of this method could generate valuable knowledge which can be utilized within human food and animal feed industries.

ABSTRAKT

Inom matindustrin och djurfoderindustrin finns det stor potential för användning av tång som näringsrikt födoämne. *Palmaria palmata* (*P. Palmata*), eller i vardagligt tal, dulse är ett rött sjögräs med högt proteininnehåll (8-35%), som innehåller många mineraler som t.ex. jod och är rikt på fiber. Den vanligaste polysackariden i dulse är xylan. Vetenskapliga artiklar föreslår att xylan är delvis bundet till proteinerna i dulse. Detta kan leda till minskad tillgänglighet av proteinerna och leda till svårigheter att smälta dessa i tarmen. Denna rapport har för avsikt att hitta och optimera metoder för extraktion av proteiner och metoder för att separera de proteiner och polysackarider som förekommer i dulse. Metoderna som används är hydrolys med proteaser och hydrolys av xylan med xylanase. De analytiska metoderna som används för att utvärdera näringsinnehållet i proverna är SDS-PAGE, Bradfordmetoden, fenol-svavelsyrametoden, TLC och HPEAC-PAD. Hydrolys med proteaser hade begränsad framgång, endast en liten ökning i proteininnehållet i provet erhölls (totalt 46.6%) när hydrolys med Umamizyme utfördes. Hydrolys med xylanase visade sig ha bäst potential med en proteinkoncentration på 53.4% i provet. Vidare optimering av denna metod, kan bidra med värdefull kunskap inom mat- och foderindustrin.

ABBREVIATIONS

Dulse – *Palmaria palmata*, a type of red seaweed
PROMAC – Energy efficient Processing of Macroalgae in blue-green value chains
HPLC – High performance liquid chromatography
BSA – Bovine serum albumin
SDS-PAGE – Sodium dodecyl sulfate polyacrylamide gel electrophoresis
PUFA – Polyunsaturated fatty acids
EPA – Eicosapentaenoic acid
Dw – Dry weight
TLC – Thin-layer chromatography
HPEAC-PAD – High-Performance Anion-Exchange Chromatography with Pulsed Amperometric detection
AMO186 – Clone with endo-1,4-beta-xylanase
AMO190 – Clone with xylanase
XylLg-A – Clone with endo-1,4-beta-xylanase
Xyl125 – Clone with endo-1,4-beta-xylanase
IPTG – Isopropyl β-D-1-thiogalactopyranoside
OD – Optical density
α-PNPX – 4-nitrophenyl-α-D-xylopyranoside
FMS – Free monosugar sample
NPN – Non-protein nitrogen

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1. INTRODUCTION

1.1. AIM

Development of convenient and easy accessible methods for separation and extraction of proteins and polysaccharides of the seaweed *P. palmata* for human food and animal feed applications is the main scope of the project.

1.2. PROMAC PROJECT BACKGROUND

This master thesis is a part of a Norwegian project called PROMAC (Energy efficient PROcessing of MACroalgae in blue-green value chains) which extends between 2015 and 2018. The project investigates three species of seaweed, with good potential for commercial cultivation in Norway, and evaluate its potential for human food and domestic animal feed applications. Since the global food and feed demands are increasing with the increasing population and living standard, there is a requisite to find new sustainable and climate friendly approaches to satisfy this increasing demand of food and feed. Cultivation of seaweed is expected to have a great potential within this field. The PROMAC project comprises six work packages; raw materials and chemical composition, product and processes -direct applications, refined products -processes and applications, nutritional and health values of macroalgae products, excess energy from industrial processes, system Life Cycle Analysis and Value Chain Modelling. This master thesis will be a part of the third work package "refined products –processes and applications" and focus on the seaweed species *P. palmata* also referred to as dulse. (PROMAC 2016)

1.3. MATÍS – FOOD AND BIOTECH R&D

Matís is a food and biotechnology research and development institute located in Reykjavik in Iceland, founded in 2007. The company focuses in research in the food and biotechnology area as well as analytical testing service for public and private authorities. Matís cooperates with several partners around the world and takes part in many international projects regarding food and biotechnology, among many, the PROMAC project. Matís is a government owned company, which by research and development, intends to improve food production and processing. The company also works to ascertain the quality and safety in food and feed production. (Matís 2016)

2. BACKGROUND

2.1. PALMARIA PALMATA

2.1.1. General facts *P. palmata*

P. palmata, commonly known as dulse is a red seaweed (phylum: Rhodophyta) which grows in the intertidal part of the coast, it grows down to a maximum depth of 20 meters. It is widely spread in the northern part of the Atlantic Sea and Pacific Ocean. The discoid shaped base of *P. palmata* grows as epiphytes on other algae species or attached to rocks, mussels etc. The branched leaves have a deep red color and- are approximately 50 cm long and 3-8 cm wide with a leathery texture. The life circle of dulse consists of two stages, an early sexual phase, the gametophyte, and a later asexual phase, the tetrasporophyte. The reproduction season for *P. palmata* reaches from November/December to March/April. (Dring 2011) Figure 1 shows the appearance of dulse.

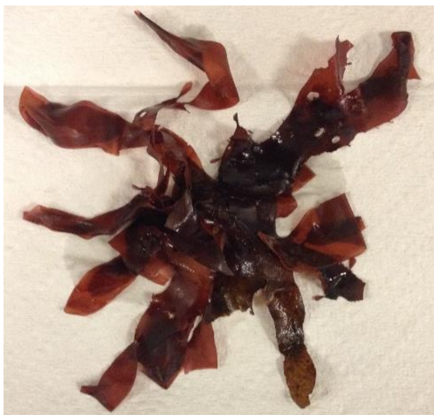

Figure 1. Thawed P. palmata sample used in the project.

2.1.2. Nutritional value of *P. palmata*

There is a seasonal difference of the nutritional content in dulse. The composition of nutrients may also vary depending on other factors such as the location where it is harvested, growth condition etc. Different analyze methods used by different sources may also have an impact on the various results regarding the nutritional content. (Morgan, Wright et al. 1980) The approximate nutritional content of dulse obtained from different studies are presented below.

Fibers/Polysaccharides:

The cell wall of *P. palmata* is composed of β-(1→4)/ β-(1→3)-linked xylan together with is β-(1→4) xylan and fibrillary cellulose, unlike other red seaweed which have galactans in their cell wall structure. Xylan is an approximately linear polysaccharide consisting of xylose units. The structure of the mixed linked xylan seems to have a repetitive pattern of four 1,4-linkages and one 1,3-linkage. A study made by Deniaud, Quemener et al., showed that the xylan in the cell wall of dulse is partly acidic,

contains slight amount of sulfate and phosphate groups, which may be associated with bonding of sulfated/phosphorylated xylogalactoprotein complexes. The mixed linked xylans seem to be attached to cell wall with H-bonds. The xylans acts as a barrier, thus decrease the accessibility of the proteins in the cell wall. (Deniaud, Quemener et al. 2003)

Lahaye, Rondeau-Mouro et al. found that the amount of xylan in whole dry alga is 34.4% of dry weight, where 19.4% of the xylan consists of 1→3 linkages and 80.6% of 1→4 linkages which confirms the pentameric pattern of the 1→4 and 1→3-linkages. Further results from this study indicates that the mixed linked xylan is both loosely and tightly attached to the cell wall. It is likely tightly attached by H-bonds as previously mentioned, which is regulated by the occurrence of 1→3 linkages and presence of water which allows a helical conformation. A small amount of short 1→4 linked xylans are also present and may be a part of the mixed-linked xylan, an own separate fibrillar network or associated with cellulose. The exact function of the fraction of 1→4 is still unknown. (Lahaye, Rondeau-Mouro et al. 2003)

Several studies have evaluated the carbohydrate and sugar content in dulse. In a study made by Jiao, Yu et al. the polysaccharide content obtained in dulse extracts, ranged between 23.25% and 68.81%. The major part of these polysaccharides, were xylan. (Jiao, Yu et al. 2012) The sugar and fiber content in samples of *P. palmata* was determined by Jard, Marfaing et al. using reverse-phase HPLC of hydrolyzed samples and enzymatic-gravimetric method. The amount of sugars was determined to be 36.9% of the total solids and the fiber content was set to 22.5% of the total solids. The amount of xylose was 23.3% of the total solids. (Jard, Marfaing et al. 2013) According to a study made by Hagen Rødde, Vårum et al. the amount of xylan in dulse varied between 24-35% of the dry weight. The amount of free sugars, xylose and galactose were low. (Hagen Rødde, Vårum et al. 2004)

Protein content:
P. palmata is a seaweed with relatively high protein content. Thus, it may be a potential candidate as protein source in human diet. The protein content of *P. palmata,* collected each month for a year during 1996 at Belle Ile on French Brittany coast, was measured and analyzed. The highest protein content was displayed in the winter-spring period (21.9 ± 3.5%) whereas the lowest protein content was shown in summer-early autumn (11.9 ± 2.0%). (Galland-Irmouli, Fleurence et al. 1999) According to Morgan et al. the protein content in dulse vary between 8-35%, the amount protein vary with season, location where it is harvested and growth conditions. (Morgan, Wright et al. 1980)

The usefulness of the protein source does not only depend on the amount of protein present, it also depends on other features such as the digestibility of the protein and the content of essential amino acids. Galland-Irmouli et al. compared the digestibility of *P. palmata* and of pork casein, the results showed that the digestibility of dulse was

significantly lower than the one of casein. The digestibility of bovine serum albumin (BSA), alone and associated with seaweed extract, using bovine trypsin, bovine chymotrypsin, pronase or human intestinal juice was performed and evaluated with SDS-PAGE. The result showed less digestibility when associated with the seaweed. The reduced digestibility may be a cause of inhibiting effects by trypsin inhibitors, phenolic compounds and/or fibers. Since *P. palmata* has a high fiber content, this may be a large factor for the reduced digestibility of proteins. Polysaccharides may interact with the proteins and reduce the availability of the proteins to degrading agents. The impact of fibers, such as polysaccharides, on protein digestibility is a field which requires further investigation. (Galland-Irmouli, Fleurence et al. 1999) There may be an interest in the discovery of methods for separation of proteins from polysaccharides in dulse. Removal of polysaccharides from proteins may increase the digestibility of the proteins which may be beneficial when applied in the food and feed industry. (R. Cian, S Drago et al. 2015)

Three factors are of importance when evaluating the nutritional value of the amino acid content of the seaweed. They are the amino acid balance, the relative content of essential amino acids (compared with egg protein) and the ratio of essential amino acids. *P. palmata* turned out promising in all three aspects. The essential amino acids hold 26-50% of the total amount of amino acids and the essential amino acid content in *P. palmata* corresponds well with the essential amino acid content in egg protein. Evaluation of the amino acid content in dulse, the occurrence and the amount, showed a high amount of aspartic acid and glycine and poor amount of methionine, hydroxyproline, proline and histidine. Cysteine was not detected at all. The more acidic amino acids were dominating. (Galland-Irmouli, Fleurence et al. 1999)

Lipid content:

According to Morgan et al., the lipid content in *P. palmata* varied slightly between time and the location where it was harvested. The amount was between 0.3-3.8 % of the dry weight. (Morgan, Wright et al. 1980) In another study of the lipid content in seaweed, the amount present in *P. palmata* was 1.57 % of the dry weight. The seaweed was collected in French Brittany coast in December 1991. *P. palmata* has a relative high amount of the beneficial polyunsaturated fatty acids (PUFAs) especially the omega-3 eicosapentaenoic acid (EPA). (Fleurence, Gutbier et al. 1994) A recent study made by Mæhre et al. shows a lipid content of 1.28-1.38% of the dry weight of dulse collected in Voldsfjorden in Norway during May and June in 2012. The method used was dichloromethane/methanol extraction. In general the lipid content of seaweeds are low, but the relative amount of beneficial omega-3 fatty acids are high in red seaweed including *P. palmata.*(Mæhre, Malde et al. 2014)

Ash and minerals/trace elements:

The ash content in freeze dried dulse was experimentally determined by Mæhre, Malde et al. to be 420 g/kg of freeze-dried dulse. The ash content represents the approximate mineral content in the seaweed. In general, seaweed has a high content of

iodine, the amount in dulse is 260 mg/kg dry weight (dw.) *P. palmata* also inhabits a relative high amount of selenium 0.14 mg/kg dw. Selenium is considered to have antioxidant effects, since it is a part of the glutathione peroxidases, which is a class of antioxidative enzymes. (Mæhre, Malde et al. 2014)

Water content:
The water content in *P. palmata* was determined to be 819.5 g/kg by Mæhre, Malde et al. (Mæhre, Malde et al. 2014)

The nutritional content obtained from the different studies are summarized in table 1. below.

Table 1. Summary of the nutritional content found in dulse by different studies.

Constituent:	Amount:	Source:
Polysaccharide	23.25-68.81% of dw, 34.4% of dw, 23.3% of total solids, 24-35% of dw	Jiao, Yu et al. 2012, Lahaye, Rondeau-Mouro et al. 2003, Jard, Marfaing et al. 2013, Hagen Rødde, Vårum et al. 2004
Protein	11.9-21.9% of dry mass, 8-35% of dw	Galland-Irmouli, Fleurence et al. 1999, Morgan, Wright et al. 1980
Lipid	0.3-3.8% of dw, 1.57% of dw, 1.28-1.38% of dw	Morgan, Wright et al. 1980, Fleurence, Gutbier et al. 1994, Mæhre, Malde et al. 2014
Ash	420 g/kg	Mæhre, Malde et al. 2014
Water	819.5 g/kg	Mæhre, Malde et al. 2014

2.2. ASSESSMENT OF METHODS AND PROCEDURE OF PROJECT

This master thesis consists of two parts, literature study which is the basis for the background part and the laboratory part which is described in material and methods and further discussed in discussion and conclusion part. In the literature study, general information regarding dulse, its components, the utilization of dulse today, suitability as raw material in the food and feed industry, information about the enzymes and assay methods used, were studied in order to get background information. This information is the basis for the design and setup of the laboratory part.

The general outline of the laboratory work is depicted in figure 2. Preparation of the samples includes techniques and methods such as wet milling of raw material, incubation with enzymes, sieving, filtration and freeze drying. A more detailed description of the preparation procedure of each batch is presented in section 3.1. The theory behind the analytical methods, used to analyze the prepared material, are described in next section.

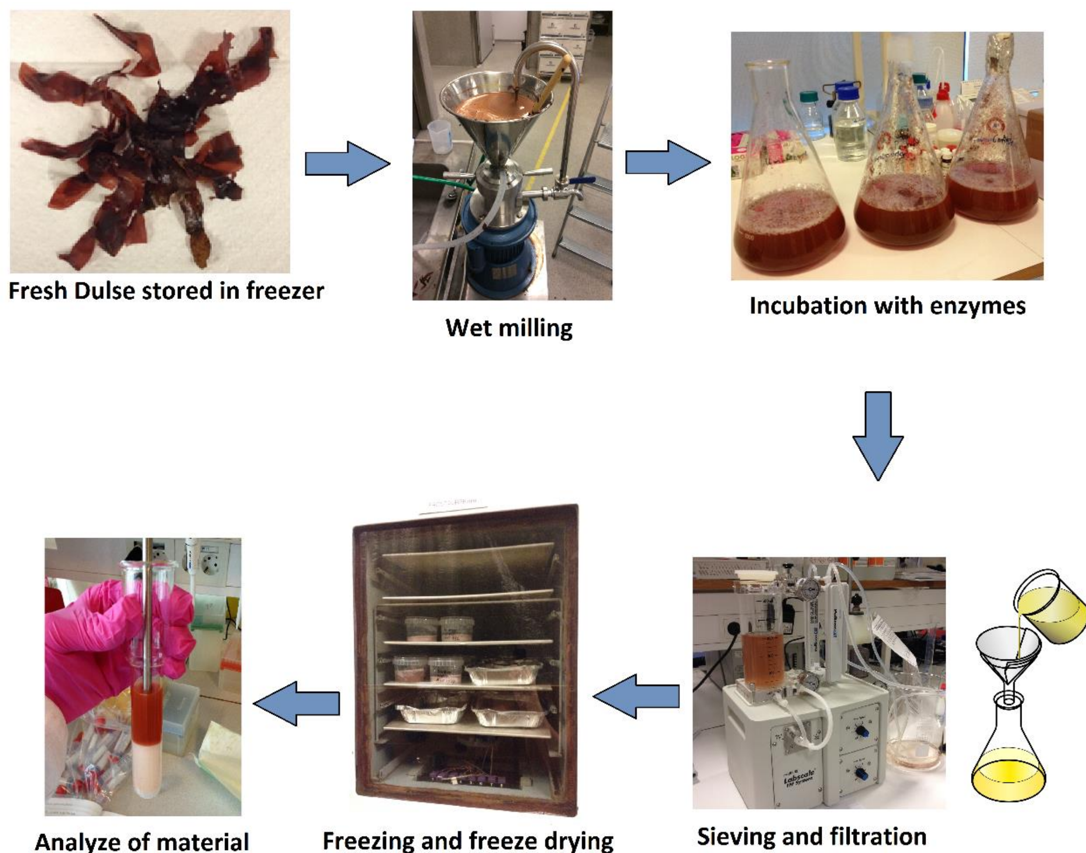

Figure 2. The general outline for preparation of the dulse material.

2.3. ANALYTICAL METHODS

The analytical methods used when analyzing the prepared seaweed material were SDS-PAGE and Bradford assay in order evaluate the protein content in the material. The phenol-sulfuric acid method, TLC and High performance anion exchange chromatography with pulsed amperometric detection (HPEAC-PAD) were used when investigating the polysaccharides and sugars in a sample. The chemical lab determined the protein, fat and ash content.

2.3.1. Membrane filtration

Membrane filtration is a method used to separate components in fluid using permeable membranes. A basic scheme of the procedure is shown below in figure 3. The feed is the liquid that is going to be filtered. The permeate is the liquid that passes through the membrane and the retentate the liquid that does not pass/is retained by the membrane.

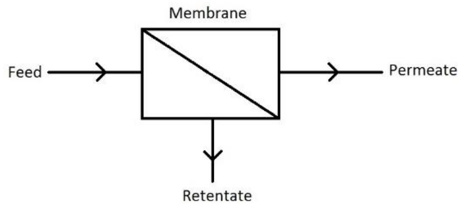

Figure 3. Scheme over the membrane procedure.

The driving force of the filtration is the flux which is often described by the transmembrane pressure.

$$p_{transmembrane} = (p_{before\ filter} + p_{after\ filter})/2$$

Darcy's equation describes the relationship between the $P_{transmembrane}$ and the flux,

$$J = \frac{P_{transmembrane}}{\mu * R_t}$$

where J is the flux, μ is the viscosity and R_t is the total resistance (both membrane and fouling). The type of filtration used is nanofiltration with a molecular cut-off value of 10 kDa. Cross-flow filtration is normally applied in order to reduce fouling. (McCabe et al. 2005) Three parallel filters were used in the laboratory work of the project, to increase the efficiency of the process.

2.3.2. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE is a method to separate proteins according to size. SDS is a detergent added to the sample which breaks most bonds in secondary and tertiary structure of the protein and put a negative charge proportional to the mass of the protein. Heating the sample with 2-mercaptoethanol will also break the disulfide bonds present. The treated sample is put in the polyacrylamide gel and an electric field is applied to the gel. The distance which the peptides migrate is logarithmic proportional to the mass of the peptide. There are different ways of staining the gels, in this experiment Coomassie Brilliant Blue was used for staining of the gel on order to make the peptide bands visible. (Berg et. al 2002)

2.3.3. Bradford assay

The Bradford assay is used to estimate the protein content in a sample. The method is based on the shift in absorption wavelength from 490 nm to 595 nm when the dye Coomassie Brilliant Blue G-250 binds to proteins in the sample. The reaction is accomplished in two steps, the Coomassie dye donate its free electron to ionizable groups on the protein which subsequently reveals its hydrophobic pocket. Thereafter, the hydrophobic dye binds to the hydrophobic part of the protein through Van der Waals bonds. The binding is strengthened by ionic binding of the negative parts of the dye and the positive amine groups of the protein. A standard curve with BSA samples is created, with the absorbance on the y-axis and the concentration on the x-axis. The standard curve is created by measuring the absorbance of BSA solutions of different known concentrations at 595 nm. The absorbance of the sample with unknown protein

content can subsequently be measured and compared with the standard curve to estimate the protein concentration. (Sapan et. al 1999)

2.3.4. Thin-layer chromatography (TLC)

In TLC, different chemical components of a sample are separated on a plate, the solid phase, by exploiting the capillary action of a liquid solvent, the mobile phase. The samples and ladder are added to one end of the plate and put in running buffer. The solid phase and mobile phase has different properties such as polarity, which makes the components in the sample adsorb more or less strongly to the stationary phase making the components ascend at different rates in the mobile phase, causing the separation. (Touchstone 1992) In the experiment, development solution containing diluted sulfuric acid is used to color the plate and thus make the result visible. TLC is used to separate and extinguish the size of different mono- and oligosaccharides in the samples of the seaweed.

2.3.5. Carbohydrate analysis with phenol-sulfuric acid method

Phenol together with sulfuric acid is a relatively easy, sensitive and reliable calorimetric method for determining the amount of sugars, oligo- and polysaccharides in small samples. The sulfuric acid, breaks down the larger molecules to monosaccharides and then reduces the monosugars within the sample. Subsequently, reduced sugars react with phenol and create a compound which turns yellow. A standard curve is created with an appropriate standard solution containing the monosugar investigated. The absorbance for the sample and the standards is measured by a spectrophotometer at 480 nm (for pentoses). The color is stable for hours and the accuracy lies within ±2%. (Nielsen 2010)

2.3.6. High performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD)

HPAEC-PAD is a type of ion exchange chromatography which is mainly used for separation and determination of carbohydrates in a sample. The method utilizes the weak acidic property of carbohydrates for highly selective separations of the different carbohydrates. The stationary phase has a strong anion exchange property, which will interact with the carbohydrates but do not interfere with neutral and cationic compounds. The differently charged carbohydrates will be retained in the column different time, hence the various compounds will have different retention times. The detection of the carbohydrates is performed by measuring the current that occurs when the sugar is oxidized on a gold electrode. (Bignardi et. al 2012)

2.3.7. Chemical assay

The methods performed in the chemical lab is described further in section 3.8 and in APPENDIX A.

2.4. GLOBAL SPREAD AND UTILIZATION OF SEAWEED TODAY

Around 145 eatable species of seaweed are used around the world (Lindsey Zemke-White and Ohno). In 2008 approximately 15.8 million tonnes live weight, with an estimated value of 6.54 billion euros, of aquatic plants were produced of which 99.6 % are seaweeds. The majority is produced in east and south east of Asia where China is the dominating country inhabiting 62.8 % of the world production of aquaculture. The major species used in production of seaweed are *Laminaria japonica*, *Kappaphycus alvarezii*, *Eucheuma* spp., *Undaria pinnatifida, Gracilaria* spp. and *Porphyra* spp. (Mathiesen 2010)

Food application of seaweed is the most important utilization area of seaweed today. It can be used as direct consumption, in functional foods and as algal hydrocolloids such as carrageen, alginates and agar. Other application areas are feed supplements, medicine and as a source for biogas etc. It has been shown in clinical studies that seaweed inhabits biological activities such as antiviral and antibacterial activities. Seaweed contains a great amount of minerals, vitamins, iodine and trace elements which makes it a valuable source of nutrients in animal feed and also in soil fertilization. (Lindsey Zemke-White and Ohno)

Low carbohydrate content will increase the availability of proteins in for instance animal feed. On the other hand, high carbohydrate content may increase properties such as rheology and flavor in food. The digestible carbohydrates may also be used as energy source in monogastric animals and indigestible carbohydrates can be used as prebiotics. (Refined products – processes and applications, 2016)

2.5. THE ENZYME UMAMIZYME®

The Umamizyme enzyme were obtained from Amano Enzyme Inc. located in Nagoya in Japan. The properties of Umamizyme are stated in table 2. The activity is measured using Sigma's enzymatic assay. The activity was measured by the release of tyrosine per min when degrading the substrate casein (1 µmol/min corresponds to 1 U) at pH 7.5 and a temperature of 37 ºC. (Adalbjörnsson 2015)

Table 2. Properties of the enzyme Umamizyme.

Enzyme	**Species of origin**	**Optimal pH**	**Optimal temperature (ºC)**	**Activity (U/mg)**
Umamizyme-K	*Aspergillus oryzae*	7-8	45-50	1.19

3. METHODOLOGY

The first step in the project was to use proteases in order to hydrolyse the proteins in dulse to separate the polysaccharides from the proteins, since studies have shown that the digestibility of proteins in dulse may be inhibited by polysaccharides attached to proteins in the cell wall (Galland-Irmouli, Fleurence et al. 1999). The aim was to cut the proteins in order to release the proteins from the polysaccharides.

Subsequently, the strategy was changed. In this approach, xylanase, an enzyme that digests xylan which is the most prominent polysaccharide in dulse, was used. The strategy included utilization of the xylanase only, together with sonication and combined with proteases.

3.1. PREPARATION OF THE DULSE

Five different batches with slightly different extraction methods have been produced. A brief description of the extraction methods for each batch is presented:

Batch 1, Protease batch: contained 50 g of dulse which had been wet milled and diluted into total 3 L. This batch was divided into three samples, treated and incubated with 20 mg of each of the different enzymes ProteAx, Umamizyme, and one control without enzymes. Overnight incubation when stirring at 100 rpm at 50 ºC was followed by sieving, Millipore ultrafiltration and finally freeze drying of the samples. The filtration part divides the samples into retentate and permeate which generated six samples in total.

Batch 2, Untreated batch: consisted of 2444 g wet milled dulse diluted to 3.9 L. This batch was incubated without enzymes at 50 ºC and 120 rpm overnight and sieved with 100 µm sieve. Subsequently the liquid and the solids collected after the sieving, generating two samples. Thereafter, the two samples were freeze dried and kept for further analyze.

Batch 3, Protease mixture batch: was prepared by wet milling 6192 g of frozen dulse and diluting it into a total volume of 14.1 L. This batch consists of three samples digested with different enzyme mixtures, one with ProteAx (224.6 mg of enzyme), a second with Umamizyme (188.3 mg) and a third with both enzymes (109.2 mg of ProteAx and 112.8 mg Umamizyme). The samples, incubated at 50 ºC and 120 rpm were followed by sieving which generated a solid and liquid part. Finally, the total of six samples were freeze dried.

Batch 4, Xylanase batch: consisted of 1335.1 g of wet milled dulse in total. The batch was divided into four samples, one treated with sonication and 10 µl of the enzyme xylanase, the second which is only sonicated, third which is treated with only 10 µl xylanase and a fourth which is untreated control sample. The samples were incubated overnight at 37 ºC and 120 rpm, sieved and filtered with Millipore ultrafiltration. The sieving generated solid and liquid samples. The filtration procedure of the liquid

generated permeate and retentate of the samples. Thus, generating in total 12 samples. Subsequently, all samples were freeze dried.

Batch 5, Xylanase/protease batch: in total 1224.2 g of dulse was wet milled with 1.2 L of added water. The batch was divided into four samples, one control without enzymes, a second with 4.5 ml of xylanase, a third with 4.5 ml of xylanase and 200 mg of Umamizyme and one with 200 mg of Umamizyme. The samples were incubated at 60 ºC and 150 rpm overnight and thereafter deactivated in a 90 ºC water bath for 30 mins. A 100 µm sieve was used to separate the liquid part from the solid part, generating in total eight samples which were all freeze dried.

3.2. ENZYMATIC PREPARATION

3.2.1. Production of xylanase

The procedure of purification of xylanase was performed using four different bacteria strains. All strains contained various plasmids with different genes and expression levels for expression of xylanase. Table 3 presents general information of the clones and it includes information of the clone, plasmid, vector, enzyme expressed, size of the xylanase, expression level, temperature optimum and deduced type of xylanase.

Table 3. The table shows information of the four different clones. The first row shows type of clone, the second shows the plasmid present in the clone, the third the vector used, the fourth the enzyme expressed, the fifth the size of the enzyme, the sixth the expression level of the plasmid and thereby the xylanase, seventh the temperature optimum for the xylanase, the eighth shows the deduced type of xylanase and the final row shows the induction compound.

Clone	**Amo186**	**Amo190**	**XylLg-A**	**Xyl125**
Plasmid	pSO304	pSO312	pLR1	HOB1
Vector	pJOE3075	pJOE3075	pJOE3075	pBTac1
Enzyme	Xyl186	Xyl190	XylLG-A	Xyl125
Size enzyme (kDa)	130	29	36	37
Expression	++	++	+++	++
T_{opt} (°C)	60-70	-	60	80
Deduced type of xylanase	endo-1,4-beta-xylanase	xylanase	endo-1,4-beta-xylanase	endo-1,4-beta-xylanase
Induction compound	Rhamnose	Rhamnose	Rhamnose	IPTG

Initially, the strain was inoculated and grown in media with ampicillin. Subsequently isolation of plasmid DNA containing the gene for xylanase was performed with a NucleoSpin® Plasmid kit from Macherey Nagel according to protocol. Following the isolation, was the transformation of the plasmid to an endotoxin free *E. coli* strain, Clear coli. This was executed by using a gene pulser. The Clear coli was considered to be endotoxin free and therefore suitable in food and feed applications.

The transformed cells were grown on plate at 37 ºC overnight and the next day inoculated in vials with media for further growth (overnight at 37 ºC). The cell culture was transferred to flasks with media and grown at 37 ºC and 200 rpm and then induced with rhamnose or IPTG (Isopropyl β-D-1-thiogalactopyranoside), depending

on the used plasmid. Inoculation occurred when the samples reached an OD around 0.8-1.0 at 600 nm wavelength. The compound used for induction in each plasmid is presented in table 3. The induction was followed by expression of the xylanase at 25 ºC overnight. Sonication was used in order to disrupt the cells and release the xylanase. The xylanase together with other proteins dissolved in the supernatant was separated from the cell broth.

The proteins in the clear coli after lysing, including the xylanase, were visualized in an SDS-PAGE gel. Samples containing xylanase (visualized in the SDS-PAGE gel) deriving from the pSO312 and pLR1 plasmid respectively were subjected to heating in order to denature other proteins in the sample. The xylanase is heat stable and was, as a result, preserved in the heating process. Another SDS-PAGE was performed in order to confirm the success in the separation of the xylanase from other proteins. Finally, the activity of the xylanase was confirmed by incubating the samples with substrate and buffer at 60 ºC overnight and the following day, performing a TLC of the samples. The activity in U/ml was determined by measuring the absorbance at 405 nm after 30 mins of reaction of the enzyme with the substrate 4-nitrophenyl-α-D-xylopyranoside (α-PNPX). More detailed protocol of the production of xylanase is presented in APPENDIX A.

3.3. ANALYTICAL METHODS: SDS-PAGE

The SDS-PAGE was performed in order to evaluate the presence and size of the proteins/peptides in the samples. In the following text, the general procedure for the SDS-PAGE is described, 2 µl sample, 8 µl of water and 3 µl of SDS buffer was mixed in PCR-tubes. The mixture was boiled at 90 °C for 5 min in a PCR machine. The treated samples, each 13 µl, were run on a 10-12% acrylamide gel together with two ladders 5-10 µl each. The gel was run for approximately 45 min at 200 V and 30 mA.

The bands were revealed using different buffer with different concentration Coomassie Blue. The buffers contained acetic acid, isopropanol and 0.05, 0.005, 0.002 and 0 % of Coomassie Blue. Initially, the strongest 0.05% buffer was added until it covered the gel, heated for 1 min at full effect in the microwave oven, placed on a shaker for 1 h and thereafter discarded. The second buffer was added and put in the microwave oven for 1 min and subsequently discarded. The same procedure followed for the third buffer. Finally, the last buffer was poured onto the gel which was followed by 1 min heating in the microwave oven. A paper was put on the top of the gel and it was placed on the shaker for 1 hour and thereafter the buffer was removed. After the buffer treatment, the bands with the proteins should be visible. The SDS-PAGE gel was put and sealed in a plastic bag and the gel was ready to be analyzed and interpreted.

3.4. ANALYTICAL METHODS: BRADFORD ASSAY

The Bradford assay is used to estimate the protein content in a sample. A standard curve with BSA samples was created, with the absorbance on the y-axis and the concentration on the x-axis. The concentration of the standard solutions varied between 0.1 – 1.4 mg/ml. Subsequently, the absorbance of the sample with unknown protein content was measured and compared with the standard curve to estimate the protein concentration. The assay was executed in a 96-well flat-bottomed plate. 5 µl of BSA/sample is properly mixed with 250 µl of Bradford solution and incubated in room temperature for approximately 10 min before measuring the absorbance. Since two different bottles of Bradford solution were used, two standard curves, one for each, was prepared. The concentration and absorbance for the standard curves are presented in table 4.

Table 4. Standard solutions prepared for the calibration curve. The concentration BSA, volume BSA and water added for x1 and x4 amount of standard solution.

Concentration (mg/mL)	Average absorbance first standard curve	Average absorbance second standard curve
0.00	0.377	0.284
0.10	0.397	0.359
0.25	0.433	0.434
0.50	0.483	0.569
0.75	0.576	0.633
1.00	0.682	0.757
1.25	0.720	0.864
1.40	0.788	0.946

3.5. ANALYTICAL METHODS: THIN-LAYER CHROMATOGRAPHY (TLC)

TLC was performed in order to separate and confirm the presence of mono- and oligosaccharides deriving from xylose, the main polysaccharide in *P. palmata* samples. The method was also used in order to confirm the activity of the xylanase which breaks down xylose to smaller units, which then can be seen in the TLC. A silica plate was used for the TLC. A baseline was drawn and the samples were marked with a pencil. The samples were added gently onto the baseline together with two ladders. The ladder consists of different sized oligomers of xylose. The plate was put in a container with running buffer. The TLC was run for approximately 4 hours before it was removed and dried. Development solution consisting of orcinol monohydrate, methanol and sulfuric acid, was poured onto the plate. The plate is then heated in an oven at 100°C to reveal the bands.

3.6. ANALYTICAL METHODS: HIGH-PERFORMANCE ANION-EXCHANGE CHROMATOGRAPHY WITH PULSED AMPEROMETRIC DETECTION (HPAEC-PAD), MODEL ICS-3000

The aim of the HPAEC-PAD method was to determine and quantify the polysaccharides present in dulse. The columns used were Dionex® CarboPac PA100 and Dionex® CarboPac PA20. The samples had to be treated according to following protocol in order to break down the polysaccharides into smaller components and thus make them detectable in the column. Two kinds of samples were prepared. The free monosugar samples (FMS) are the ones which are not treated with sulfuric acid and thus acts as a reference, to take into account the already free monosugars present in the sample. The other sample was treated with sulfuric acid in order to break down the polysaccharides present in dulse into monosaccharides which were detected in the ion exchange chromatography. Some of the water diluted samples were saved and kept as FMS. The remaining sample was treated with 72% sulfuric acid and put in a heating block at 100 ºC for 3 h. Subsequently the pH was adjusted to approximately 5-6 by using $Ba(OH)_2 \cdot H_2O$. The centrifuged samples were then collected in a vial after being filtered through a 0.45 µm filter. Also, the FMS were filtered through a 0.45 µm filter. The samples and FMS were run through the columns, the running time for PA100 and PA20 were 43 and 25 min respectively.

3.7. ANALYTICAL METHODS: CARBOHYDERATE ANALYSIS WITH PHENOL-SULFURIC ACID METHOD

Another method to estimate the total carbohydrate content in the samples, is the phenol-sulfuric acid method. The protocol was obtained from Masuko et al. (Masuko, 2005) Initially, the standard solutions for the standard curve was prepared. The standard solutions were prepared from a stock solution with 1 mg/ml xylose. Xylose is the type of sugar used as standard, since it is the building block of xylan which is considered to be the most abundant polysaccharide in dulse. The standard solutions were prepared by diluting xylose in water in different concentrations, according to table 5.

Table 5. Concentration of standard solution, amount of stock solution and the amount of water added when preparing the standard solutions.

Concentration of standard solution (mg/mL)	Amount of stock solution added (µl)	Amount of water added (µl)
0.05	50	950
0.10	100	900
0.15	150	850
0.20	200	800
0.25	250	750
0.30	300	700

Approximately 1 mg/mL solutions of the samples were prepared and different dilutions from this concentration were prepared, x10, x80, x100 and x1000 dilutions were done. Subsequently the standards and the samples were prepared in a 96-well PCR plate, in triplicates. 96 % sulfuric acid and 5 w/v% phenol were added to the wells containing the samples. The plate was heated to 90 ºC for 5 min in a PCR machine. Thereafter, the samples were transferred to another plate for absorbance measurements. The absorbance was measured at 490 nm in a spectrophotometer. The absorbance for the standard solutions was plotted against the concentration. The concentration for the samples can be estimated by using the standard curve.

3.8. ALYTICAL METHODS: CHEMICAL ANALYSIS

3.8.1. Protein content

When measuring the protein content, combustion of the sample with a steady supply of oxygen was performed. The compounds containing nitrogen will form residues such as N_2, NO_X. Other residues such as volatile halogens, SO_2, H_2O and CO_2 were also formed. The N_2 content in the gas was measured in a thermal conductivity detector. The method was done according to the procedure performed by the chemical lab and is presented in APPENDIX B.

The amount protein was calculated using a nitrogen conversion factor of 6.25. *Ref ISO 1663-1 (2008)* This conversion factor is based on early determinations of protein content where the amount protein was estimated to approximately 16%. This is a rough approximation and it has been suggested that specific conversion factor should be used, depending on what kind of sample that is analyzed. This is because not all nitrogen in a sample derange from protein, these so called non-protein nitrogen (NPN) could be free amino acids, nucleotides, creatine etc. Also, not all amino acids contain the same proportion of nitrogen (percentage of weight) since not all amino acid have the same molecular weight and the number of nitrogen in the amino acid varies. (Food and Agriculture Organization of the United Nations, 2003)

In a study made by Lourenço S. O. et. al, the nitrogen content and amino acid content for 19 species of red seaweed was determined in order to estimate the nitrogen conversion factor for red seaweed. The conversion factor for red seaweed was suggested to 4.59 ± 0.54 in this study which is lower than the commonly used 6.25.

3.8.2. Water content

The sample is heated in an oven at 103 °C for four hours. The water content corresponds to the weight loss after four hours. *Ref. ISO 6496 (1983).* (APPENDIX B)

3.8.3. Ash content:

At 550 °C, the samples are turned into ash which is subsequently weighed. *Ref. ISO 5984-1978 (E).* (APPENDIX B)

3.8.4. Fat content:

The sample is extracted with petroleum ether, boiling range 40-60 °C. The extraction apparatus is 2050 Soxtec Avanti Automatic System. *Ref. AOCS Official Method Ba-3-38 with modifications according to Application note Tecator no AN 301.* (APPENDIX B)

4. RESULTS

The results for batch 1-5 are presented in the following paragraphs. The results for each different analyze method are presented, these methods are SDS-PAGE, Bradford assay, TLC, HPAEC-PAD, carbohydrate analysis with phenol-sulfuric acid method and chemical analysis of the components. Moisture analysis of pure dulse from freezer showed that the seaweed contains 85% water and 15% dry weight.

4.1. BATCH 1, Protease batch.

4.1.1. Batch 1, protease batch: SDS-PAGE

In the protease batch, none of the samples showed any result regarding the protein content in the SDS-PAGE. The coomassie dye, used for staining, is larger than the dipeptides and most tripeptides and these are thus not visible in the gel. Smaller peptides have most likely migrated past the gel.

4.1.2. Batch 1, protease batch: Bradford assay

The result from the Bradford assay of the samples in batch 1 is presented in table 6.

Table 6. The table presents the absorbance obtained from the Bradford assay performed on the samples in batch 1.

Sample:	**Absorbance:**
Control unfiltered	0.369
Umamizyme unfiltered	0.376
ProteAx unfiltered	0.382
Control retentate	0.386
Umamizyme retentate	0.451
ProteAx retentate	0.450
Control permeate	0.381
Umamizyme permeate	0.372
ProteAx permeate	0.371

The Bradford assay showed absorbance between 0.369 and 0.386 in all samples (except retentate Umamizyme and ProteAx) which is similar to the absorbance for the blank in the standard solutions (0.377). This indicates that there are no or very little proteins in these samples. This could be due to, too diluted samples. The absorbance for the Umamizyme and ProteAx retentate were 0.451 and 0.450 this may indicate a protein concentration between 0.25 and 0.50 mg/mL. This low protein concentration in the retentate for Umamizyme and ProteAx samples could be from the presence of the enzymes themselves. The standard curve used for the Bradford assay is presented in figure 4. The blank with the absorbance 0.377 has been subtracted from the values data points in the curve.

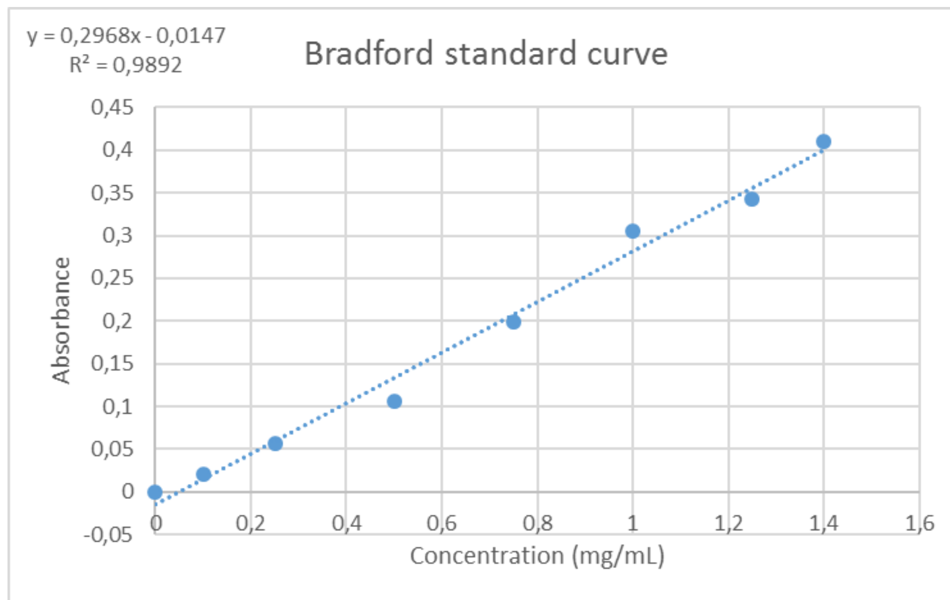

Figure 4. The figure shows the standard curve used in the Bradford assay.

4.1.3. Batch 1, protease batch: TLC

Figure 5 depicts the results of the TLC performed on the samples of the protease batch. The TLC shows the sugars present in nondigested dulse, dulse digested with ProteAx and Umamizyme. Both permeate and retentate, were evaluated in the analysis. The figure shows xylose ladder in the first and the last lane. The ladder consists of oligomers of xylose. The least migrated band contains pentose, followed by tetrose, triose and biose respectively. The second and third lane holds the retentate and permeate of dulse treated with ProteAx. The fourth and fifth lane from left shows the retentate and permeate of dulse treated with Umamizyme. The sixth and seventh lane holds the retentate and permeate of the undigested dulse without enzyme. In the wells of the retentates, in all samples, most of the liquid did not migrate at all and stayed at the bottom of the plate, this indicates presence of long polysaccharides, such as the expected xylan. Some vague bands which have migrated various distances can also be extinguished in all samples. By comparing these bands with the ladder, it can be concluded that the samples also contain some shorter sugars such as mono- di- and trisaccharides.

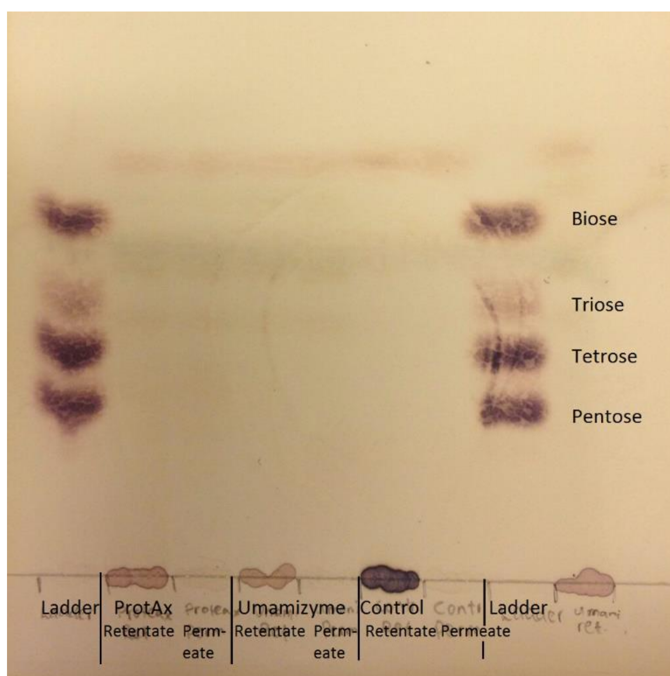

Figure 5. Results of the TLC of batch 1. The first lane to the left shows the ladder consisting of oligomers of xylose, the second ProteAx retentate, the third ProteAx permeate, the fourth Umamizyme retentate, the fifth Umamizyme permeate, the sixth without enzyme retentate, the seventh without enzyme permeate and the last, another ladder.

4.1.4. Batch 1, protease batch: HPAEC-PAD

The HPAEC-PAD chromatograms are shown in APPENDIX C. The analyze was performed two times with different retention times and columns, 25 min, 43 min using columns CarboPac PA20 and CarboPac PA100 respectively. The CarboPac PA20 column is efficient when separating monosaccharides and CarboPac P100 is mainly used for oligosaccharides. The reason for using different times between the columns was to obtain good separation between the different sugars (peaks in chromatogram) in each column. The relative areas of xylose, glucose and sucrose in each sample for CarboPac PA20 and CarboPac P100 respectively, are summarized in table 7 and 8 respectively. The results show presence of xylose, the primary sugar in dulse. Sugars expected in dulse are xylose (forming 1,3/1,4-linked xylose), galactose (forming floridoside) and glucose (forming cellulose) (Lahaye, Vigouroux 1992). Sucrose is not a sugar that is expected in *P. palmata.* It is possible that there is some other disaccharide present or that sucrose is present because some kind of contamination has occurred.

Table 7. The relative amount of xylose, glucose and sucrose compared to the total amount of sugar in each sample when using CarboPac PA20.

Sample	Relative amount xylose of total amount sugar (%)	Relative amount glucose of total amount sugar (%)	Relative amount sucrose of total amount sugar (%)
Umamizyme permeate	0.94	8.60	24.5
Umamizyme retentate	5.48	16.8	30.1
Umamizyme unfiltered	2.46	18.8	38.4
ProteAx permeate	1.87	9.14	28.2
ProteAx retentate	2.99	7.26	12.6
ProteAx unfiltered	3.13	14.0	30.4
Control permeate	2.83	37.8	6.91
Control retentate	1.69	11.2	67.9
Control unfiltered	0.80	9.88	26.0

b

Table 8. The relative amount of xylose, glucose and sucrose compared to the total amount of sugar in each sample when using CarboPac P100.

Sample	Relative amount xylose of total amount sugar (%)	Relative amount glucose of total amount sugar (%)	Relative amount sucrose of total amount sugar (%)
Umamizyme permeate	9.99	-	3.98
Umamizyme retentate	14.9	0.48	7.78
Umamizyme unfiltered	11.6	-	7.67
ProteAx permeate	17.7	-	6.63
ProteAx retentate	9.23	0.51	4.94
ProteAx unfiltered	6.50	-	7.54
Control permeate	18.1	-	6.81
Control retentate	18.2	-	5.39
Control unfiltered	12.6	-	10.4

4.2. BATCH 2 AND 3, untreated batch and protease mixture batch.

4.2.1. Batch 2 and 3: SDS-PAGE

SDS-PAGE was performed in order to evaluate the protein content in the samples. The result is presented in figure 6. Lane 1 contains ProteAx sieved, 2 ProteAx and Umamizyme sieved, 3 Umamizyme sieved, 4 ProteAx solids, 5 ProteAx and Umamizyme solids, 6 Umamizyme solids, 7 undigested sieved and well 8 undigested solids (1-6 from batch 2 and 7-8 from batch 3). The ladder and its size is presented in figure 7.

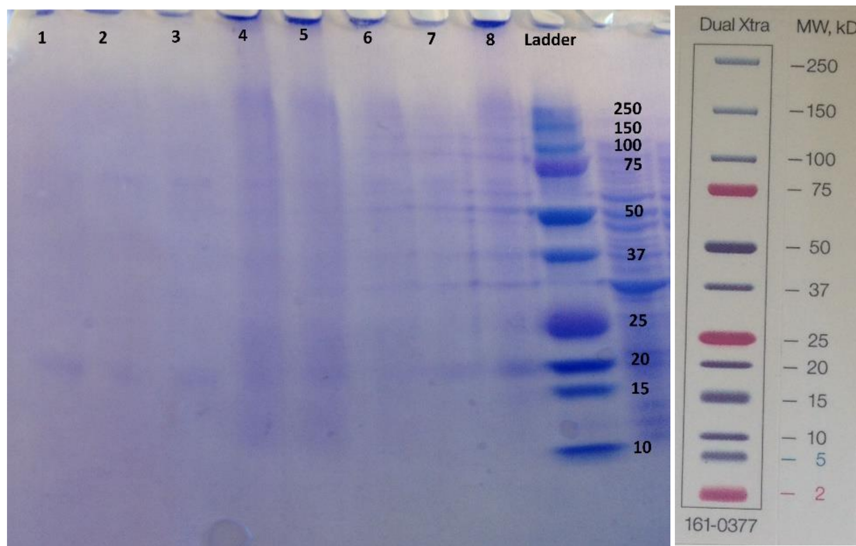

Figure 6. An SDS-PAGE of sample 1-8 and the ladder, is shown in the figure.

Figure 7. The ladder used in the SDS-PAGE.

Lane 1-3 with the sieved samples, seem to contain small amounts of proteins. 4-6 contain the solids, the color is slightly stronger in lane 4 and 5. Lane 6-8 seem to have been slightly contaminated by the ladder. Bands around 60 kDa and around 20 kDa are faintly visible in all wells. This indicates a similar protein profile in all samples. Thus, it seems like the enzyme digestion did not work as expected, since the desired outcome would generate samples with different size of the proteins and peptides. The enzyme was expected to cut the proteins into smaller fragments compared to the undigested samples, this cannot be distinguished either.

4.2.2. Batch 2 and 3, untreated batch and protease mixture batch: Bradford assay

The results from the Bradford assay in batch 2 and 3, untreated batch and protease mixture batch are presented in table 9. The samples named control are from the untreated batch 2 and the other samples from protease mixture batch. It seems to be higher protein content in the solids compared to the sieved except for the controls. The concentration in the control samples are quite similar (control sieved is slightly higher). The low protein concentration in the control may be because the proteins are bound to xylan, which makes it difficult for the dye to bind to the aromatic amino acids in the protein. The difference in protein concentration between the different enzymes used are not large. Thus, it seems like it does not matter which type of protease/protease mixture that is used.

Table 9. Results from the Bradford protein assay. The average absorbance of three replicates, the concentration, weight and percentage of initial weight are displayed in the table.

	Absorbance (average)	Concentration (mg/mL)	Weight of protein(mg)	Percentage of initial weight (%)
ProteAX solids	0.323	1.14	11.4	26.4
ProteAX + Umamizyme solids	0.305	1.08	10.8	19.2
Umamizyme solids	0.255	0.907	9.07	18.5
Control solids	0.010	0.386	3.86	8.11
ProteAX sieved	0.160	0.589	5.89	12.0
ProteAX + Umamizyme sieved	0.234	0.836	8.36	17.1
Umamizyme sieved	0.256	0.912	9.12	16.0
Control sieved	0.126	0.475	4.75	8.91

4.2.3. Batch 2 and 3, untreated batch and protease mixture batch: Chemical analysis

The results from the chemical analysis for batch 2 and 3, untreated batch and protease mixture batch are presented in table 10 and 11 respectively. For the untreated batch 2, water, protein, fat and ash content were measured whereas only protein content was measured in the protease mixture batch.

Table 10. Protein, fat, water and ash content of the control samples in the untreated batch.

	Protein (%)	Fat (%)	Water (%)	Ash (%)
Control solids	35.8	0.50	3.10	12.9
Control sieved	20.6	0.40	5.70	29.1

Table 11. Protein content of the enzyme digested samples in protease mixture batch.

Samples	Protein (%)
ProteAX solids	42.7
ProteAX + Umamizyme solids	45.2
Umamizyme solids	46.6
ProteAX sieved	21.6
ProteAX + Umamizyme sieved	19.7
Umamizyme sieved	20.5

The solid fraction in both untreated batch and protease mixture batch, have a larger protein content compared to the sieved part. It seems like most of the proteins are retained in the solid fraction even though there is a considerable large amount of proteins in the sieved part too.

4.2.4. Batch 2 and 3, untreated batch and protease mixture batch: TLC

Figure 8 shows the TLC of the solids and sieved of the second and third batch. First well to the left shows ProteAx solids, the second solids of ProteAx and Umamizyme, the third solids of Umamizyme and the fourth is the control (solid from batch 2). Well 5 contains the ladder which consist small oligosaccharides of the monosugar xylose. The oligosaccharides are triose (migrated furthest), tetrose, pentose and hexose

respectively. Well 6-9 contains sieved from ProteAx, ProteAx and Umamizyme, Umamizyme and control (sieved from batch 2) respectively.

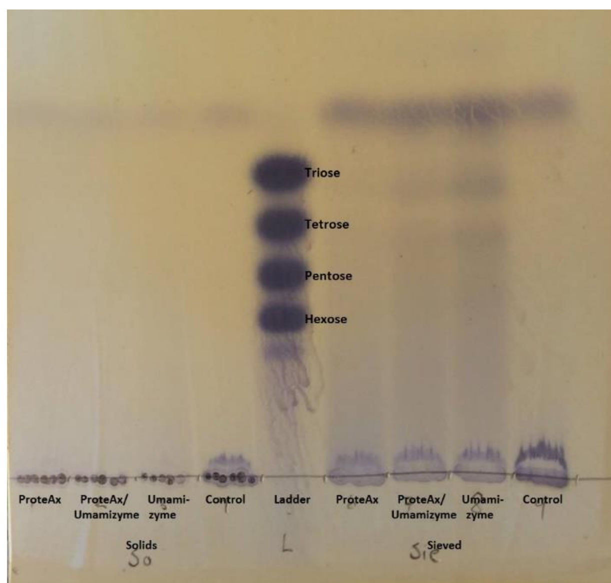

Figure 8. The image shows TLC of ProteAx, ProteAx and Umamizyme, Umamizyme and control, of both solids and sieved samples. A xylose ladder is also included in the TLC in order to compare the size of the bands in the samples.

According to the TLC, there are some monosugars and oligosugars in both sieved and solid parts. There are also samples left in the wells, which indicates that there are larger polysaccharides present too.

4.2.5. Batch 2 and 3, untreated batch and protease mixture batch: HPAEC-PAD

The results for the HPAEC-PAD performed on the samples in the untreated batch are presented in table 12 and 13. Table 12 shows the 25-min run when using CarboPac PA20 and table 13 shows the 43-min run when using CarboPac PA100.

Table 12. The relative amount of xylose, glucose and sucrose compared to the total amount of sugar in each sample for the 25-min run.

Sample	Relative amount xylose of total amount sugar (%)	Relative amount glucose of total amount sugar (%)	Relative amount sucrose of total amount sugar (%)
Solids	16.4	6.76	-
Sieved	70.5	2.89	-

Table 13. The relative amount of xylose, glucose and sucrose compared to the total amount of sugar in each sample for the 43-min run.

Sample	Relative amount xylose of total amount sugar (%)	Relative amount glucose of total amount sugar (%)	Relative amount sucrose of total amount sugar (%)
Solids	37.1	-	-
Sieved	22.6	-	4.00

4.2.6. Batch 2 and 3, untreated batch and protease mixture batch: Carbohydrate analysis with phenol-sulfuric acid method

The standard curve for the phenol-sulfuric acid method was plotted according to the data presented in table 14.

Table 14. Xylose concentration used for standard curve and absorbance measured at 490 nm.

Xylose concentration (mg/ml)	Absorbance (490 nm)
0.00	0.0643
0.05	0.212
0.10	0.371
0.15	0.588
0.20	0.772
0.25	1.01
0.30	1.21

The results of the total sugar determination of the samples in the untreated batch and the protease mixture batch are summarized in table 15. The table shows the amount sugars in the initial undiluted samples when using x10, x50 and x100 dilution of the samples when measuring of the absorbance. The results are also illustrated in a plot, together with the standard curve, in figure 9 and 10 for the solids and sieved samples respectively.

Table 15. The concentration sugars in the samples. The concentration for x10, x50 and x100 dilutions and the average concentration of the three dilutions are shown in the table.

Samples	Original amount sugar when using the x10 dilution (mg sugar/mg dry dulse)	Original amount sugar when using the x50 dilution (mg sugar/mg dry dulse)	Original amount sugar when using the x100 dilution (mg sugar/mg dry dulse))	Average amount (mg sugar/mg dry dulse)
ProteAx, solids	0.584	0.814	0.779	0.726
ProteAx +Umamizyme, solids	0.509	0.788	0.587	0.628
Umamizyme solids	0.711	1.09	0.893	0.897
Control, solids	0.609	0.753	0.725	0.696
ProteAx, sieved	0.586	0.695	0.803	0.694
ProteAx +Umamizyme, sieved	0.549	0.852	0.799	0.733
Umamizyme, sieved	0.635	0.764	0.663	0.688
Control, sieved	0.453	0.484	0.630	0.522

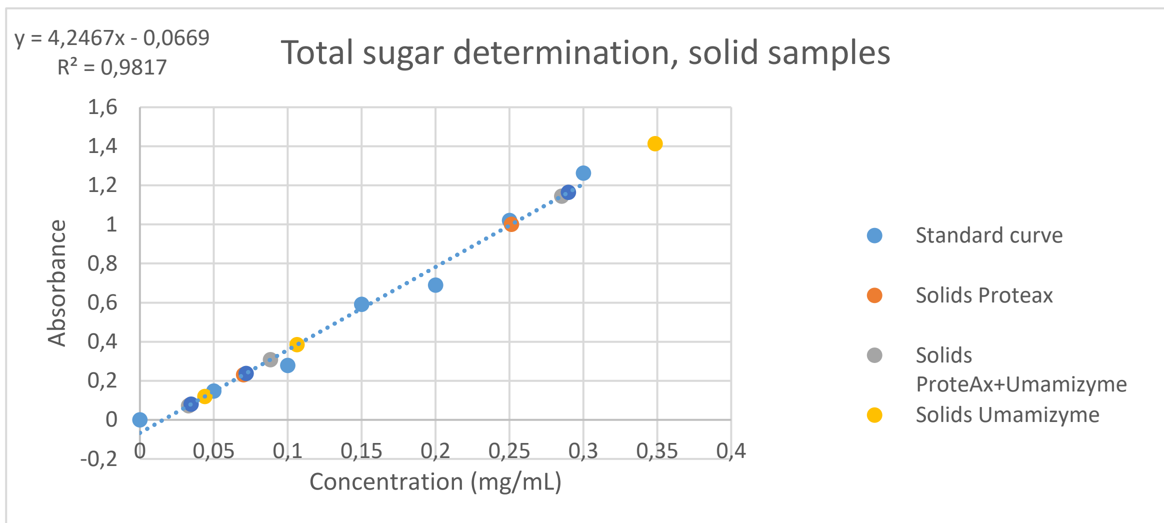

Figure 9. The curve shows the average absorbance and the concentration for the diluted samples when using x10, x50 and x100 dilutions. This graph shows the solid samples.

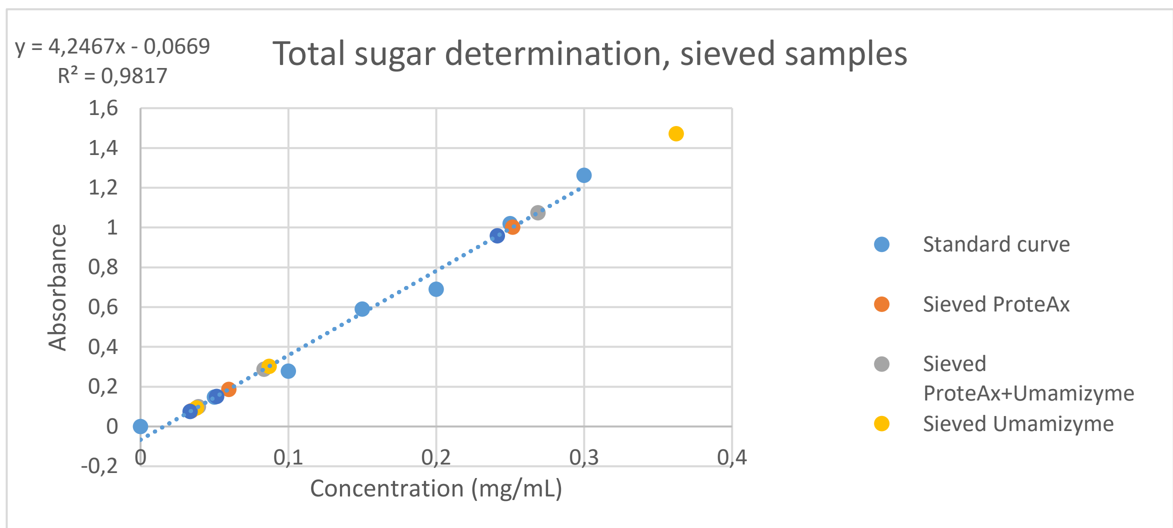

Figure 10. The curve shows the average absorbance and the concentration for the diluted samples when using x10, x50 and x100 dilutions. This graph shows the sieved samples.

4.3. BATCH 4.

4.3.1. Batch 4, xylanase batch: Bradford assay

A Bradford assay was done to estimate the amount proteins in the samples in the xylanase batch. The results from the Bradford assay is presented in table 16. In this assay, the retentate seem to hold most of the proteins. The amount protein in the permeate is approximately half of the amount in the retentate. The Bradford assay does not seem to show any difference in protein content between the different samples.

Table 16. The amount protein per amount dry weight dulse obtained from the Bradford assay.

Sample	Protein content (% of initial weight)
Sonicated+xylanase retentate	14.4
Sonicated+xylanase permeate	7.26
Sonicated, retentate	15.3
Sonicated, permeate	7.61
Xylanase, retentate	15.7
Xylanase, permeate	7.13
Control, retentate	16.9
Control, parmeate	8.02

4.3.2. Batch 4, xylanase batch: Chemical assay

The results obtained from chemical analysis of the protein content of the samples are shown in table 17. According to this result, the amount of protein seems to be similar in all samples. Thus, there is no difference in protein content in permeate or retentate, enzyme or without enzyme and neither in samples where sonication has been used or not.

Table 17. The protein content in the samples from batch 4.

Sample	Protein content (%)
Sonicated+xylanase, retentate	16.8
Sonicated, retentate	16.2
Xylanase, retentate	16.0
Control, retentate	16.9
Sonicated+xylanase, permeate	15.9
Sonicated, permeate	16.1
Xylanase, permeate	16.2
Control, permeate	15.6

Chemical analysis of the protein content was also performed on the solids of the samples in the xylanase batch and on pure dried seaweed in order to compare. These results are presented in table 18. It seems like the solids contain similar and relatively high amounts of proteins, around 38 %. The protein share in pure dried dulse seem to be lower, in average 27.8 %.

Table 18. Chemical analysis of protein content in the solids of the samples and in pure seaweed.

Sample	Protein content (%)
Sonicated+xylanase, solids	38.1
Sonicated, solids	37.5
Xylanase, solids	38.2
Control, solids	38.8
Pure dulse, middle of bag 1	27.2
Pure dulse, end of bag 1	28.2
Pure dulse, end of bag 2	27.9

4.3.3. Batch 4, xylanase batch: TLC

The result of the TLC performed on the samples in the xylanase batch are shown in figure 11. In general, A which is the retentate has stronger band in the bottom and a

weaker band in the top compared to B, the permeate. This may be an indication that the retentate contains more of the larger polysaccharides, less monosaccharides and oligosaccharides compared to the permeate. Sample 1 is the one treated with both sonication and xylanase, 2 is the sonicated sample, 3 the sample treated with only xylanase and 4 the control.

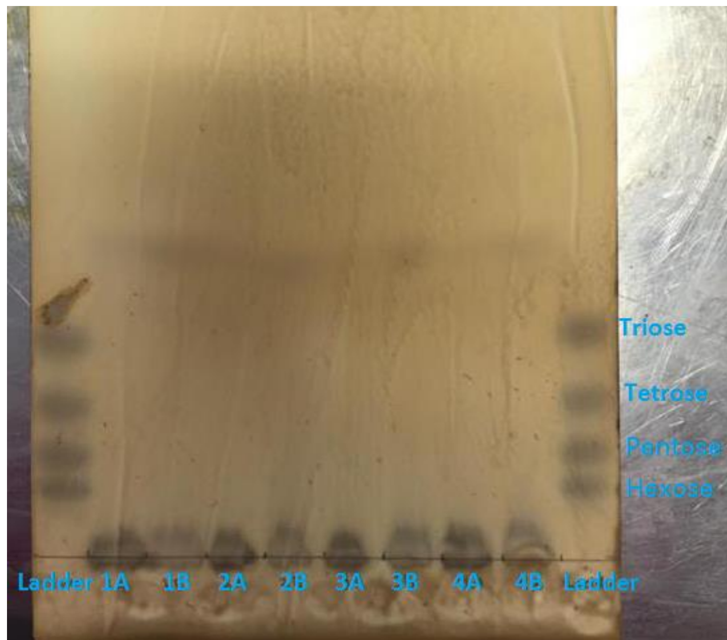

Figure 11. TLC on the samples in the xylanase batch. Sample 1 is the sonication + xylanase, 2 is sonication, 3 is xylanase and 4 is control. A and B are retentate and permeate respectively.

4.3.4. Batch 4, xylanase batch: Carbohydrate analysis with phenol-sulfuric acid method

The carbohydrate concentration with the phenol-sulfuric acid method, for x10, x50, x100 dilutions and the average concentration are displayed in table 19. The data for the samples fitted to the standard curve are shown in figure 12-15 for sample 1-4 respectively. This method did not seem to work properly since, in most cases, the average concentration of protein seems to exceed the amount of dry matter of sample used. Thus, the results are not reasonable. The reason for these inadequate results are not established. Though, an explanation could be that the spectrophotometer equipment did not work properly, since it has been problems with it previously or that something unexpected occurred when preparing the samples.

Table 19. The total sugar concentration (percentage) when using x10, x50 and x100 dilution. The average of the three concentrations are also shown.

Sample	Concentration x10 dilution (mg/mg)	Concentration x50 dilution (mg/mg)	Concentration x100 dilution (mg/mg)	Average concentration (mg/mg)
Sonicated+xylanase, retentate	0.845	1.314	1.68	1.28
Sonicated, retentate	0.526	0.975	1.35	0.948
Xylanase, retentate	0.826	1.22	1.57	1.20
Control, retentate	0.563	0.984	1.57	1.04
Sonicated+xylanase, permeate	0.801	1.33	1.67	1.27
Sonicated, permeate	0.457	0.912	1.28	0.884
Xylanase, permeate	0.636	1.47	1.62	1.24
Control, permeate	0.577	1.04	1.23	0.947

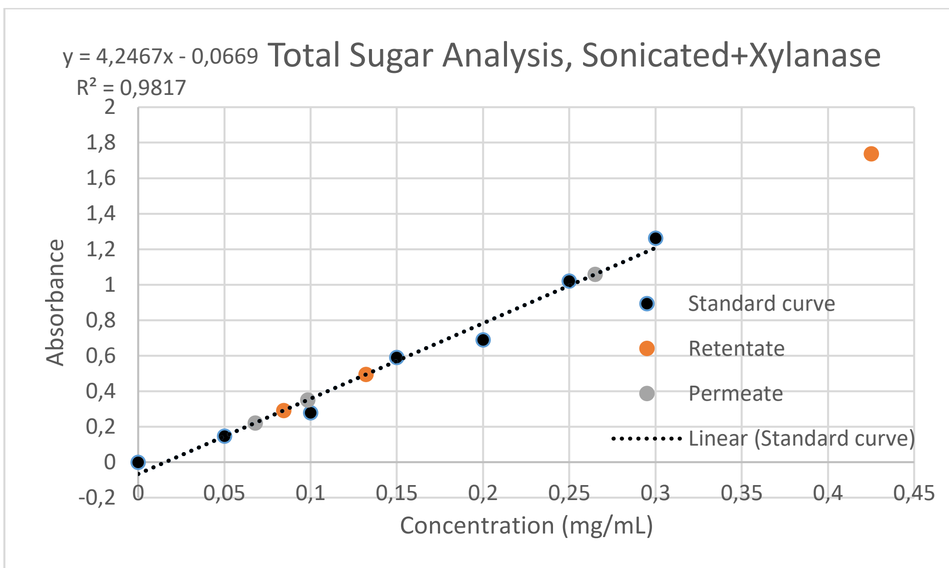

Figure 12. Total sugar analysis with phenol-sulfuric acid method on sonicated/xylanase sample. The black dots show the standard curve, the orange dots the retentate and grey the permeate.

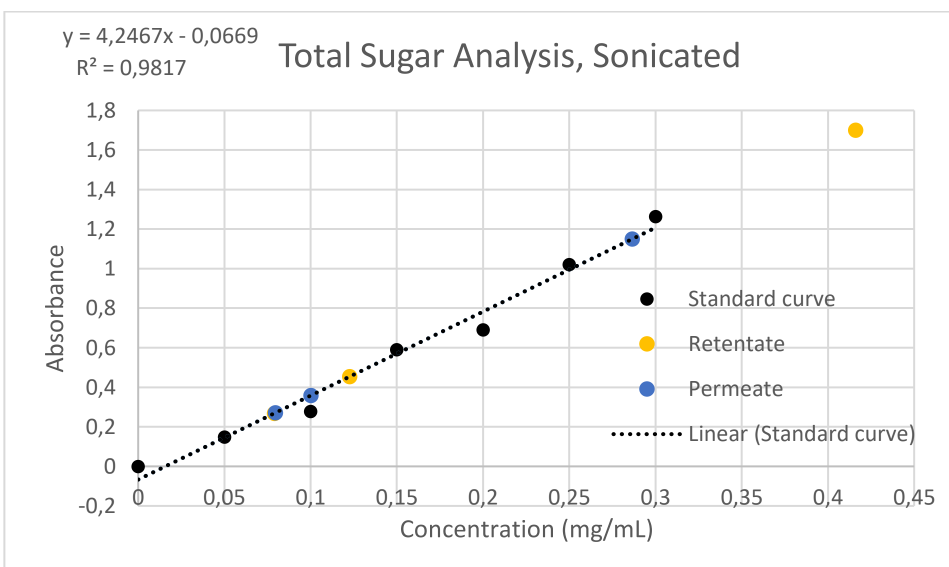

Figure 13. Total sugar analysis with phenol-sulfuric acid method on sonicated sample. The black dots show the standard curve, the yellow dots the retentate and blue the permeate.

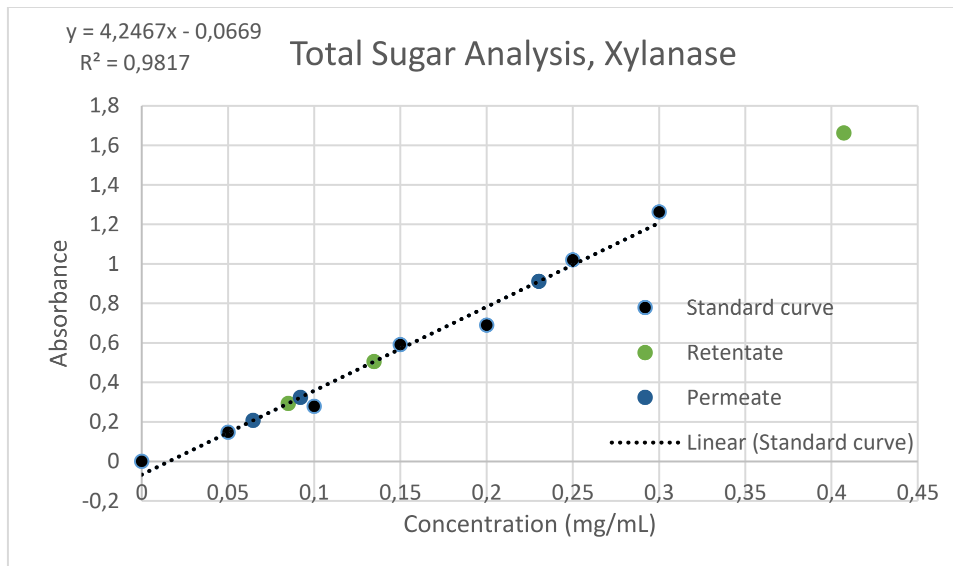

Figure 14. Total sugar analysis with phenol-sulfuric acid method on the xylanase sample. The black dots show the standard curve, the green dots the retentate and the blue the permeate.

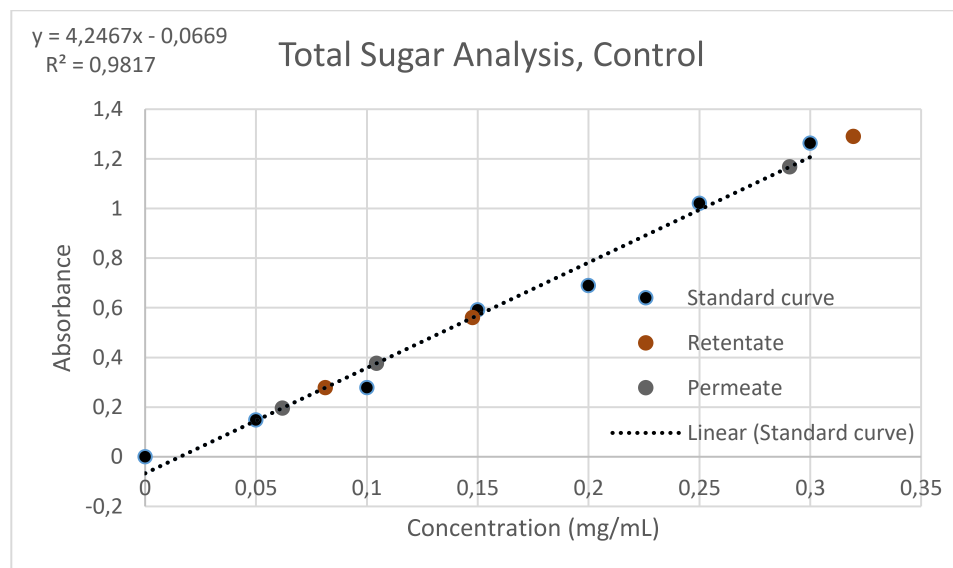

Figure 15. Total sugar analysis with phenol-sulfuric acid method on the control sample. The black dots show the standard curve, the red dots the retentate and the grey the permeate.

4.4. BATCH 5.

4.4.1. Batch 5, xylanase/protease batch: SDS-PAGE

The results from the SDS-PAGE analyze of batch 5 is presented in figure 16. The wells with “L” shows the liquid samples which passed through the sieve and the “S” shows the solids which remained in the sieve. Number 1 is the control, 2 is xylanase, 3 is xylanase and Umamizyme and 4 is only Umamizyme. The results show no presence or very little amount of protein in the liquid sample. The solids show faint bands and thus occurrence of proteins in the samples. Interestingly, the sample containing only xylanase shows strongest bands and hence it indicates highest protein concentration followed by the sample treated with both enzymes. The different protein

profiles in the solids are a bit hard to distinguish, but it seems like the control and the xylanase have somewhat similar protein profile. It also seems like the Umamizyme/xylanase and the Umamizyme have to some extent similar protein profile.

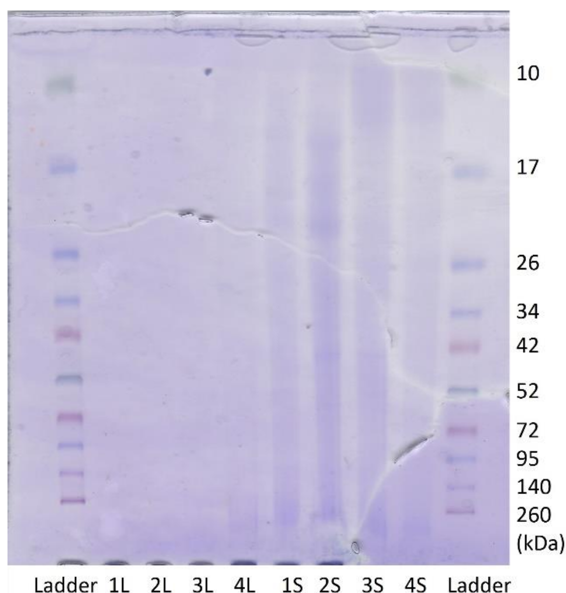

Figure 16. SDS-PAGE on batch 5. 1-4 are control, xylanase, xylanase/Umamizyme and Umamizyme. "L" represents the sieved part and "S" the solid part.

4.4.2. Batch 5, xylanase/protease batch: Bradford assay

The result in the Bradford assay is presented in the table 20. The protein content obtained in the Bradford is very low. The liquid samples are lower than the first value of the standard curve this implies that these results are not so accurate. According to the Bradford assay the protein concentration is higher in the solids compared to the liquid sample. The solid sample with the Umamizyme enzyme shows highest protein concentration, followed by the solid sample with xylanase enzyme.

Table 20. The protein content according to the Bradford assay of the samples in batch 5. The percentage of the dry weight of dulse is shown in the table.

Sample	Protein content (%)
1L	1.31
2L	0.610
3L	1.61
4L	1.52
1S	5.18
2S	5.78
3S	4.92
4S	6.63

4.4.3. Batch 5, xylanase/protease batch: Chemical assay

The results from the combined xylanase/protease batch, performed by the chemical lab is presented in table 21. It shows considerable higher amount of protein in the solid part compared to the liquid. The protein content is highest in the sample treated

with xylanase. Accordingly, the sample with lowest protein concentration is the liquid sample treated with xylanase only. This indicates good separation of the protein in dulse.

Table 21. The table shows the chemical composition of the samples when analyzed in the chemical lab.

Sample	Water (%)	Fat (%)	Salt (NaCl) (%)	Ash (%)	Protein (%)
1L	7.29	0.14	21.5	34.7	15.1
2L	63.0	0.14	7.92	12.9	12.1
3L	62.3	0.08	6.72	11.1	29.9
4L	14.4	0.15	15.6	25.8	26.9
1S	6.42	0.44	6.43	12.6	40.2
2S	6.70	1.60	4.42	8.74	53.4
3S	6.86	5.41	4.04	9.48	36.0
4S	7.23	3.86	5.44	11.8	32.0

The samples 2L and 3L were diluted in 5 ml of water in order to dissolve the sugars in the samples in all analyzes except for the protein analyze. Thus, the dilution should be taken into consideration, the samples are recalculated to show the percentage of each component for the undiluted samples (for all components except the protein since it will be the same). The results for the undiluted sample 2L and 3L are shown in table 22.

Table 22. The chemical composition of undiluted 2L and 3L samples obtained from the chemical assay.

Sample	Water (%)	Fat (%)	Salt (NaCl) (%)	Ash (%)	Protein (%)
2L	11.0	0.240	19.0	31.0	12.1
3L	11.4	0.235	15.8	26.1	29.9

4.4.4. Batch 5, xylanase/protease batch: TLC

The result from the TLC is depicted in figure 17. The samples are labeled as the previous assays of batch 5. The liquid fraction, which passed the sieve, shows higher presence of smaller sugars such as monosugars, diose, triose and tetrose compared to the solids. The solids show only some of the smaller sugars but seem to have larger polysaccharides left in the bottom of the plate. In the control of the liquid fraction, without enzyme treatment, there are only few monosugars visible. This can be seen as a band in the top. Most of the polysaccharides in the sample have remained visible as a band in the bottom of the plate. The xylanase and both xylanase and Umamizyme in the liquid part have somewhat similar pattern showing no polysaccharides left in the bottom of the plate and quite a lot of oligosaccharides in the top. The sample with only Umamizyme in the liquid part seem to have triose, tetrose and smaller sugars present.

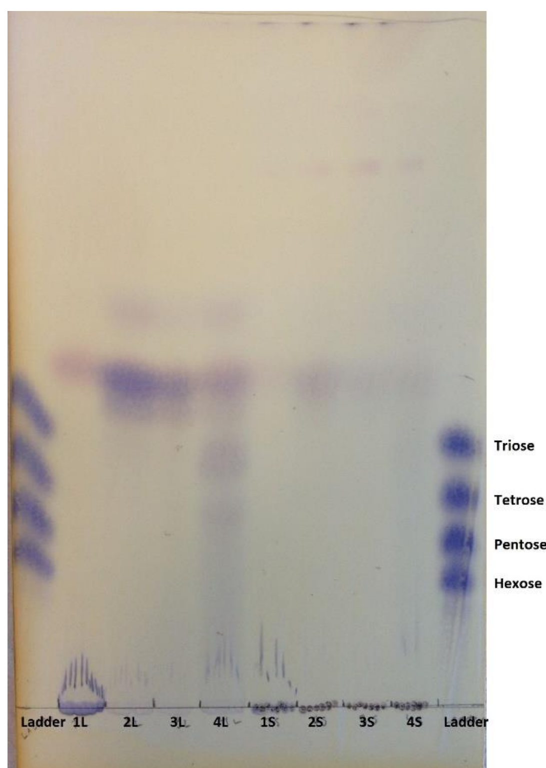

Figure 17. TLC performed on the samples in batch 5. Number 1 is the control, 2 xylanase, 3 xylanase/Umamizyme and 4 Umamizyme only. "L" are the sieved samples and "S" are the solid ones.

4.4.5. Batch 5, xylanase/protease batch: HPAEC-PAD

The results from HPAEC-PAD showed no presence of xylose in any sample except in 2L, which is the sieved sample treated with xylanase. The relative area in this case was 71.01%, which is very high. These results seem to be inaccurate and are therefore excluded. The chromatograms are presented in APPENDIX C.

5. DISCUSSION

The first step in the project was to use proteases, these results showed limited success. Hence, the strategy was changed and xylanase which hydrolyse xylan was used instead, alone and in combination with proteases and other treatment. The most successful approach according to this study was utilization of the xylanase only. In another study by Fleurence et al., *P. palmata* was incubated 14 h with xylanase in order to extract the proteins. The results in this study was proved successful (Fleurence et al. 1995)

In the first protease batch the Bradford did not show any or very low results. Similar results (absence of protein) were the case in the SDS-PAGE. An explanation to the absence of proteins in the samples could be that they were too diluted, and thus have too low concentration of proteins to be detected by these methods. Succeeding batches were designed in order to get higher concentrations.

In the untreated batch 2 and protease mixture batch 3, both results from Bradford assay and chemical assay (table 9-11) show that the protease digested samples have a higher protein content compared to the undigested samples. It seems like the utilization of proteases increase the protein content in the samples, even though there seem to be no difference in concentration between the different enzymes/enzyme mixture used. Most proteins seem to end up in the solid part after sieving, although there is a considerable part of proteins left in the sieved fraction. For batch 3 the aim was to use the proteases to hydrolyse the proteins into smaller pieces and thus let the proteins/peptides into the sieved part and remove the proteins from the solids. This was not the case. The conclusion is that the enzymatic digestion of the proteins did not work as expected. One explanation for this could be that the proteins are bound to the xylan and the proteases have a limited access to them. Nevertheless, the highest protein concentration was found in the solids for the sample treated with Umamizyme, 46.6%

In the fourth xylanase batch, the results from chemical analysis and Bradford assay (table 16 and 17) showed inconsistency when comparing the permeate results. Despite this, the protein content was a bit low in both permeate and retentate when comparing with literature. This may be explained by the higher protein content in the solids, almost 40 %. It seems like most of the protein and peptides stayed in the solid part. There is no significant difference between the different xylanase and sonication treatments. An explanation could be the amount of xylanase was very low compared to the amount xylanase used in the subsequent combined xylanase/protease batch 5.

The amount of protein in xylanase/protease batch, reveal interesting results. The Bradford assay (table 20) showed low protein content in comparison to the chemical analysis (table 21), indicating possible errors in the measurements. According to chemical analysis of the protein content, the solids of the xylanase sample showed highest value, 53.4%, compared to the other results in previous batches. This result may indicate that extraction and

separation of proteins from polysaccharides by using xylanase is possible and a method with potential for further research and development.

When comparing the protein content when using Bradford assay and chemical analysis, the amount of protein is lower in the Bradford assay. An explanation for this could be that the coefficient used in the chemical assay is 6.25 which is considered to be too high for red seaweed. Thus, the estimated protein content in the chemical analysis is likely slightly high. On the other hand, the Bradford only gives reading for whole proteins and excludes smaller peptides, which possibly have been cut by the enzymes. In general, free amino acids, peptides and proteins below 3 kDa are not detected with this method (Thermo Fisher 2016). Thus, the Bradford may show an underestimation of the protein/peptide content in the samples treated with proteases.

The SDS-PAGE showed in all cases (except the first batch, the protease batch, where no bands were visible) that the solid fraction contained more proteins than the sieved part. When using proteases only, the protein profile seems to be similar in all cases, no matter which protease or combination that was used. However, when xylanase was used (figure 16) there was a difference between the samples. Xylanase only showed the strongest bands indicating highest concentration protein. This is further confirmed in the chemical analysis discussed above. A reason for this could be that the xylanase cut polysaccharides attached to proteins allowing them to separate and thus get a higher protein concentration. The SDS-PAGE also showed that xylanase and control had a similar protein profile whereas both xylanase and Umamizyme showed similar profile as Umamizyme only. An explanation to this could be that Umamizyme cut the proteins generating different bands compared to the xylanase which only cuts the polysaccharides.

The TLC shows that all samples in the untreated batch and the protease mixture batch contains both, polysaccharides, oligosaccharides and monosaccharides (figure 8). The amount sugar in the samples is quite similar, it does not vary much between solids and sieved. It does not seem to vary much between the different samples either. Hence, the utilization of proteases does not seem to be an efficient method to separate polysaccharides from proteins. In order to solve this problem, utilization of xylanases to cut xylan, which is the most predominant polysaccharide in dulse, was suggested.

The TLC in the first batch (figure 5) showed presence of oligo- and monosaccharides in all samples, the retentate also contained larger polysaccharides visible in the bottom of the plate. Thus, the there is no difference in the polysaccharide content between the different enzymes. The filtration seems to separate the polysaccharides in the retentate from smaller oligo- and monosaccharides in both permeate and retentate.

In the second untreated batch and the third protease mixture batch, the smaller sugars are present in both the sieved and solid part. The bands are more distinct in the sieved part it may be due to higher concentration of sugars in these samples. There are also samples left in the well, which indicates that there are larger polysaccharides present in the samples.

Another noticeable thing is that the protease digested sample contain more of smaller sugars than the control sample. Again, the different appearance in these batches seem to lie in the filtration process and not the different enzymes used.

In the fourth xylanase batch, it is assumably, more of the smaller mono- and oligosaccharides and less of the polysaccharides in the enzyme treated samples compared to the untreated ones. According to the TLC performed on the samples in this batch (figure 11), this seems not to be the case; the different samples with different enzyme and sonication treatments show similar results. However, it seems like there is a difference between the retentate and permeate of the samples which is, like previously, likely due to the filtration process. Digestion with xylanase in the xylanase batch did not provide the desired results, which arise the question whether the xylanase works and is active. TLC of the xylanase with xylan from birch and dulse extract confirms that the enzyme is active. Another explanation could be that the amount of enzyme used in this batch is too low, resulting in poor or no activity of the enzyme. In the subsequent fifth batch, a larger quantity of the xylanase is produced and used in order to increase the share of enzyme and thereby increase the total xylanase activity performed by the enzyme.

The TLC in xylanase/protease batch (figure 17) shows that the monosugars and oligosugars are separated by sieving. The liquid fraction contains more oligosugars and less polysaccharides compared to the solid part. The liquid samples containing xylanase (2L and 3L) contain more of the smaller oligosaccharides compared to the other samples, implying that the utilization of this enzyme generates smaller units from the larger polysaccharides. The sample with only xylanase seems to work best. Thus, the enzyme works as desired.

The results from HPEAC-PAD showed inconsistency, which complicates to make conclusions from this assay. A reason for this could be that no amino trap was used which may have let free amino acids interfere with the result. Another explanation could be that the pretreatment of the sample might not completely degrade the polysaccharides into monosaccharides. Normally, the method is a good way to detect and quantify polysaccharides within a sample but in this case, the method did not work as desired.

The phenol-sulfuric acid method does not seem to show any difference in polysaccharide content between the samples in the second and third batch. All samples in these batches seem to have a polysaccharide content between 52 and 89.7%. According to literature, this is a bit high. In the studies mentioned in the introduction, *P. palmata* has a polysaccharide content between approximately 23-67%. Seasonal and location where it is harvested could have an impact on the result. The result obtained from the xylanase batch show unreasonably high values and are thus not reliable. Previous problems with the spectrophotometer could have contributed to the deviating results.

6. CONCLUSION

Utilization of xylanases in order to separate the proteins from the most abundant polysaccharide, xylan, in dulse seem to be a successful method. Both according to analyses performed in this study and in previous studies.

Further research is required in order to optimize the amount (and activity) of the xylanase and also additional methods for separation of the two components are needed. The findings in this research are a good start for future research which may lead to optimized utilization of dulse which has a great potential within the feed and human food industries.

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8. APPENDICES

APPENDIX A

Procedure of purification of xylanase from *E. coli*

1. Isolation of plasmid DNA
    - 4 different xylanase clones will be used.
    - Clones from storage (freezer) will be cultured on plate (Solveig does this).
    - Colonies will be picked and cultivated in 5-10 ml ampicillin (overnight). (DAY 1)
    - Isolation of plasmid DNA (according to protocol).

2. Transform plasmid DNA into endotoxin free *E. coli* strain
    - Transform plasmid DNA into endotoxin free strain (BL21 derivate). Put on plates overnight
    - Pick colonies from transformation plate, overnight culture 5-10 mL. (DAY 2)
    - Dilute overnight culture 1/50 dilution.

3. Induction of plasmid with IPTG and rhamnose
    - Induce expression of the plasmid in the bacteria and cultivate, in order to express the xylanase (Intracellular).
    - Induce pHoB1with IPTG at ~OD 0.6
    - Induce the other plasmids with rhamnose at ~OD 1.0
    - After induction, cultivate at 25°C overnight (DAY 3)
    - Centrifuge the culture.
    - Harvest the cells (the pellet) and dissolve in H_2O or KP buffer (10 mM). Use approximately 100 mL cells in ~ 3 mL buffer.
    - Lyse the cells with sonication
    - Centrifuge the samples to separate the insoluble part from the soluble part. (The insoluble part, the pellet, should contain the intact xylanase) ➐ crude extract
    - Dissolve the insoluble part in buffer (~3 mL)

4. Control presence of the xylanase with SDS-PAGE.
    - Run samples, 10 µl on SDS-PAGE.
    - (Dilute the sample x10) 1 µl sample and 10 µl water
    - Add 1 µl sample, 9 µl water and 3 µl SDS buffer, to a PCR plate.
    - Boil in 90 °C for 5 minutes in a PCR machine.
    - Take gel (12 % acrylamide), take of strip and place in holder. The plastic plate thing should face the holder. Put in SDS container.
    - Take out comb.
    - Fill PCR container with buffer, to mark “2 gels”
    - Add sample and ladders carefully to the wells in the gel.
    - Attach cords to SDS device.
    - Adjust settings on the SDS device. (200 V, 30 mA (one gel) for 45 mins)
    - Press stop, remove cords, empty buffer.
    - Open holder with green device.
    - Put gel in plastic box. Wash with water and throw away the water.
    - Add buffer A for 1 minute in microwave. Leave to shake for 1 hour.
    - Remove buffer A and add buffer B, heat 1 minute in microwave oven. Discard buffer.
    - Add buffer C to gel, microwave for 1 minute. Discard buffer.

- Add buffer D to gel put in microwave for 1 minute. Put a piece of paper on top and put in shaker for one hour. Remove buffer. Proteins visible!
- Put gel in plastic bag and seal.

5. Denaturation of other proteins with heating to separate xylanase from other proteins
    - Heat precipitate and incubate at 60 °C for 30 minutes (If possible check temperature optimum). The designated xylanase is thermostable.

6. Activity test of enzyme extract
    - Use enzyme extract for activity tests. Use xylane as substrate. Pure xylane from E.S. Sigma is used as positive control and the dulse extract is used as sample.

Time schedule

Week 14. 4-8/4

Monday: Preparation for plasmid isolation. To the step when cultivating with ampicillin.

Tuesday: Isolation of plasmid DNA, Transformation of plasmid to overnight culture.

Wednesday: Dilution of overnight culture. Induction of plasmids to cultivation at 25 °C overnight.

Thursday: Centrifuge, lyse cells, separate soluble from insoluble, SDS-PAGE, denaturation, activity tests.

Friday: Activity tests. If necessary repeats. Evaluate results.

APPENDIX B

Protein content analysis with DUMAS method:
The sample is burned in a combustion tube. Oxygen is pumped into the tube, and nitrogen compounds will burn and form N_2 and NO_x. Halogen compounds in the samples form volatile halogen compounds and sulfur forms SO_2 of SO_3. Hydrogen forms H_2O and carbon forms CO_2.
CO_2 transfers the gas in the sample from the combustion tube to the post-combustion tube and reduction tube. The gas is freeze-dried in a condenser after the post-combustion tube and then the remaining liquid is dried in a dry tube that contains sicapent. In reduction tube, NO_x compounds are transferred to tungsten to form N_2 and remaining oxygen is bound. The sulfur is linked with tungsten, and volatile halogen compounds are linked with silver wool. Nitrogen in the gas current is measured in a thermal conductivity detector that sends electrical poles to a computer where the results are reported with a graph, and the amount is calculated using a nitrogen factor of 6,25.
Ref ISO 1663-1 (2008)

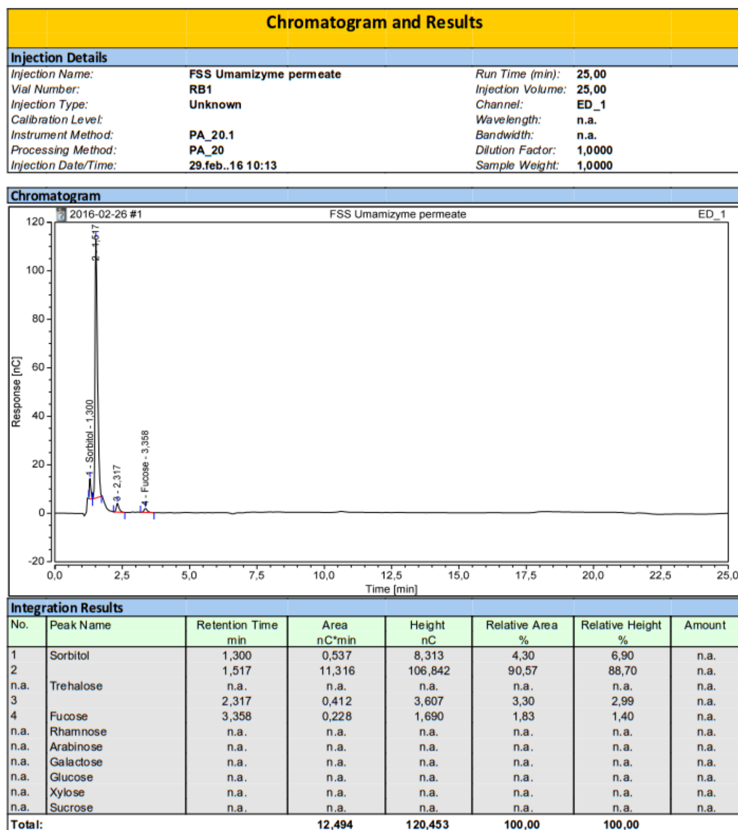
Water content analysis:
The sample is heated in a heating oven at 103°C +/-2°C for four hours. Water corresponds to the weight loss.
Ref. ISO 6496 (1983).

Ash content analysis:
The sample is ashed at 550°C, and the residue is weighed.
Ref. ISO 5984-1978 (E).

Fat content analysis:
The sample is extracted with petroleum ether, boiling range 40-60°C. The extraction apparatus is 2050 Soxtec Avanti Automatic System.
Ref. AOCS Official Method Ba-3-38 with modifications according to Application note Tecator no AN 301.

APPENDIX C

HPAEC-PAD using PA20 column:



Chromatogram and Results

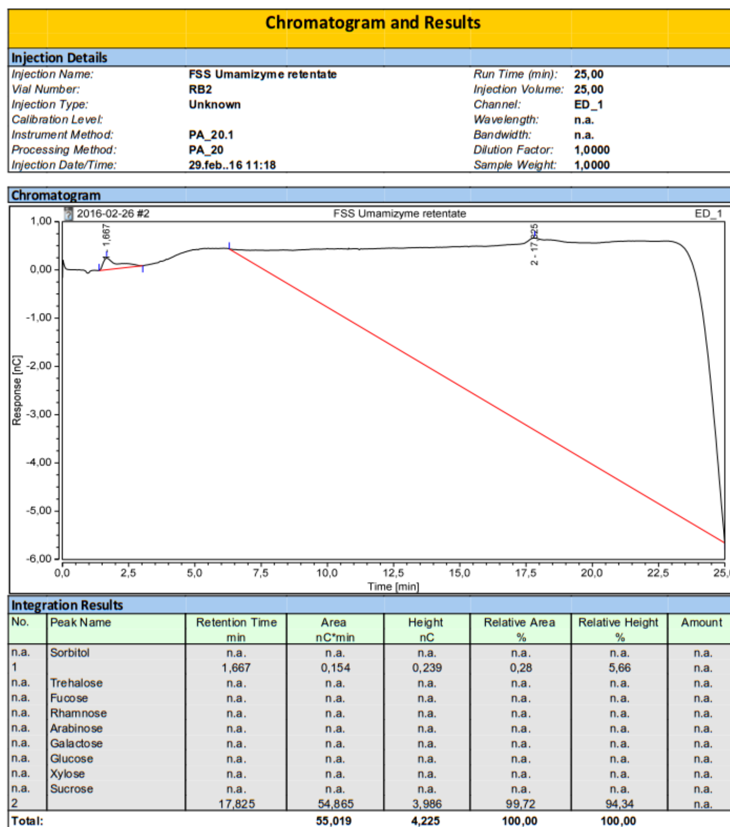
Injection Details

Injection Name:	**FSS Umamizyme permeate**	*Run Time (min):*	**25,00**
Vial Number:	**RB1**	*Injection Volume:*	**25,00**
Injection Type:	**Unknown**	*Channel:*	**ED_1**
Calibration Level:		*Wavelength:*	**n.a.**
Instrument Method:	**PA_20.1**	*Bandwidth:*	**n.a.**
Processing Method:	**PA_20**	*Dilution Factor:*	**1,0000**
Injection Date/Time:	**29.feb..16 10:13**	*Sample Weight:*	**1,0000**

Chromatogram

Integration Results

No.	Peak Name	Retention Time min	Area nC*min	Height nC	Relative Area %	Relative Height %	Amount
1	Sorbitol	1,300	0,537	8,313	4,30	6,90	n.a.
2		1,517	11,316	106,842	90,57	88,70	n.a.
n.a.	Trehalose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
3		2,317	0,412	3,607	3,30	2,99	n.a.
4	Fucose	3,358	0,228	1,690	1,83	1,40	n.a.
n.a.	Rhamnose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Arabinose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Galactose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Glucose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Xylose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Sucrose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Total:			**12,494**	**120,453**	**100,00**	**100,00**	



Chromatogram and Results

Injection Details

Injection Name:	**FSS Umamizyme retentate**	*Run Time (min):*	**25,00**
Vial Number:	**RB2**	*Injection Volume:*	**25,00**
Injection Type:	**Unknown**	*Channel:*	**ED_1**
Calibration Level:		*Wavelength:*	**n.a.**
Instrument Method:	**PA_20.1**	*Bandwidth:*	**n.a.**
Processing Method:	**PA_20**	*Dilution Factor:*	**1,0000**
Injection Date/Time:	**29.feb..16 11:18**	*Sample Weight:*	**1,0000**

Chromatogram

Integration Results

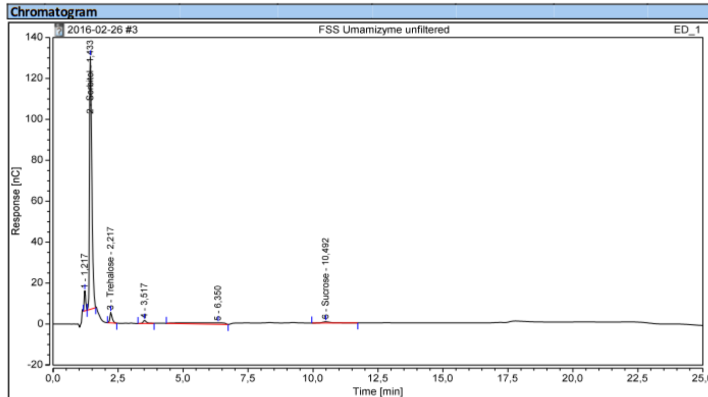
No.	Peak Name	Retention Time min	Area nC*min	Height nC	Relative Area %	Relative Height %	Amount
n.a.	Sorbitol	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
1		1,667	0,154	0,239	0,28	5,66	n.a.
n.a.	Trehalose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Fucose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Rhamnose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Arabinose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Galactose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Glucose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Xylose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Sucrose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
2		17,825	54,865	3,986	99,72	94,34	n.a.
Total:			**55,019**	**4,225**	**100,00**	**100,00**	

Chromatogram and Results

Injection Details

Injection Name:	**FSS Umamizyme unfiltered**	*Run Time (min):*	**25,00**
Vial Number:	**RB3**	*Injection Volume:*	**25,00**
Injection Type:	**Unknown**	*Channel:*	**ED_1**
Calibration Level:		*Wavelength:*	**n.a.**
Instrument Method:	**PA_20.1**	*Bandwidth:*	**n.a.**
Processing Method:	**PA_20**	*Dilution Factor:*	**1,0000**
Injection Date/Time:	**29.feb..16 12:16**	*Sample Weight:*	**1,0000**

Chromatogram



Integration Results

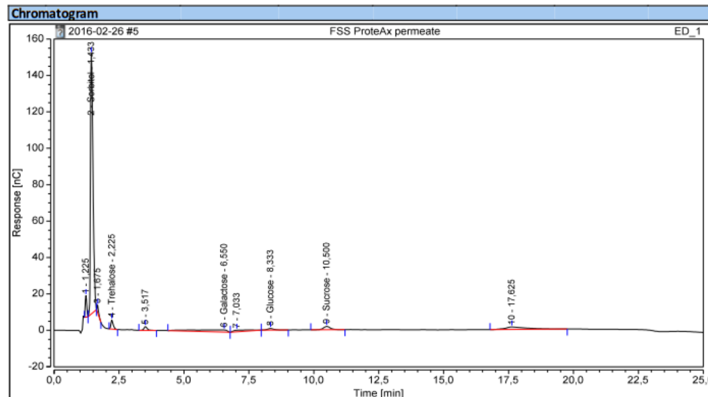
No.	Peak Name	Retention Time min	Area nC*min	Height nC	Relative Area %	Relative Height %	Amount
1		1,217	0,654	9,822	4,25	6,97	n.a.
2	Sorbitol	1,433	12,725	123,264	82,70	87,48	n.a.
3	Trehalose	2,217	0,516	5,013	3,35	3,56	n.a.
n.a.	Fucose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
4		3,517	0,211	1,520	1,37	1,08	n.a.
n.a.	Rhamnose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Arabinose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
5		6,350	1,043	0,701	6,78	0,50	n.a.
n.a.	Galactose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Glucose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Xylose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
6	Sucrose	10,492	0,237	0,588	1,54	0,42	n.a.
Total:			**15,387**	**140,908**	**100,00**	**100,00**	

Chromatogram and Results

Injection Details

Injection Name:	**FSS ProteAx permeate**	*Run Time (min):*	**25,00**
Vial Number:	**RB4**	*Injection Volume:*	**25,00**
Injection Type:	**Unknown**	*Channel:*	**ED_1**
Calibration Level:		*Wavelength:*	**n.a.**
Instrument Method:	**PA_20.1**	*Bandwidth:*	**n.a.**
Processing Method:	**PA_20**	*Dilution Factor:*	**1,0000**
Injection Date/Time:	**29.feb..16 15:25**	*Sample Weight:*	**1,0000**

Chromatogram



Integration Results

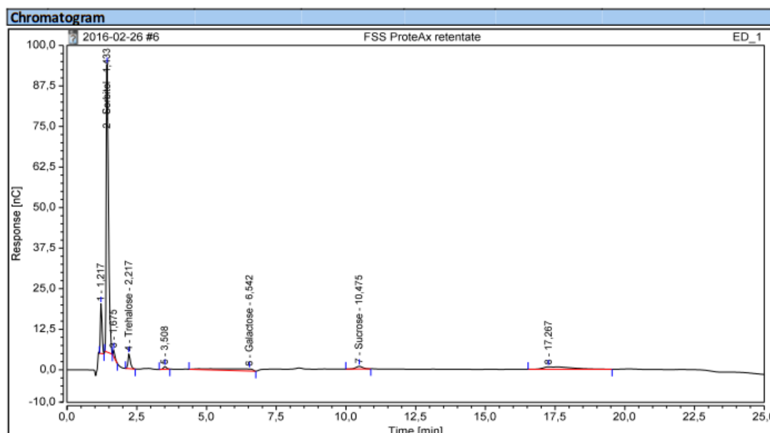
No.	Peak Name	Retention Time min	Area nC*min	Height nC	Relative Area %	Relative Height %	Amount
1		1,225	0,789	11,746	3,55	6,83	n.a.
2	Sorbitol	1,433	15,930	142,999	71,59	83,17	n.a.
3		1,675	0,259	4,122	1,17	2,40	n.a.
4	Trehalose	2,225	0,508	4,963	2,28	2,89	n.a.
n.a.	Fucose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
5		3,517	0,307	2,139	1,38	1,24	n.a.
n.a.	Rhamnose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Arabinose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
6	Galactose	6,550	1,649	1,121	7,41	0,65	n.a.
7		7,033	0,570	0,834	2,56	0,48	n.a.
8	Glucose	8,333	0,238	0,835	1,07	0,49	n.a.
n.a.	Xylose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
9	Sucrose	10,500	0,584	1,823	2,63	1,06	n.a.
10		17,625	1,417	1,350	6,37	0,78	n.a.
Total:			**22,251**	**171,932**	**100,00**	**100,00**	

Chromatogram and Results

Injection Details

Injection Name:	**FSS ProteAx retentate**	*Run Time (min):*	**25,00**
Vial Number:	**RB5**	*Injection Volume:*	**25,00**
Injection Type:	**Unknown**	*Channel:*	**ED_1**
Calibration Level:		*Wavelength:*	**n.a.**
Instrument Method:	**PA_20.1**	*Bandwidth:*	**n.a.**
Processing Method:	**PA_20**	*Dilution Factor:*	**1,0000**
Injection Date/Time:	**29.feb..16 16:23**	*Sample Weight:*	**1,0000**

Chromatogram



Integration Results

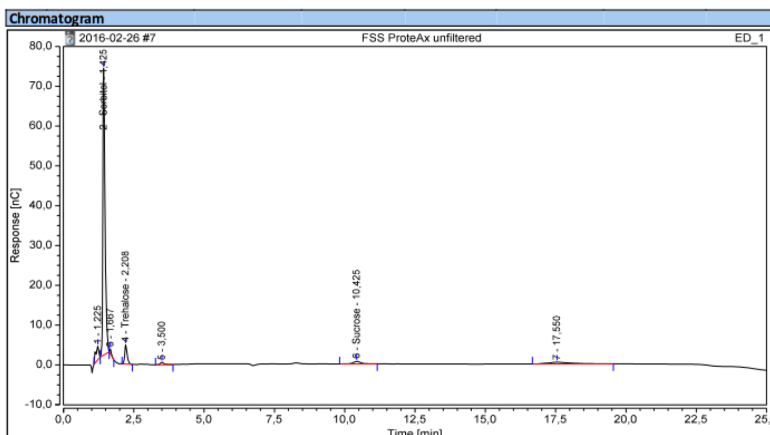
No.	Peak Name	Retention Time min	Area nC*min	Height nC	Relative Area %	Relative Height %	Amount
1		1,217	1,091	15,305	9,05	13,49	n.a.
2	Sorbitol	1,433	8,254	88,598	68,50	78,07	n.a.
3		1,675	0,136	2,080	1,13	1,83	n.a.
4	Trehalose	2,217	0,452	4,526	3,75	3,99	n.a.
n.a.	Fucose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
5		3,508	0,100	0,821	0,83	0,72	n.a.
n.a.	Rhamnose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Arabinose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
6	Galactose	6,542	0,905	0,613	7,51	0,54	n.a.
n.a.	Glucose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Xylose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
7	Sucrose	10,475	0,224	0,798	1,86	0,70	n.a.
8		17,267	0,888	0,746	7,37	0,66	n.a.
Total:			**12,049**	**113,486**	**100,00**	**100,00**	

Chromatogram and Results

Injection Details

Injection Name:	**FSS ProteAx unfiltered**	*Run Time (min):*	**25,00**
Vial Number:	**RB6**	*Injection Volume:*	**25,00**
Injection Type:	**Unknown**	*Channel:*	**ED_1**
Calibration Level:		*Wavelength:*	**n.a.**
Instrument Method:	**PA_20.1**	*Bandwidth:*	**n.a.**
Processing Method:	**PA_20**	*Dilution Factor:*	**1,0000**
Injection Date/Time:	**29.feb..16 17:21**	*Sample Weight:*	**1,0000**

Chromatogram



Integration Results

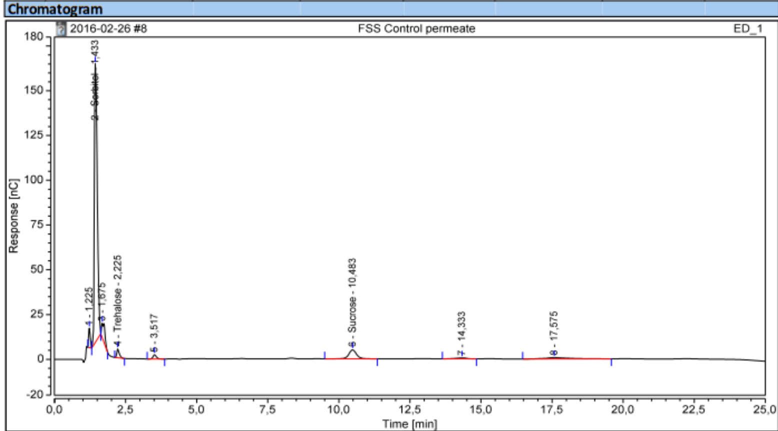
No.	Peak Name	Retention Time min	Area nC*min	Height nC	Relative Area %	Relative Height %	Amount
1		1,225	0,428	3,352	5,13	4,04	n.a.
2	Sorbitol	1,425	6,607	71,967	79,28	86,69	n.a.
3		1,667	0,075	1,224	0,90	1,47	n.a.
4	Trehalose	2,208	0,482	4,785	5,78	5,76	n.a.
n.a.	Fucose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
5		3,500	0,088	0,627	1,06	0,76	n.a.
n.a.	Rhamnose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Arabinose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Galactose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Glucose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Xylose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
6	Sucrose	10,425	0,194	0,605	2,33	0,73	n.a.
7		17,550	0,460	0,453	5,52	0,55	n.a.
Total:			**8,333**	**83,013**	**100,00**	**100,00**	

Chromatogram and Results

Injection Details

Injection Name:	**FSS Control permeate**	*Run Time (min):*	**25,00**
Vial Number:	**RB7**	*Injection Volume:*	**25,00**
Injection Type:	**Unknown**	*Channel:*	**ED_1**
Calibration Level:		*Wavelength:*	**n.a.**
Instrument Method:	**PA_20.1**	*Bandwidth:*	**n.a.**
Processing Method:	**PA_20**	*Dilution Factor:*	**1,0000**
Injection Date/Time:	**29.feb..16 18:20**	*Sample Weight:*	**1,0000**

Chromatogram



Integration Results

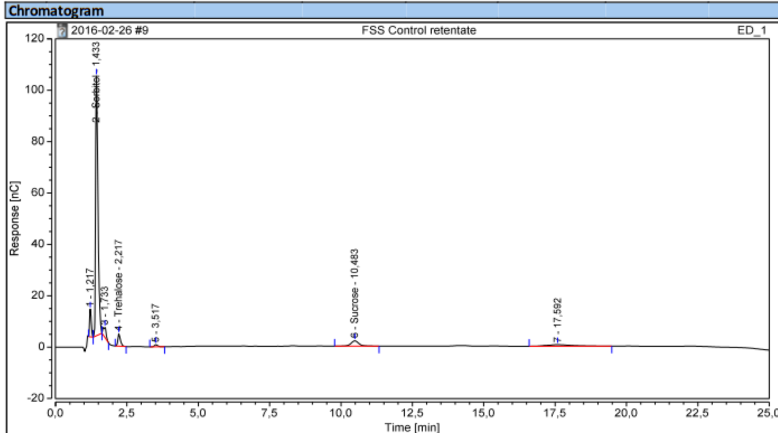
No.	Peak Name	Retention Time min	Area nC*min	Height nC	Relative Area %	Relative Height %	Amount
1		1,225	0,711	10,865	3,01	5,78	n.a.
2	Sorbitol	1,433	18,237	155,673	77,15	82,76	n.a.
3		1,675	1,224	7,913	5,18	4,21	n.a.
4	Trehalose	2,225	0,498	4,875	2,11	2,59	n.a.
n.a.	Fucose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
5		3,517	0,331	2,464	1,40	1,31	n.a.
n.a.	Rhamnose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Arabinose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Galactose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Glucose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Xylose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
6	Sucrose	10,483	1,627	5,049	6,88	2,68	n.a.
7		14,333	0,264	0,540	1,12	0,29	n.a.
8		17,575	0,746	0,721	3,16	0,38	n.a.
Total:			**23,638**	**188,098**	**100,00**	**100,00**	

Chromatogram and Results

Injection Details

Injection Name:	**FSS Control retentate**	*Run Time (min):*	**25,00**
Vial Number:	**RB8**	*Injection Volume:*	**25,00**
Injection Type:	**Unknown**	*Channel:*	**ED_1**
Calibration Level:		*Wavelength:*	**n.a.**
Instrument Method:	**PA_20.1**	*Bandwidth:*	**n.a.**
Processing Method:	**PA_20**	*Dilution Factor:*	**1,0000**
Injection Date/Time:	**29.feb..16 19:18**	*Sample Weight:*	**1,0000**

Chromatogram



Integration Results

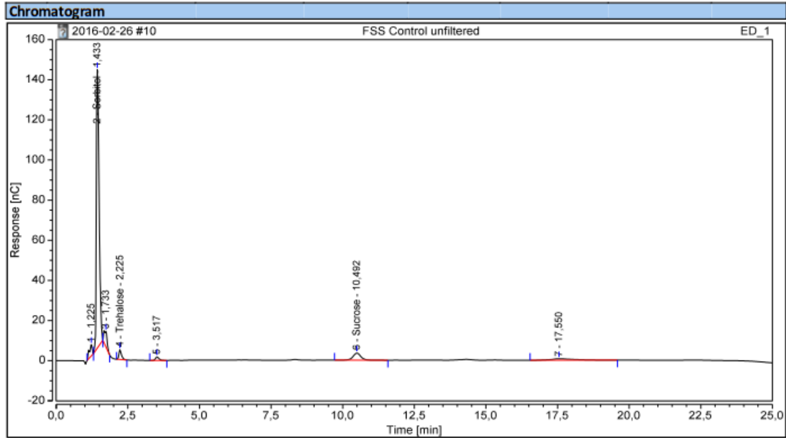
No.	Peak Name	Retention Time min	Area nC*min	Height nC	Relative Area %	Relative Height %	Amount
1		1,217	0,738	10,805	5,72	8,70	n.a.
2	Sorbitol	1,433	9,778	101,023	75,85	81,34	n.a.
3		1,733	0,469	4,021	3,64	3,24	n.a.
4	Trehalose	2,217	0,487	4,789	3,78	3,86	n.a.
n.a.	Fucose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
5		3,517	0,116	0,882	0,90	0,71	n.a.
n.a.	Rhamnose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Arabinose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Galactose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Glucose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Xylose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
6	Sucrose	10,483	0,679	2,086	5,27	1,68	n.a.
7		17,592	0,625	0,589	4,85	0,47	n.a.
Total:			**12,892**	**124,195**	**100,00**	**100,00**	

Chromatogram and Results

Injection Details

Injection Name:	**FSS Control unfiltered**	*Run Time (min):*	**25,00**
Vial Number:	**RC1**	*Injection Volume:*	**25,00**
Injection Type:	**Unknown**	*Channel:*	**ED_1**
Calibration Level:		*Wavelength:*	**n.a.**
Instrument Method:	**PA_20.1**	*Bandwidth:*	**n.a.**
Processing Method:	**PA_20**	*Dilution Factor:*	**1,0000**
Injection Date/Time:	**29.feb..16 20:16**	*Sample Weight:*	**1,0000**

Chromatogram



Integration Results

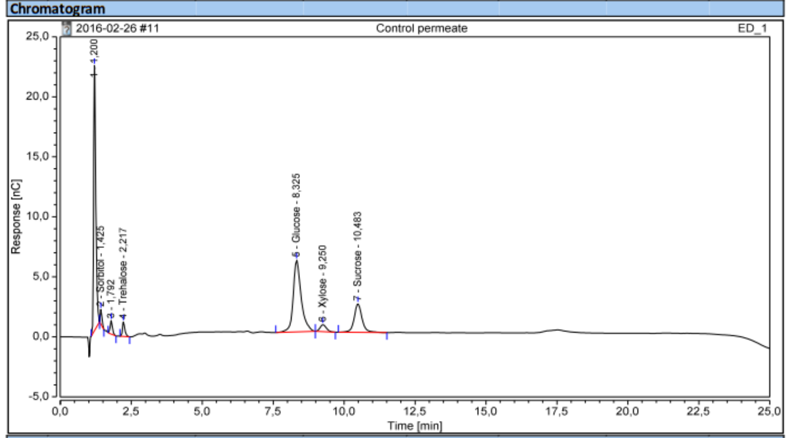
No.	Peak Name	Retention Time min	Area nC*min	Height nC	Relative Area %	Relative Height %	Amount
1		1,225	0,683	5,574	3,52	3,42	n.a.
2	Sorbitol	1,433	15,197	138,719	78,29	85,09	n.a.
3		1,733	0,947	7,797	4,88	4,78	n.a.
4	Trehalose	2,225	0,496	4,816	2,55	2,95	n.a.
n.a.	Fucose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
5		3,517	0,245	1,836	1,26	1,13	n.a.
n.a.	Rhamnose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Arabinose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Galactose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Glucose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Xylose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
6	Sucrose	10,492	1,130	3,546	5,82	2,18	n.a.
7		17,550	0,714	0,734	3,68	0,45	n.a.
Total:			**19,411**	**163,022**	**100,00**	**100,00**	

Chromatogram and Results

Injection Details

Injection Name:	**Control permeate**	*Run Time (min):*	**25,00**
Vial Number:	**RC2**	*Injection Volume:*	**25,00**
Injection Type:	**Unknown**	*Channel:*	**ED_1**
Calibration Level:		*Wavelength:*	**n.a.**
Instrument Method:	**PA_20.1**	*Bandwidth:*	**n.a.**
Processing Method:	**PA_20**	*Dilution Factor:*	**100,0000**
Injection Date/Time:	**29.feb..16 21:14**	*Sample Weight:*	**1,0000**

Chromatogram



Integration Results

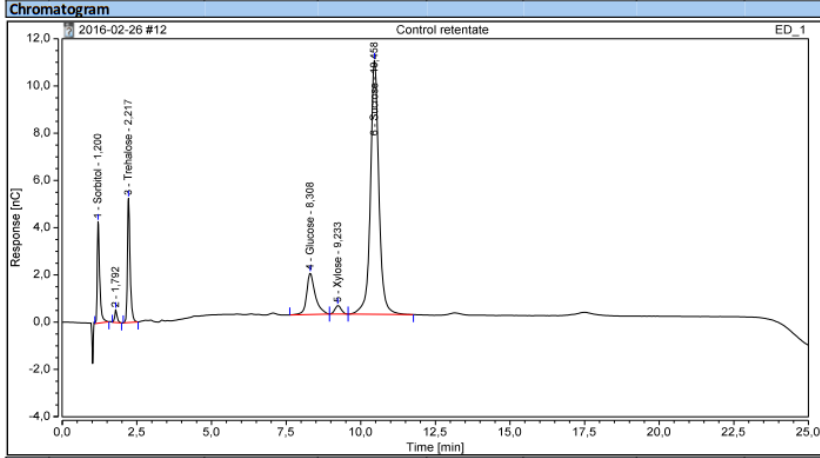
No.	Peak Name	Retention Time min	Area nC*min	Height nC	Relative Area %	Relative Height %	Amount
1		1,200	1,966	22,103	38,32	63,94	n.a.
2	Sorbitol	1,425	0,084	1,204	1,63	3,48	n.a.
3		1,792	0,105	1,125	2,05	3,25	n.a.
4	Trehalose	2,217	0,121	1,198	2,36	3,47	n.a.
n.a.	Fucose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Rhamnose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Arabinose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Galactose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
5	Glucose	8,325	1,938	5,981	37,77	17,30	n.a.
6	Xylose	9,250	0,145	0,569	2,83	1,65	n.a.
7	Sucrose	10,483	0,771	2,387	15,03	6,91	n.a.
Total:			**5,130**	**34,566**	**100,00**	**100,00**	

Chromatogram and Results

Injection Details

Injection Name:	**Control retentate**	*Run Time (min):*	**25,00**
Vial Number:	**RC3**	*Injection Volume:*	**25,00**
Injection Type:	**Unknown**	*Channel:*	**ED_1**
Calibration Level:		*Wavelength:*	**n.a.**
Instrument Method:	**PA_20.1**	*Bandwidth:*	**n.a.**
Processing Method:	**PA_20**	*Dilution Factor:*	**100,0000**
Injection Date/Time:	**29.feb..16 22:13**	*Sample Weight:*	**1,0000**

Chromatogram



Integration Results

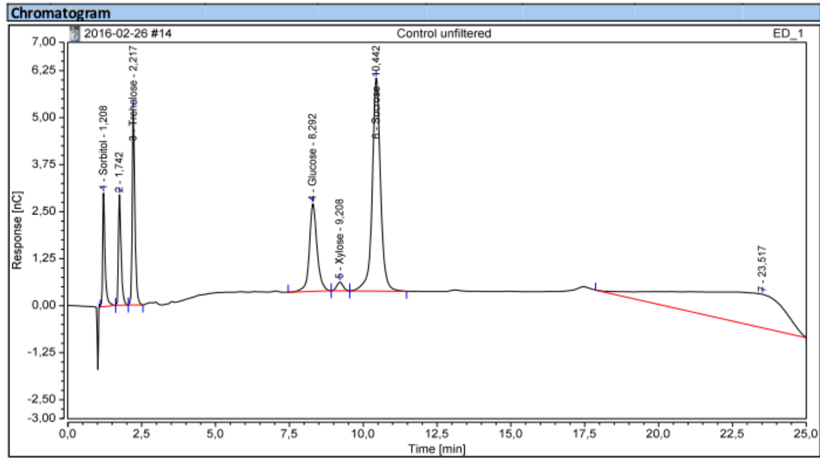
No.	Peak Name	Retention Time min	Area nC*min	Height nC	Relative Area %	Relative Height %	Amount
1	Sorbitol	1,200	0,405	4,300	7,79	18,72	n.a.
2		1,792	0,049	0,539	0,95	2,35	n.a.
3	Trehalose	2,217	0,547	5,258	10,53	22,89	n.a.
n.a.	Fucose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Rhamnose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Arabinose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Galactose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
4	Glucose	8,308	0,580	1,758	11,17	7,65	n.a.
5	Xylose	9,233	0,088	0,360	1,69	1,57	n.a.
6	Sucrose	10,458	3,528	10,754	67,88	46,82	n.a.
Total:			**5,198**	**22,970**	**100,00**	**100,00**	

Chromatogram and Results

Injection Details

Injection Name:	**Control unfiltered**	*Run Time (min):*	**25,00**
Vial Number:	**RC4**	*Injection Volume:*	**25,00**
Injection Type:	**Unknown**	*Channel:*	**ED_1**
Calibration Level:		*Wavelength:*	**n.a.**
Instrument Method:	**PA_20.1**	*Bandwidth:*	**n.a.**
Processing Method:	**PA_20**	*Dilution Factor:*	**100,0000**
Injection Date/Time:	**01.mar..16 00:09**	*Sample Weight:*	**1,0000**

Chromatogram



Integration Results

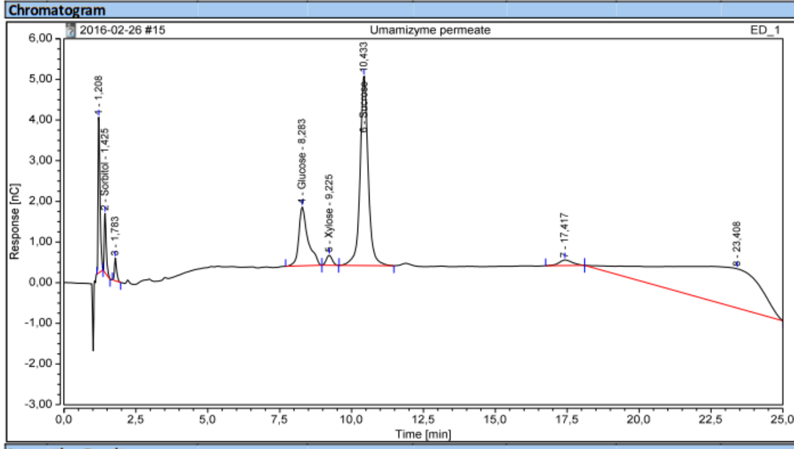
No.	Peak Name	Retention Time min	Area nC*min	Height nC	Relative Area %	Relative Height %	Amount
1	Sorbitol	1,208	0,283	3,020	3,98	14,92	n.a.
2		1,742	0,312	2,935	4,39	14,50	n.a.
3	Trehalose	2,217	0,545	5,187	7,68	25,62	n.a.
n.a.	Fucose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Rhamnose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Arabinose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Galactose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
4	Glucose	8,292	0,701	2,322	9,88	11,47	n.a.
5	Xylose	9,208	0,057	0,231	0,80	1,14	n.a.
6	Sucrose	10,442	1,845	5,658	25,99	27,95	n.a.
7		23,517	3,357	0,892	47,28	4,41	n.a.
Total:			**7,100**	**20,244**	**100,00**	**100,00**	

Chromatogram and Results

Injection Details

Injection Name:	**Umamizyme permeate**	*Run Time (min):*	**25,00**
Vial Number:	**RC5**	*Injection Volume:*	**25,00**
Injection Type:	**Unknown**	*Channel:*	**ED_1**
Calibration Level:		*Wavelength:*	**n.a.**
Instrument Method:	**PA_20.1**	*Bandwidth:*	**n.a.**
Processing Method:	**PA_20**	*Dilution Factor:*	**100,0000**
Injection Date/Time:	**01.mar..16 01:07**	*Sample Weight:*	**1,0000**

Chromatogram



Integration Results

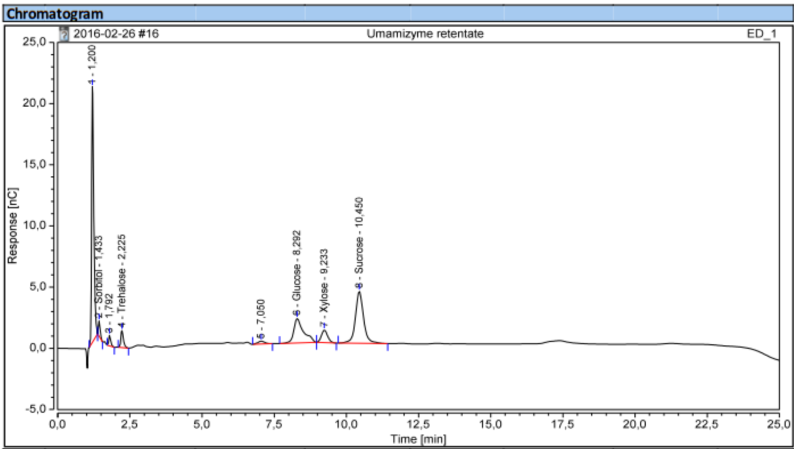
No.	Peak Name	Retention Time min	Area nC*min	Height nC	Relative Area %	Relative Height %	Amount
1		1,208	0,295	3,826	4,72	28,80	n.a.
2	Sorbitol	1,425	0,114	1,451	1,83	10,92	n.a.
3		1,783	0,048	0,558	0,77	4,20	n.a.
n.a.	Trehalose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Fucose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Rhamnose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Arabinose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Galactose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
4	Glucose	8,283	0,537	1,452	8,60	10,93	n.a.
5	Xylose	9,225	0,058	0,245	0,94	1,85	n.a.
6	Sucrose	10,433	1,531	4,650	24,54	35,00	n.a.
7		17,417	0,075	0,138	1,20	1,04	n.a.
8		23,408	3,580	0,964	57,39	7,26	n.a.
Total:			**6,238**	**13,284**	**100,00**	**100,00**	

Chromatogram and Results

Injection Details

Injection Name:	**Umamizyme retentate**	*Run Time (min):*	**25,00**
Vial Number:	**RC6**	*Injection Volume:*	**25,00**
Injection Type:	**Unknown**	*Channel:*	**ED_1**
Calibration Level:		*Wavelength:*	**n.a.**
Instrument Method:	**PA_20.1**	*Bandwidth:*	**n.a.**
Processing Method:	**PA_20**	*Dilution Factor:*	**100,0000**
Injection Date/Time:	**01.mar..16 02:05**	*Sample Weight:*	**1,0000**

Chromatogram



Integration Results

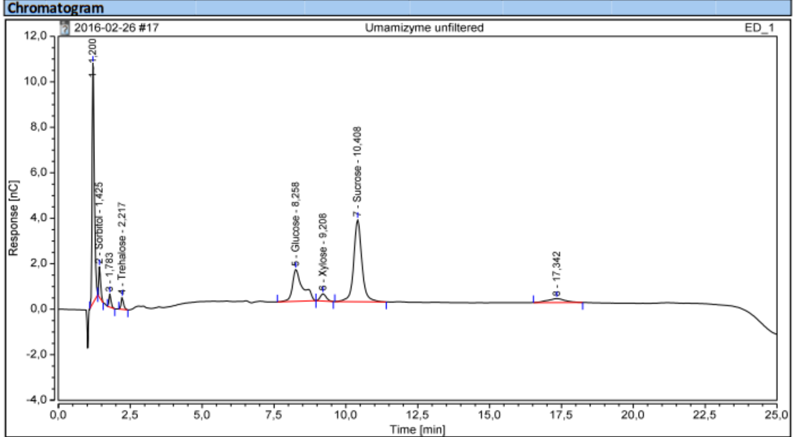
No.	Peak Name	Retention Time min	Area nC*min	Height nC	Relative Area %	Relative Height %	Amount
1		1,200	1,847	20,881	39,82	65,55	n.a.
2	Sorbitol	1,433	0,085	1,237	1,84	3,88	n.a.
3		1,792	0,071	0,853	1,52	2,68	n.a.
4	Trehalose	2,225	0,141	1,383	3,03	4,34	n.a.
n.a.	Fucose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Rhamnose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Arabinose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Galactose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
5		7,050	0,066	0,238	1,42	0,75	n.a.
6	Glucose	8,292	0,781	1,997	16,83	6,27	n.a.
7	Xylose	9,233	0,254	1,015	5,48	3,19	n.a.
8	Sucrose	10,450	1,395	4,253	30,07	13,35	n.a.
Total:			**4,639**	**31,858**	**100,00**	**100,00**	

Chromatogram and Results

Injection Details

Injection Name:	**Umamizyme unfiltered**	*Run Time (min):*	**25,00**
Vial Number:	**RC7**	*Injection Volume:*	**25,00**
Injection Type:	**Unknown**	*Channel:*	**ED_1**
Calibration Level:		*Wavelength:*	**n.a.**
Instrument Method:	**PA_20.1**	*Bandwidth:*	**n.a.**
Processing Method:	**PA_20**	*Dilution Factor:*	**100,0000**
Injection Date/Time:	**01.mar..16 03:04**	*Sample Weight:*	**1,0000**

Chromatogram



Integration Results

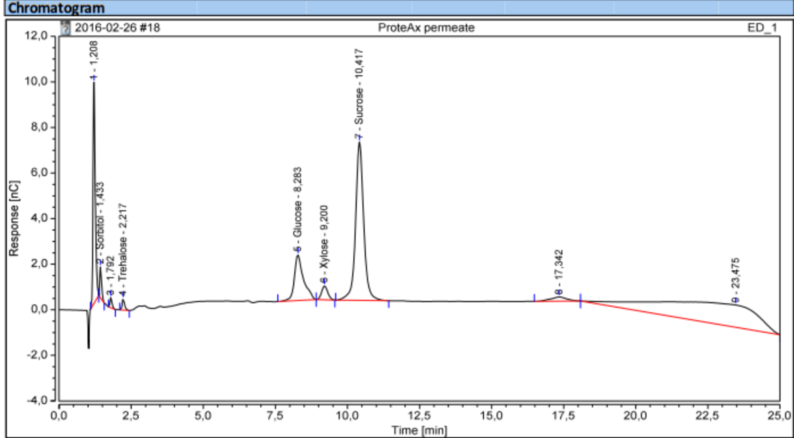
No.	Peak Name	Retention Time min	Area nC*min	Height nC	Relative Area %	Relative Height %	Amount
1		1,200	0,930	10,546	30,03	57,04	n.a.
2	Sorbitol	1,425	0,098	1,335	3,16	7,22	n.a.
3		1,783	0,048	0,574	1,53	3,11	n.a.
4	Trehalose	2,217	0,053	0,526	1,71	2,85	n.a.
n.a.	Fucose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Rhamnose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Arabinose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Galactose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
5	Glucose	8,258	0,582	1,405	18,79	7,60	n.a.
6	Xylose	9,208	0,076	0,317	2,46	1,71	n.a.
7	Sucrose	10,408	1,189	3,614	38,40	19,55	n.a.
8		17,342	0,121	0,170	3,92	0,92	n.a.
Total:			**3,097**	**18,487**	**100,00**	**100,00**	

Chromatogram and Results

Injection Details

Injection Name:	**ProteAx permeate**	*Run Time (min):*	**25,00**
Vial Number:	**RC8**	*Injection Volume:*	**25,00**
Injection Type:	**Unknown**	*Channel:*	**ED_1**
Calibration Level:		*Wavelength:*	**n.a.**
Instrument Method:	**PA_20.1**	*Bandwidth:*	**n.a.**
Processing Method:	**PA_20**	*Dilution Factor:*	**100,0000**
Injection Date/Time:	**01.mar..16 04:02**	*Sample Weight:*	**1,0000**

Chromatogram



Integration Results

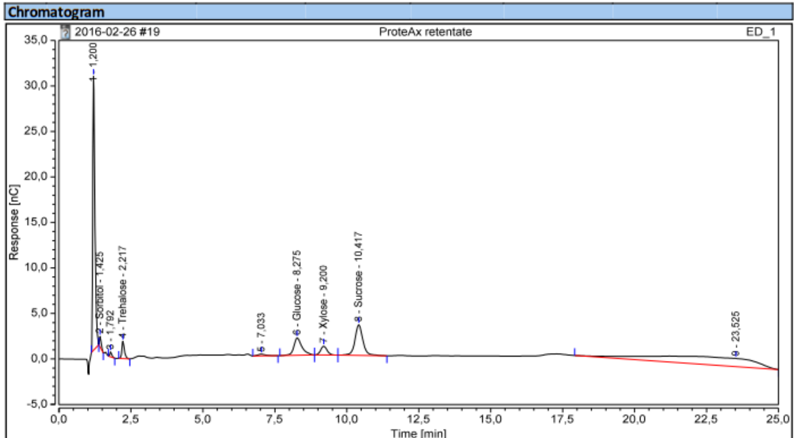
No.	Peak Name	Retention Time min	Area nC*min	Height nC	Relative Area %	Relative Height %	Amount
1		1,208	0,866	9,706	10,74	42,70	n.a.
2	Sorbitol	1,433	0,099	1,357	1,23	5,97	n.a.
3		1,792	0,037	0,450	0,46	1,98	n.a.
4	Trehalose	2,217	0,049	0,490	0,61	2,15	n.a.
n.a.	Fucose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Rhamnose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Arabinose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Galactose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
5	Glucose	8,283	0,737	2,007	9,14	8,83	n.a.
6	Xylose	9,200	0,150	0,607	1,87	2,67	n.a.
7	Sucrose	10,417	2,270	6,953	28,15	30,59	n.a.
8		17,342	0,123	0,185	1,52	0,81	n.a.
9		23,475	3,734	0,977	46,29	4,30	n.a.
Total:			**8,066**	**22,732**	**100,00**	**100,00**	

Chromatogram and Results

Injection Details

Injection Name:	**ProteAx retentate**	*Run Time (min):*	**25,00**
Vial Number:	**RD1**	*Injection Volume:*	**25,00**
Injection Type:	**Unknown**	*Channel:*	**ED_1**
Calibration Level:		*Wavelength:*	**n.a.**
Instrument Method:	**PA_20.1**	*Bandwidth:*	**n.a.**
Processing Method:	**PA_20**	*Dilution Factor:*	**100,0000**
Injection Date/Time:	**01.mar..16 05:00**	*Sample Weight:*	**1,0000**

Chromatogram



Integration Results

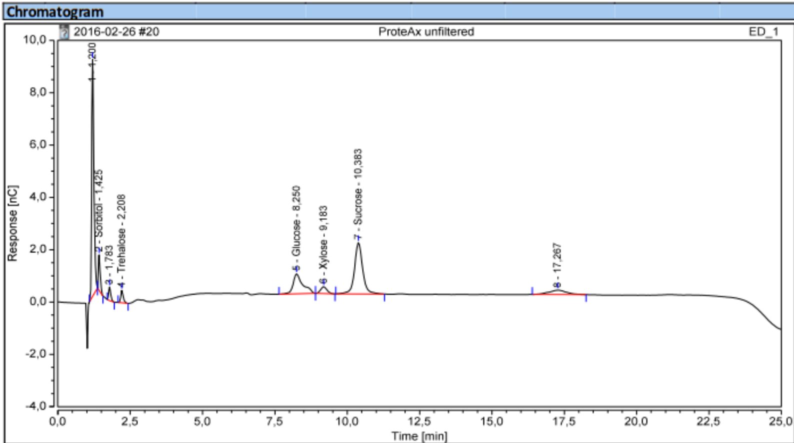
No.	Peak Name	Retention Time min	Area nC*min	Height nC	Relative Area %	Relative Height %	Amount
1		1,200	2,607	30,108	30,37	73,30	n.a.
2	Sorbitol	1,425	0,080	1,154	0,93	2,81	n.a.
3		1,792	0,044	0,552	0,52	1,34	n.a.
4	Trehalose	2,217	0,193	1,896	2,25	4,62	n.a.
n.a.	Fucose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Rhamnose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Arabinose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Galactose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
5		7,033	0,057	0,185	0,67	0,45	n.a.
6	Glucose	8,275	0,623	1,914	7,26	4,66	n.a.
7	Xylose	9,200	0,256	0,981	2,99	2,39	n.a.
8	Sucrose	10,417	1,083	3,378	12,62	8,22	n.a.
9		23,525	3,641	0,905	42,41	2,20	n.a.
Total:			**8,585**	**41,073**	**100,00**	**100,00**	

Chromatogram and Results

Injection Details

Injection Name:	**ProteAx unfiltered**	*Run Time (min):*	**25,00**
Vial Number:	**RD2**	*Injection Volume:*	**25,00**
Injection Type:	**Unknown**	*Channel:*	**ED_1**
Calibration Level:		*Wavelength:*	**n.a.**
Instrument Method:	**PA_20.1**	*Bandwidth:*	**n.a.**
Processing Method:	**PA_20**	*Dilution Factor:*	**100,0000**
Injection Date/Time:	**01.mar..16 05:58**	*Sample Weight:*	**1,0000**

Chromatogram



Integration Results

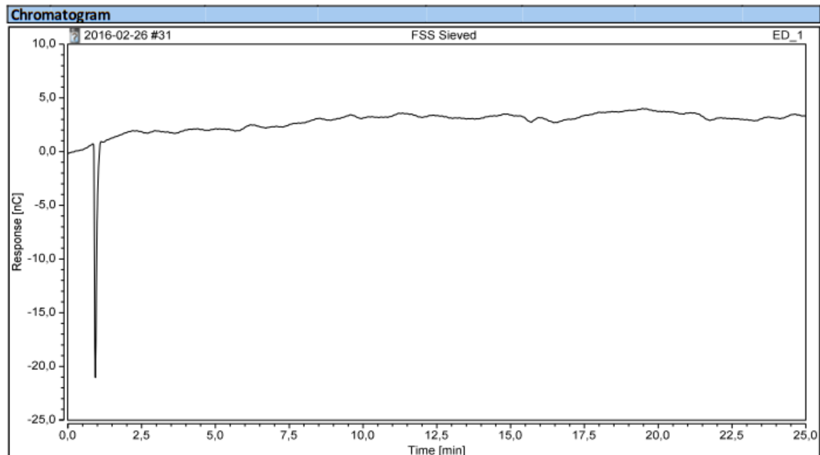
No.	Peak Name	Retention Time min	Area nC*min	Height nC	Relative Area %	Relative Height %	Amount
1		1,200	0,797	9,050	37,76	62,13	n.a.
2	Sorbitol	1,425	0,101	1,364	4,78	9,36	n.a.
3		1,783	0,043	0,518	2,03	3,55	n.a.
4	Trehalose	2,208	0,047	0,473	2,23	3,25	n.a.
n.a.	Fucose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Rhamnose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Arabinose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Galactose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
5	Glucose	8,250	0,296	0,763	14,01	5,24	n.a.
6	Xylose	9,183	0,066	0,264	3,13	1,81	n.a.
7	Sucrose	10,383	0,642	1,966	30,41	13,50	n.a.
8		17,267	0,119	0,168	5,65	1,16	n.a.
Total:			**2,111**	**14,566**	**100,00**	**100,00**	

Chromatogram and Results

Injection Details

Injection Name:	**FSS Sieved**	*Run Time (min):*	**25,00**
Vial Number:	**GE2**	*Injection Volume:*	**25,00**
Injection Type:	**Unknown**	*Channel:*	**ED_1**
Calibration Level:		*Wavelength:*	**n.a.**
Instrument Method:	**PA_20.1**	*Bandwidth:*	**n.a.**
Processing Method:	**PA_20**	*Dilution Factor:*	**1,0000**
Injection Date/Time:	**09.mar..16 17:56**	*Sample Weight:*	**1,0000**

Chromatogram



Integration Results

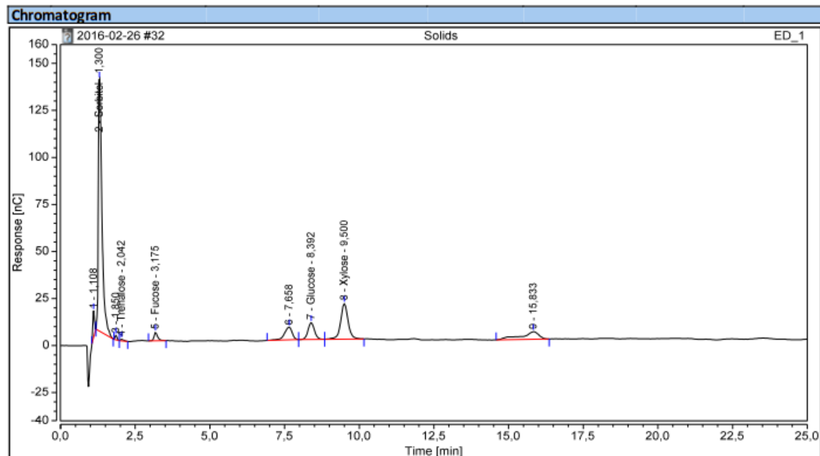
No.	Peak Name	Retention Time min	Area nC*min	Height nC	Relative Area %	Relative Height %	Amount
n.a.	Sorbitol	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Trehalose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Fucose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Rhamnose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Arabinose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Galactose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Glucose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Xylose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Sucrose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Total:			**0,000**	**0,000**	**0,00**	**0,00**	

Chromatogram and Results

Injection Details

Injection Name:	**Solids**	*Run Time (min):*	**25,00**
Vial Number:	**GE3**	*Injection Volume:*	**25,00**
Injection Type:	**Unknown**	*Channel:*	**ED_1**
Calibration Level:		*Wavelength:*	**n.a.**
Instrument Method:	**PA_20.1**	*Bandwidth:*	**n.a.**
Processing Method:	**PA_20**	*Dilution Factor:*	**1,0000**
Injection Date/Time:	**09.mar..16 18:55**	*Sample Weight:*	**1,0000**

Chromatogram



Integration Results

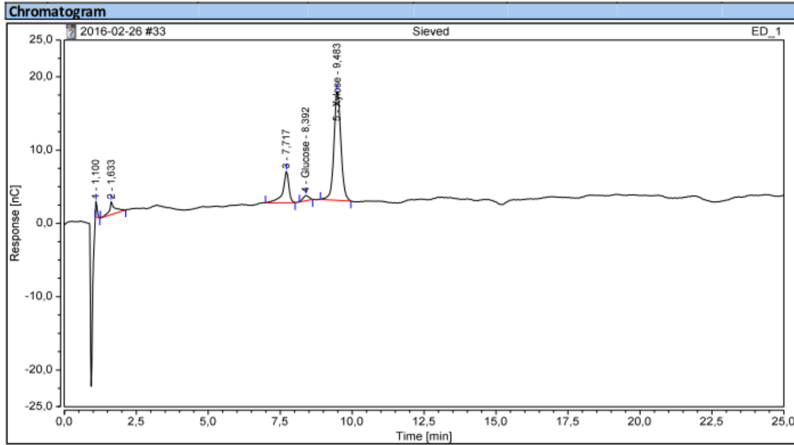
No.	Peak Name	Retention Time min	Area nC*min	Height nC	Relative Area %	Relative Height %	Amount
1		1,108	0,915	14,058	2,76	7,23	n.a.
2	Sorbitol	1,300	18,772	133,916	56,59	68,87	n.a.
3		1,850	0,170	2,406	0,51	1,24	n.a.
4	Trehalose	2,042	0,097	0,847	0,29	0,44	n.a.
5	Fucose	3,175	0,629	4,575	1,90	2,35	n.a.
n.a.	Rhamnose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Arabinose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Galactose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
6		7,658	2,063	6,751	6,22	3,47	n.a.
7	Glucose	8,392	2,241	8,934	6,76	4,59	n.a.
8	Xylose	9,500	5,434	18,885	16,38	9,71	n.a.
n.a.	Sucrose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
9		15,833	2,849	4,068	8,59	2,09	n.a.
Total:			**33,169**	**194,442**	**100,00**	**100,00**	

Chromatogram and Results

Injection Details

Injection Name:	**Sieved**	*Run Time (min):*	**25,00**
Vial Number:	**GE4**	*Injection Volume:*	**25,00**
Injection Type:	**Unknown**	*Channel:*	**ED_1**
Calibration Level:		*Wavelength:*	**n.a.**
Instrument Method:	**PA_20.1**	*Bandwidth:*	**n.a.**
Processing Method:	**PA_20**	*Dilution Factor:*	**1,0000**
Injection Date/Time:	**09.mar..16 19:53**	*Sample Weight:*	**1,0000**

Chromatogram



Integration Results

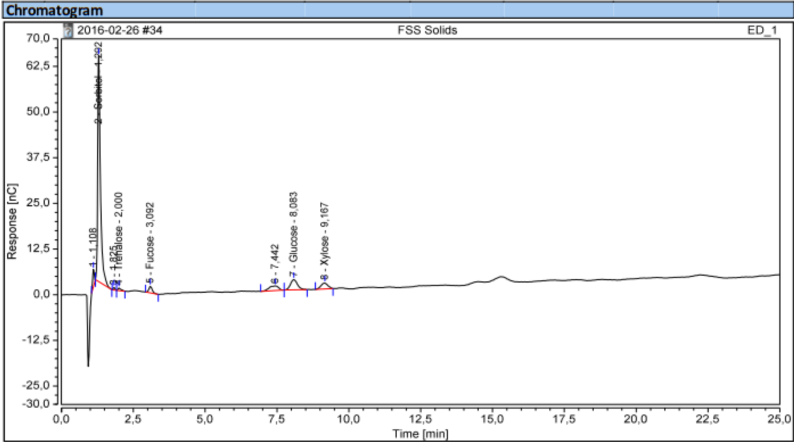
No.	Peak Name	Retention Time min	Area nC*min	Height nC	Relative Area %	Relative Height %	Amount
1		1,100	0,128	2,137	2,18	8,97	n.a.
n.a.	Sorbitol	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
2		1,633	0,403	1,784	6,85	7,49	n.a.
n.a.	Trehalose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Fucose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Rhamnose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Arabinose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Galactose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
3		7,717	1,035	4,319	17,57	18,13	n.a.
4	Glucose	8,392	0,170	0,729	2,89	3,06	n.a.
5	Xylose	9,483	4,152	14,851	70,51	62,35	n.a.
n.a.	Sucrose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Total:			**5,889**	**23,820**	**100,00**	**100,00**	

Chromatogram and Results

Injection Details

Injection Name:	**FSS Solids**	*Run Time (min):*	**25,00**
Vial Number:	**GE1**	*Injection Volume:*	**25,00**
Injection Type:	**Unknown**	*Channel:*	**ED_1**
Calibration Level:		*Wavelength:*	**n.a.**
Instrument Method:	**PA_20.1**	*Bandwidth:*	**n.a.**
Processing Method:	**PA_20**	*Dilution Factor:*	**1,0000**
Injection Date/Time:	**10.mar..16 11:12**	*Sample Weight:*	**1,0000**

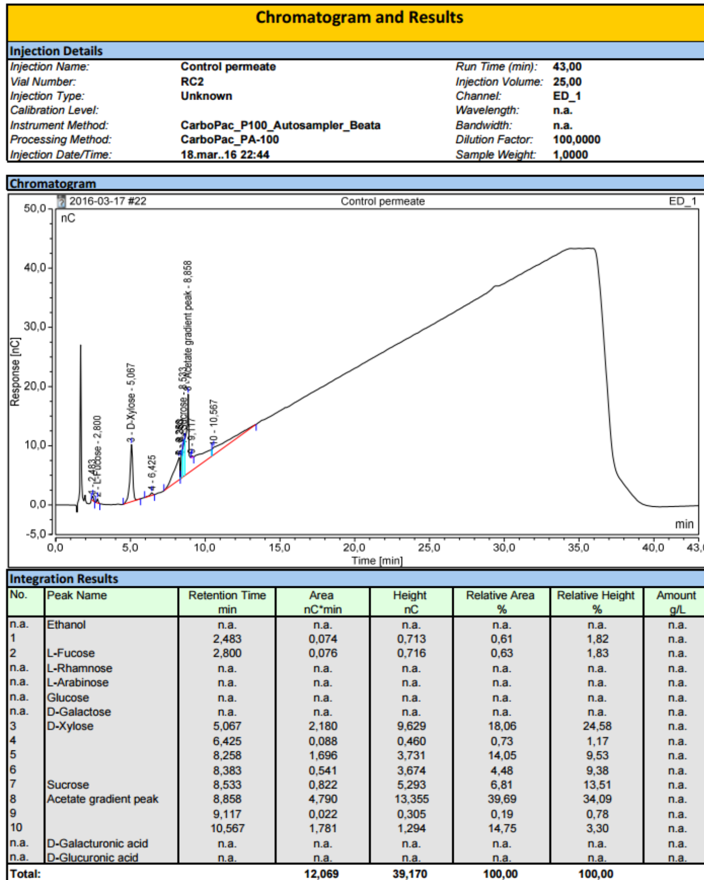
Chromatogram



Integration Results

No.	Peak Name	Retention Time min	Area nC*min	Height nC	Relative Area %	Relative Height %	Amount
1		1,108	0,312	4,890	3,41	6,47	n.a.
2	Sorbitol	1,292	6,714	61,762	73,30	81,72	n.a.
3		1,825	0,050	0,722	0,55	0,95	n.a.
4	Trehalose	2,000	0,086	0,709	0,94	0,94	n.a.
5	Fucose	3,092	0,249	1,825	2,72	2,41	n.a.
n.a.	Rhamnose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Arabinose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Galactose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
6		7,442	0,487	1,257	5,32	1,66	n.a.
7	Glucose	8,083	0,795	2,794	8,68	3,70	n.a.
8	Xylose	9,167	0,465	1,618	5,08	2,14	n.a.
n.a.	Sucrose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Total:			**9,159**	**75,576**	**100,00**	**100,00**	

HPAEC-PAD using PA100 column



Chromatogram and Results

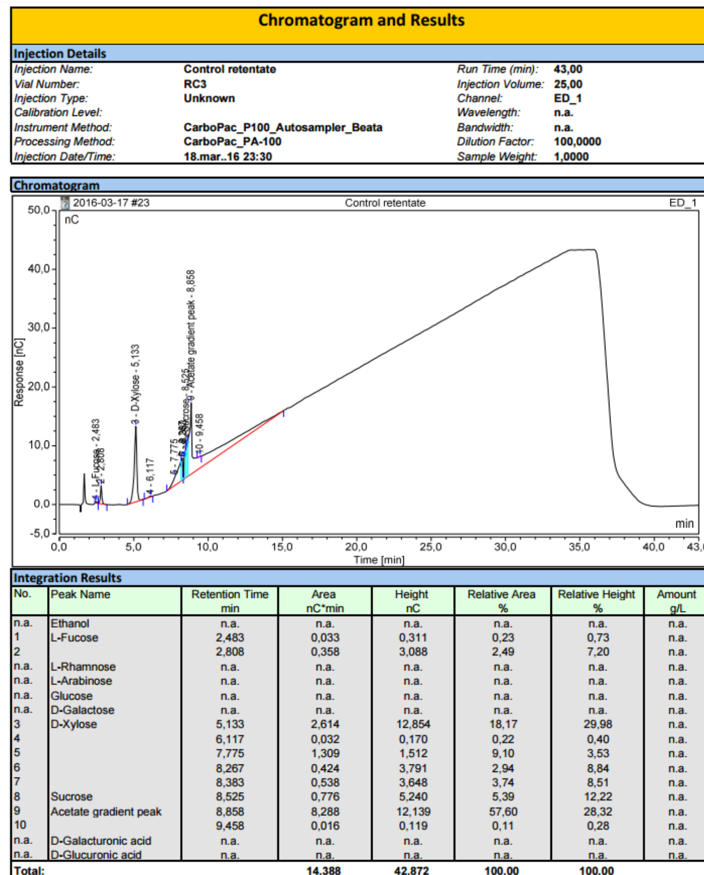
Injection Details

Injection Name:	**Control permeate**	*Run Time (min):*	**43,00**
Vial Number:	**RC2**	*Injection Volume:*	**25,00**
Injection Type:	**Unknown**	*Channel:*	**ED_1**
Calibration Level:		*Wavelength:*	**n.a.**
Instrument Method:	**CarboPac_P100_Autosampler_Beata**	*Bandwidth:*	**n.a.**
Processing Method:	**CarboPac_PA-100**	*Dilution Factor:*	**100,0000**
Injection Date/Time:	**18.mar..16 22:44**	*Sample Weight:*	**1,0000**

Chromatogram

Integration Results

No.	Peak Name	Retention Time min	Area nC*min	Height nC	Relative Area %	Relative Height %	Amount g/L
n.a.	Ethanol	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
1		2,483	0,074	0,713	0,61	1,82	n.a.
2	L-Fucose	2,800	0,076	0,716	0,63	1,83	n.a.
n.a.	L-Rhamnose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	L-Arabinose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Glucose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	D-Galactose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
3	D-Xylose	5,067	2,180	9,629	18,06	24,58	n.a.
4		6,425	0,088	0,460	0,73	1,17	n.a.
5		8,258	1,696	3,731	14,05	9,53	n.a.
6		8,383	0,541	3,674	4,48	9,38	n.a.
7	Sucrose	8,533	0,822	5,293	6,81	13,51	n.a.
8	Acetate gradient peak	8,858	4,790	13,355	39,69	34,09	n.a.
9		9,117	0,022	0,305	0,19	0,78	n.a.
10		10,567	1,781	1,294	14,75	3,30	n.a.
n.a.	D-Galacturonic acid	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	D-Glucuronic acid	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Total:			**12,069**	**39,170**	**100,00**	**100,00**	



Chromatogram and Results

Injection Details

Injection Name:	**Control retentate**	*Run Time (min):*	**43,00**
Vial Number:	**RC3**	*Injection Volume:*	**25,00**
Injection Type:	**Unknown**	*Channel:*	**ED_1**
Calibration Level:		*Wavelength:*	**n.a.**
Instrument Method:	**CarboPac_P100_Autosampler_Beata**	*Bandwidth:*	**n.a.**
Processing Method:	**CarboPac_PA-100**	*Dilution Factor:*	**100,0000**
Injection Date/Time:	**18.mar..16 23:30**	*Sample Weight:*	**1,0000**

Chromatogram

Integration Results

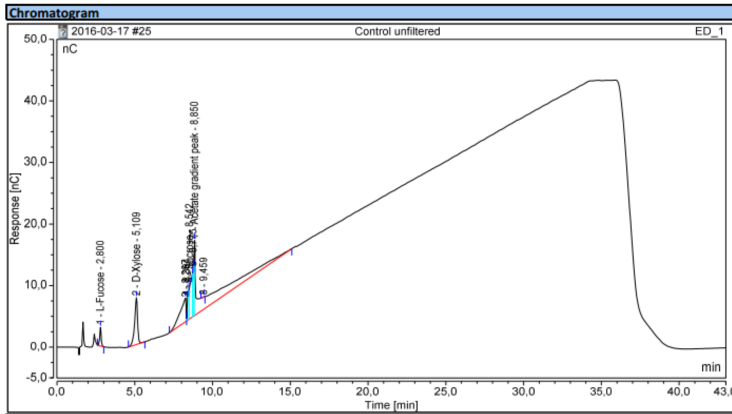
No.	Peak Name	Retention Time min	Area nC*min	Height nC	Relative Area %	Relative Height %	Amount g/L
n.a.	Ethanol	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
1	L-Fucose	2,483	0,033	0,311	0,23	0,73	n.a.
2		2,808	0,358	3,088	2,49	7,20	n.a.
n.a.	L-Rhamnose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	L-Arabinose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Glucose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	D-Galactose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
3	D-Xylose	5,133	2,614	12,854	18,17	29,98	n.a.
4		6,117	0,032	0,170	0,22	0,40	n.a.
5		7,775	1,309	1,512	9,10	3,53	n.a.
6		8,267	0,424	3,791	2,94	8,84	n.a.
7		8,383	0,538	3,648	3,74	8,51	n.a.
8	Sucrose	8,525	0,776	5,240	5,39	12,22	n.a.
9	Acetate gradient peak	8,858	8,288	12,139	57,60	28,32	n.a.
10		9,458	0,016	0,119	0,11	0,28	n.a.
n.a.	D-Galacturonic acid	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	D-Glucuronic acid	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Total:			**14,388**	**42,872**	**100,00**	**100,00**	

Chromatogram and Results

Injection Details

Injection Name:	**Control unfiltered**	*Run Time (min):*	**43,00**
Vial Number:	**RC4**	*Injection Volume:*	**25,00**
Injection Type:	**Unknown**	*Channel:*	**ED_1**
Calibration Level:		*Wavelength:*	**n.a.**
Instrument Method:	**CarboPac_P100_Autosampler_Beata**	*Bandwidth:*	**n.a.**
Processing Method:	**CarboPac_PA-100**	*Dilution Factor:*	**100,0000**
Injection Date/Time:	**19.mar..16 01:03**	*Sample Weight:*	**1,0000**

Chromatogram



Integration Results

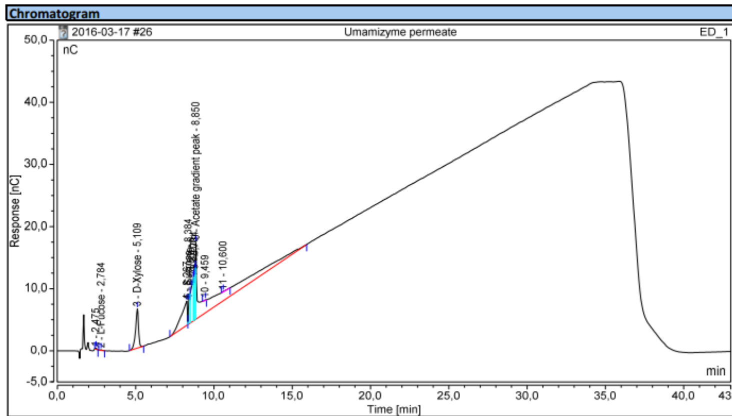
No.	Peak Name	Retention Time min	Area nC*min	Height nC	Relative Area %	Relative Height %	Amount g/L
n.a.	Ethanol	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
1	L-Fucose	2,800	0,331	3,015	2,49	6,90	n.a.
n.a.	L-Rhamnose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	L-Arabinose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Glucose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	D-Galactose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
2	D-Xylose	5,109	1,682	7,583	12,63	17,35	n.a.
3		8,267	1,775	3,834	13,33	8,77	n.a.
4		8,384	0,550	3,722	4,13	8,51	n.a.
5	Sucrose	8,542	1,387	5,452	10,42	12,47	n.a.
6		8,775	0,694	7,787	5,21	17,82	n.a.
7	Acetate gradient peak	8,850	6,881	12,195	51,68	27,90	n.a.
8		9,459	0,014	0,119	0,11	0,27	n.a.
n.a.	D-Galacturonic acid	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	D-Glucuronic acid	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Total:			**13,314**	**43,708**	**100,00**	**100,00**	

Chromatogram and Results

Injection Details

Injection Name:	**Umamizyme permeate**	*Run Time (min):*	**43,00**
Vial Number:	**RC5**	*Injection Volume:*	**25,00**
Injection Type:	**Unknown**	*Channel:*	**ED_1**
Calibration Level:		*Wavelength:*	**n.a.**
Instrument Method:	**CarboPac_P100_Autosampler_Beata**	*Bandwidth:*	**n.a.**
Processing Method:	**CarboPac_PA-100**	*Dilution Factor:*	**100,0000**
Injection Date/Time:	**19.mar..16 01:49**	*Sample Weight:*	**1,0000**

Chromatogram



Integration Results

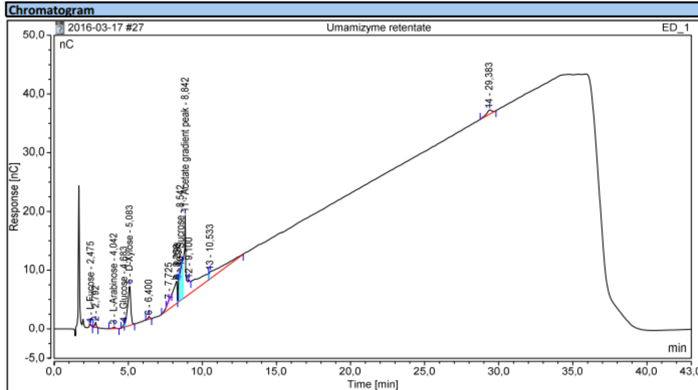
No.	Peak Name	Retention Time min	Area nC*min	Height nC	Relative Area %	Relative Height %	Amount g/L
n.a.	Ethanol	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
1		2,475	0,024	0,224	0,18	0,47	n.a.
2	L-Fucose	2,784	0,014	0,088	0,10	0,19	n.a.
n.a.	L-Rhamnose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	L-Arabinose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Glucose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	D-Galactose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
3	D-Xylose	5,109	1,382	6,305	9,99	13,35	n.a.
4		8,267	1,806	3,880	13,05	8,22	n.a.
5	Sucrose	8,384	0,550	3,711	3,98	7,86	n.a.
6		8,559	1,221	5,642	8,82	11,95	n.a.
7		8,700	0,234	6,953	1,69	14,72	n.a.
8		8,784	0,705	7,916	5,10	16,76	n.a.
9	Acetate gradient peak	8,850	7,859	12,298	56,80	26,04	n.a.
10		9,459	0,014	0,121	0,10	0,26	n.a.
11		10,600	0,026	0,088	0,19	0,19	n.a.
n.a.	D-Galacturonic acid	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	D-Glucuronic acid	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Total:			**13,837**	**47,227**	**100,00**	**100,00**	

Chromatogram and Results

Injection Details

Injection Name:	**Umamizyme retentate**	*Run Time (min):*	**43,00**
Vial Number:	**RC6**	*Injection Volume:*	**25,00**
Injection Type:	**Unknown**	*Channel:*	**ED_1**
Calibration Level:		*Wavelength:*	**n.a.**
Instrument Method:	**CarboPac_P100_Autosampler_Beata**	*Bandwidth:*	**n.a.**
Processing Method:	**CarboPac_PA-100**	*Dilution Factor:*	**100,0000**
Injection Date/Time:	**19.mar..16 02:35**	*Sample Weight:*	**1,0000**

Chromatogram



Integration Results

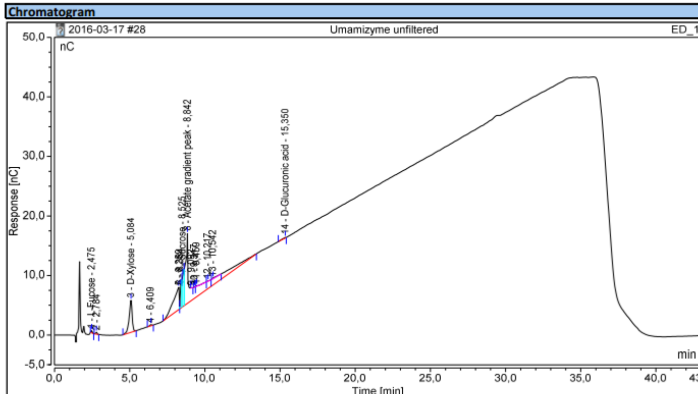
No.	Peak Name	Retention Time min	Area nC*min	Height nC	Relative Area %	Relative Height %	Amount g/L
n.a.	Ethanol	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
1	L-Fucose	2,475	0,025	0,248	0,22	0,65	n.a.
2		2,792	0,095	0,869	0,85	2,27	n.a.
n.a.	L-Rhamnose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
3	L-Arabinose	4,042	0,058	0,290	0,52	0,76	n.a.
4	Glucose	4,683	0,054	0,482	0,48	1,26	n.a.
n.a.	D-Galactose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
5	D-Xylose	5,083	1,660	6,645	14,87	17,38	n.a.
6		6,400	0,076	0,494	0,68	1,29	n.a.
7		7,725	0,027	0,168	0,24	0,44	n.a.
8		8,258	1,735	3,751	15,54	9,81	n.a.
9		8,383	0,511	3,722	4,58	9,73	n.a.
10	Sucrose	8,542	0,868	5,420	7,78	14,17	n.a.
11	Acetate gradient peak	8,842	4,445	13,909	39,81	36,37	n.a.
12		9,100	0,025	0,324	0,23	0,85	n.a.
13		10,533	1,282	1,192	11,48	3,12	n.a.
n.a.	D-Galacturonic acid	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	D-Glucuronic acid	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
14		29,383	0,303	0,728	2,71	1,90	n.a.
Total:			**11,166**	**38,243**	**100,00**	**100,00**	

Chromatogram and Results

Injection Details

Injection Name:	**Umamizyme unfiltered**	*Run Time (min):*	**43,00**
Vial Number:	**RC7**	*Injection Volume:*	**25,00**
Injection Type:	**Unknown**	*Channel:*	**ED_1**
Calibration Level:		*Wavelength:*	**n.a.**
Instrument Method:	**CarboPac_P100_Autosampler_Beata**	*Bandwidth:*	**n.a.**
Processing Method:	**CarboPac_PA-100**	*Dilution Factor:*	**100,0000**
Injection Date/Time:	**19.mar..16 03:21**	*Sample Weight:*	**1,0000**

Chromatogram



Integration Results

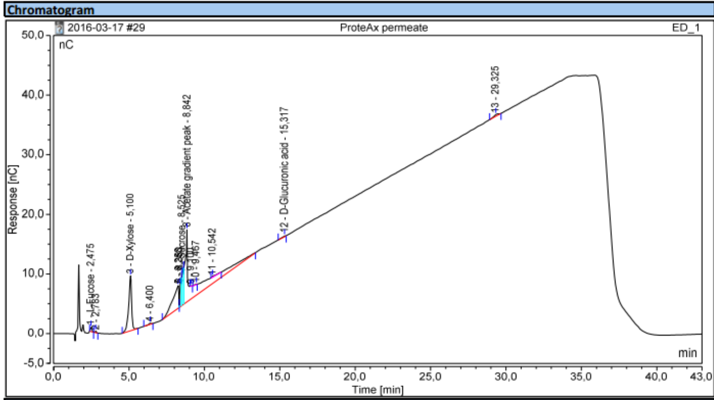
No.	Peak Name	Retention Time min	Area nC*min	Height nC	Relative Area %	Relative Height %	Amount g/L
n.a.	Ethanol	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
1	L-Fucose	2,475	0,037	0,391	0,35	1,24	n.a.
2		2,784	0,038	0,339	0,36	1,08	n.a.
n.a.	L-Rhamnose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	L-Arabinose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Glucose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	D-Galactose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
3	D-Xylose	5,084	1,239	5,371	11,59	17,06	n.a.
4		6,409	0,032	0,196	0,30	0,62	n.a.
5		8,259	1,748	3,768	16,34	11,97	n.a.
6		8,384	0,523	3,779	4,90	12,01	n.a.
7	Sucrose	8,525	0,820	5,259	7,67	16,71	n.a.
8	Acetate gradient peak	8,842	6,134	11,700	57,36	37,17	n.a.
9		9,092	0,012	0,144	0,11	0,46	n.a.
10		9,317	0,007	0,089	0,06	0,28	n.a.
11		9,459	0,012	0,118	0,12	0,37	n.a.
12		10,217	0,013	0,076	0,12	0,24	n.a.
13		10,542	0,044	0,137	0,41	0,44	n.a.
n.a.	D-Galacturonic acid	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
14	D-Glucuronic acid	15,350	0,034	0,109	0,32	0,35	n.a.
Total:			**10,694**	**31,475**	**100,00**	**100,00**	

Chromatogram and Results

Injection Details

Injection Name:	**ProteAx permeate**	*Run Time (min):*	**43,00**
Vial Number:	**RC8**	*Injection Volume:*	**25,00**
Injection Type:	**Unknown**	*Channel:*	**ED_1**
Calibration Level:		*Wavelength:*	**n.a.**
Instrument Method:	**CarboPac_P100_Autosampler_Beata**	*Bandwidth:*	**n.a.**
Processing Method:	**CarboPac_PA-100**	*Dilution Factor:*	**100,0000**
Injection Date/Time:	**19.mar..16 04:08**	*Sample Weight:*	**1,0000**

Chromatogram



Integration Results

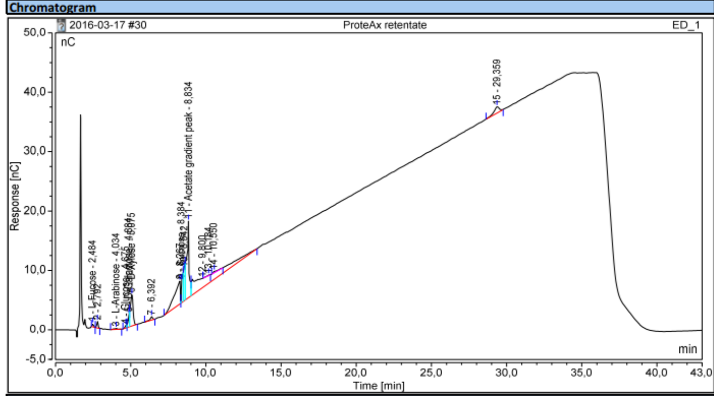
No.	Peak Name	Retention Time min	Area nC*min	Height nC	Relative Area %	Relative Height %	Amount g/L
n.a.	Ethanol	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
1	L-Fucose	2,475	0,076	0,724	0,64	2,00	n.a.
2		2,783	0,029	0,280	0,24	0,78	n.a.
n.a.	L-Rhamnose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	L-Arabinose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Glucose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	D-Galactose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
3	D-Xylose	5,100	2,079	9,179	17,69	25,42	n.a.
4		6,400	0,040	0,212	0,34	0,59	n.a.
5		8,258	1,766	3,787	15,03	10,49	n.a.
6		8,383	0,552	3,742	4,70	10,36	n.a.
7	Sucrose	8,525	0,779	5,256	6,63	14,55	n.a.
8	Acetate gradient peak	8,842	6,212	12,089	52,85	33,48	n.a.
9		9,100	0,010	0,130	0,08	0,36	n.a.
10		9,467	0,019	0,106	0,16	0,29	n.a.
11		10,542	0,052	0,165	0,44	0,46	n.a.
n.a.	D-Galacturonic acid	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
12	D-Glucuronic acid	15,317	0,033	0,139	0,28	0,38	n.a.
13		29,325	0,109	0,302	0,93	0,84	n.a.
Total:			**11,754**	**36,111**	**100,00**	**100,00**	

Chromatogram and Results

Injection Details

Injection Name:	**ProteAx retentate**	*Run Time (min):*	**43,00**
Vial Number:	**RD1**	*Injection Volume:*	**25,00**
Injection Type:	**Unknown**	*Channel:*	**ED_1**
Calibration Level:		*Wavelength:*	**n.a.**
Instrument Method:	**CarboPac_P100_Autosampler_Beata**	*Bandwidth:*	**n.a.**
Processing Method:	**CarboPac_PA-100**	*Dilution Factor:*	**100,0000**
Injection Date/Time:	**19.mar..16 04:54**	*Sample Weight:*	**1,0000**

Chromatogram



Integration Results

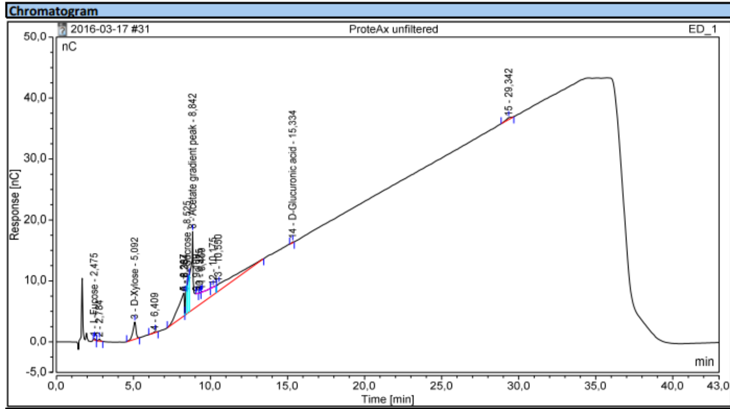
No.	Peak Name	Retention Time min	Area nC*min	Height nC	Relative Area %	Relative Height %	Amount g/L
n.a.	Ethanol	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
1	L-Fucose	2,484	0,050	0,477	0,42	1,22	n.a.
2		2,792	0,120	1,142	1,01	2,92	n.a.
n.a.	L-Rhamnose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
3	L-Arabinose	4,034	0,044	0,182	0,37	0,47	n.a.
4	Glucose	4,675	0,061	0,433	0,51	1,11	n.a.
5	D-Galactose	4,884	0,246	2,102	2,07	5,38	n.a.
6	D-Xylose	5,075	1,097	5,216	9,23	13,35	n.a.
7		6,392	0,112	0,573	0,94	1,47	n.a.
8		8,267	1,815	3,830	15,27	9,80	n.a.
9	Sucrose	8,384	0,587	3,699	4,94	9,47	n.a.
10		8,542	0,749	5,493	6,30	14,06	n.a.
11	Acetate gradient peak	8,834	2,308	12,908	19,42	33,04	n.a.
12		9,800	4,146	1,543	34,88	3,95	n.a.
13		10,184	0,024	0,121	0,20	0,31	n.a.
14		10,550	0,073	0,272	0,62	0,70	n.a.
n.a.	D-Galacturonic acid	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	D-Glucuronic acid	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
15		29,359	0,454	1,075	3,82	2,75	n.a.
Total:			**11,885**	**39,067**	**100,00**	**100,00**	

Chromatogram and Results

Injection Details

Injection Name:	**ProteAx unfiltered**	*Run Time (min):*	**43,00**
Vial Number:	**RD2**	*Injection Volume:*	**25,00**
Injection Type:	**Unknown**	*Channel:*	**ED_1**
Calibration Level:		*Wavelength:*	**n.a.**
Instrument Method:	**CarboPac_P100_Autosampler_Beata**	*Bandwidth:*	**n.a.**
Processing Method:	**CarboPac_PA-100**	*Dilution Factor:*	**100,0000**
Injection Date/Time:	**19.mar..16 05:40**	*Sample Weight:*	**1,0000**

Chromatogram



Integration Results

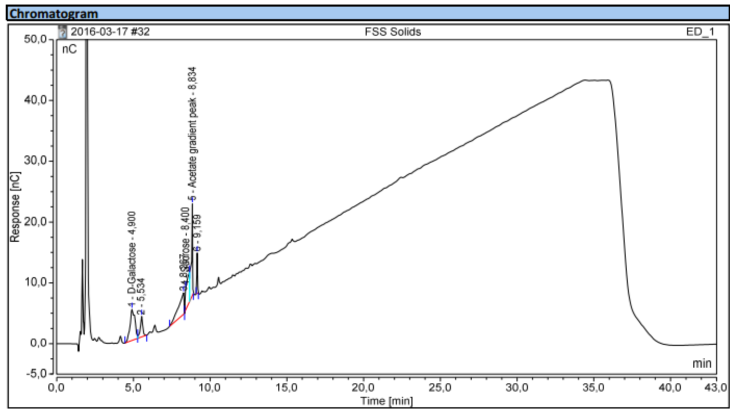
No.	Peak Name	Retention Time min	Area nC*min	Height nC	Relative Area %	Relative Height %	Amount g/L
n.a.	Ethanol	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
1	L-Fucose	2,475	0,027	0,270	0,26	0,87	n.a.
2		2,784	0,043	0,350	0,42	1,13	n.a.
n.a.	L-Rhamnose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	L-Arabinose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Glucose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	D-Galactose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
3	D-Xylose	5,092	0,664	2,818	6,50	9,09	n.a.
4		6,409	0,036	0,184	0,35	0,59	n.a.
5		8,267	1,755	3,751	17,19	12,10	n.a.
6		8,384	0,533	3,619	5,22	11,67	n.a.
7	Sucrose	8,525	0,770	5,193	7,54	16,75	n.a.
8	Acetate gradient peak	8,842	4,450	12,805	43,58	41,31	n.a.
9		9,109	0,010	0,120	0,09	0,39	n.a.
10		9,325	0,007	0,075	0,07	0,24	n.a.
11		9,459	0,002	0,094	0,02	0,30	n.a.
12		10,175	0,015	0,084	0,15	0,27	n.a.
13		10,550	1,756	1,183	17,20	3,82	n.a.
n.a.	D-Galacturonic acid	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
14	D-Glucuronic acid	15,334	0,017	0,124	0,16	0,40	n.a.
15		29,342	0,128	0,327	1,25	1,06	n.a.
Total:			**10,211**	**30,998**	**100,00**	**100,00**	

Chromatogram and Results

Injection Details

Injection Name:	**FSS Solids**	*Run Time (min):*	**43,00**
Vial Number:	**GE1**	*Injection Volume:*	**25,00**
Injection Type:	**Unknown**	*Channel:*	**ED_1**
Calibration Level:		*Wavelength:*	**n.a.**
Instrument Method:	**CarboPac_P100_Autosampler_Beata**	*Bandwidth:*	**n.a.**
Processing Method:	**CarboPac_PA-100**	*Dilution Factor:*	**1,0000**
Injection Date/Time:	**19.mar..16 06:26**	*Sample Weight:*	**1,0000**

Chromatogram



Integration Results

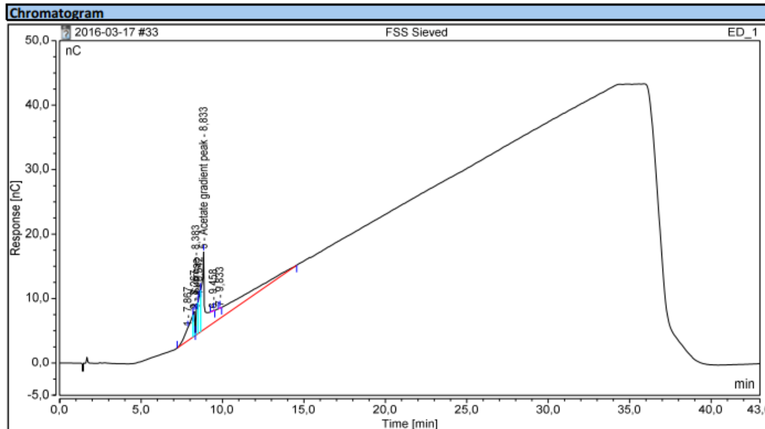
No.	Peak Name	Retention Time min	Area nC*min	Height nC	Relative Area %	Relative Height %	Amount g/L
n.a.	Ethanol	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	L-Fucose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	L-Rhamnose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	L-Arabinose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Glucose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
1	D-Galactose	4,900	1,850	5,122	26,59	13,73	n.a.
n.a.	D-Xylose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
2		5,534	0,623	3,360	8,95	9,01	n.a.
3		8,267	1,507	3,489	21,65	9,35	n.a.
4	Sucrose	8,400	1,293	3,622	18,58	9,71	n.a.
5	Acetate gradient peak	8,834	1,286	15,101	18,48	40,49	n.a.
6		9,159	0,400	6,606	5,75	17,71	n.a.
n.a.	D-Galacturonic acid	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	D-Glucuronic acid	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Total:			**6,959**	**37,300**	**100,00**	**100,00**	

Chromatogram and Results

Injection Details

Injection Name:	**FSS Sieved**	*Run Time (min):*	**43,00**
Vial Number:	**GE2**	*Injection Volume:*	**25,00**
Injection Type:	**Unknown**	*Channel:*	**ED_1**
Calibration Level:		*Wavelength:*	**n.a.**
Instrument Method:	**CarboPac_P100_Autosampler_Beata**	*Bandwidth:*	**n.a.**
Processing Method:	**CarboPac_PA-100**	*Dilution Factor:*	**1,0000**
Injection Date/Time:	**19.mar..16 07:13**	*Sample Weight:*	**1,0000**

Chromatogram



Integration Results

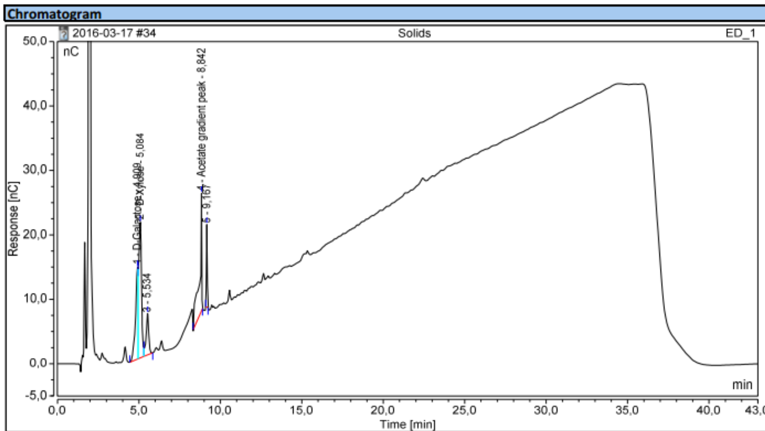
No.	Peak Name	Retention Time min	Area nC*min	Height nC	Relative Area %	Relative Height %	Amount g/L
n.a.	Ethanol	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	L-Fucose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	L-Rhamnose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	L-Arabinose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Glucose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	D-Galactose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	D-Xylose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
1		7,867	1,344	1,997	12,69	7,33	n.a.
2		8,267	0,452	3,826	4,27	14,05	n.a.
3	Sucrose	8,383	0,587	3,711	5,54	13,63	n.a.
4		8,542	0,957	5,483	9,03	20,14	n.a.
5	Acetate gradient peak	8,833	7,236	12,058	68,31	44,29	n.a.
6		9,458	0,014	0,113	0,13	0,41	n.a.
7		9,833	0,003	0,041	0,03	0,15	n.a.
n.a.	D-Galacturonic acid	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	D-Glucuronic acid	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Total:			**10,593**	**27,228**	**100,00**	**100,00**	

Chromatogram and Results

Injection Details

Injection Name:	**Solids**	*Run Time (min):*	**43,00**
Vial Number:	**GE3**	*Injection Volume:*	**25,00**
Injection Type:	**Unknown**	*Channel:*	**ED_1**
Calibration Level:		*Wavelength:*	**n.a.**
Instrument Method:	**CarboPac_P100_Autosampler_Beata**	*Bandwidth:*	**n.a.**
Processing Method:	**CarboPac_PA-100**	*Dilution Factor:*	**1,0000**
Injection Date/Time:	**19.mar..16 07:59**	*Sample Weight:*	**1,0000**

Chromatogram



Integration Results

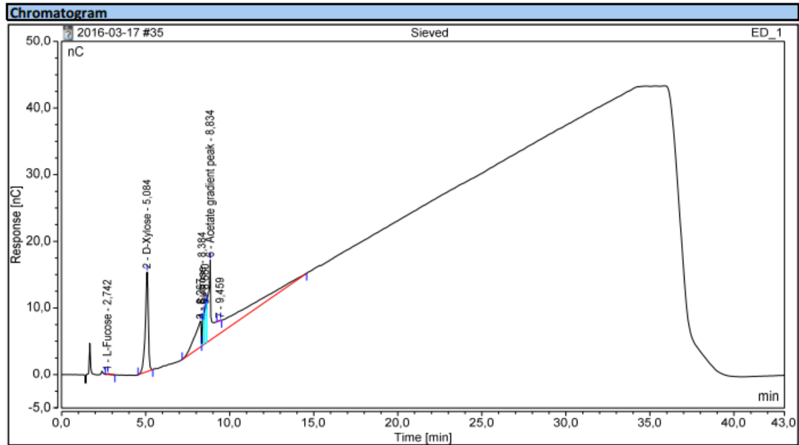
No.	Peak Name	Retention Time min	Area nC*min	Height nC	Relative Area %	Relative Height %	Amount g/L
n.a.	Ethanol	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	L-Fucose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	L-Rhamnose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	L-Arabinose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Glucose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
1	D-Galactose	4,909	2,546	14,245	22,56	19,58	n.a.
2	D-Xylose	5,084	4,188	21,011	37,11	28,88	n.a.
3		5,534	1,180	6,394	10,46	8,79	n.a.
n.a.	Sucrose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
4	Acetate gradient peak	8,842	2,599	18,257	23,03	25,10	n.a.
5		9,167	0,773	12,841	6,85	17,65	n.a.
n.a.	D-Galacturonic acid	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	D-Glucuronic acid	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Total:			**11,287**	**72,748**	**100,00**	**100,00**	

Chromatogram and Results

Injection Details

Injection Name:	**Sieved**	*Run Time (min):*	**43,00**
Vial Number:	**GE4**	*Injection Volume:*	**25,00**
Injection Type:	**Unknown**	*Channel:*	**ED_1**
Calibration Level:		*Wavelength:*	**n.a.**
Instrument Method:	**CarboPac_P100_Autosampler_Beata**	*Bandwidth:*	**n.a.**
Processing Method:	**CarboPac_PA-100**	*Dilution Factor:*	**1,0000**
Injection Date/Time:	**19.mar..16 08:45**	*Sample Weight:*	**1,0000**

Chromatogram



Integration Results

No.	Peak Name	Retention Time min	Area nC*min	Height nC	Relative Area %	Relative Height %	Amount g/L
n.a.	Ethanol	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
1	L-Fucose	2,742	0,032	0,120	0,24	0,30	n.a.
n.a.	L-Rhamnose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	L-Arabinose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	Glucose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	D-Galactose	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
2	D-Xylose	5,084	3,082	14,941	22,63	37,13	n.a.
3		8,267	1,796	3,835	13,19	9,53	n.a.
4	Sucrose	8,384	0,544	3,685	4,00	9,16	n.a.
5		8,550	0,731	5,532	5,37	13,75	n.a.
6	Acetate gradient peak	8,834	7,419	12,004	54,46	29,83	n.a.
7		9,459	0,017	0,121	0,12	0,30	n.a.
n.a.	D-Galacturonic acid	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	D-Glucuronic acid	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Total:			**13,621**	**40,237**	**100,00**	**100,00**	