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Extraction and Chemical Modification of Hemicellulose

Bachelor of Science Thesis

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Department of Chemistry and Chemical Engineering
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ABSTRACT

As society gradually shift away from fossil fuels and petroleum-based products towards more renewable substitutes the demand for economically feasible bio-based materials increase. Hemicelluloses, the world's second most abundant plant polymers have barely been industrially utilized in this quest for new bio-based products. This study presents knowledge about the method for arabinoxylan (AX) extraction from oat and wheat bran, method for hydroxypropyl methylation of these arabinoxylans and the influence of empty reactor space on the final modification result. Carbohydrate composition of oat and wheat bran and their respective AX fractions were analysed with an ionic chromatograph. The different arabinoxylans and hydroxypropyl methylated AX were further analysed with FT-IR spectroscopy and finally their clouding point were determined by UV-Vis spectroscopy. Analysis of the carbohydrate composition showed that the carbohydrate composition was very similar between oat bran and wheat bran as well as between their respective AX fractions but the AX yield from oat bran was significantly higher compared to wheat bran. The FT-IR result showed structural differences between AX from oat and wheat bran. Finally, the FT-IR and clouding result showed that both arabinoxylan from oat bran and wheat bran were successfully hydroxypropyl methylated and that the degree of substitution increase as the empty reactor space decreased.

Keywords: Hemicellulose, arabinoxylan, hydroxypropyl methylated arabinoxylan, bio-based materials.

Sammanfattning

Allteftersom samhället gradvis flyttar sig från fossila bränslen och petroleumbaserade produkter till mer förnyelsebara substitut, ökar efterfrågan på ekonomiskt genomförbara bio-baserade material. Hemicellulosa, världens näst mest omfattande växtpolymerer, har knappt använts industriellt i strävan efter nya bio-baserade produkter. Denna studie presenterar kunskap om metoden för arabinoxylan (AX) extraktion från havre och vetekli, metod för hydroxipropyl metylering av dessa arabinoxylaner samt hur det tomma reaktionsutrymmet påverkar det slutliga modifierings resultatet. Kolhydratkompositionen av havre och vetekli och deras respektive AX fraktioner analyserades med en jonbyteskromatograf. De olika arabinoxylanerna och hydroxipropyl metylerade AX analyserades ytterligare med FT-IR spektroskopi och slutligen bestämdes deras grumlinghetspunkt med hjälp av UV-Vis spektroskopi. Analys av kolhydratkompositionen visade att det var väldigt liten skillnad i kolhydratkompositionen mellan havre och vetekli samt mellan deras respektive AX fraktioner men AX utbytet från havrekli var betydligt högre jämfört med vetekli. FT-IR resultatet visade på strukturella skillnader mellan AX från havre och vetekli. Slutligen visade FT-IR och grumlighets resultatet att både arabinoxylan från havre och vetekli framgångsrikt hydroxipropyl metylerades och att graden av substitution ökade allteftersom det tomma reaktorutrymmet minskade.

Abbreviation

AX	Arabinoxylan
AXW	Arabinoxylan from wheat bran
AXO	Arabinoxylan from oat bran
HPAE	High-performance anion-exchange chromatography.
HPMC	Hydroxypropyl methylated cellulose
HPMAX	Hydroxypropyl methylated arabinoxylan
HPMAXW	Hydroxypropyl methylated arabinoxylan from wheat
HPMAXO	Hydroxypropyl methylated arabinoxylan from oat
LCC	Lining-carbohydrate complex

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1. INTRODUCTION

1.1. Background

The exhausted availability of fossil fuels in the foreseeable future as well as the environmental impact of petroleum-based product has brought about intense research towards the development of new bio-based products. Furthermore, there is of great interest for biorefineries to develop new streams of valuable co-products from unused side-streams that can improve the overall value derived from biomass feedstock. Hemicelluloses, one of the world's most abundant plant polymers have lately attracted increasing attention, appearing promising in a step towards co-product processing and a highly integrated biorefinery concept.[1-3] They are accessible from the paper industry as black liquor and different forestry and agricultural low-value by-products such as wood, husk and straw but although hemicelluloses represent an extensive raw material recourse they have so far hardly been industrially utilized.[2, 4]

Hemicelluloses are found in complex organization with cellulose and lignin in plant cell walls. They are branched heterogenous polysaccharides composed of many different monosaccharide and make up for approximately half of the biomass of annual and perennial plants.[2, 5] The composition and structure of hemicelluloses varies depending upon the isolation method used and the plant source, making it a complicated issue to determining which hemicellulose that should be modified to acquire a desired property for a specific product.[3, 4, 6]

The potential uses of hemicelluloses are not fully investigated but they are regarded as possessing interesting properties for industrial applications as supplements to cellulose and plastic replacements in many products.[1, 7, 8] However, for hemicelluloses to be considered as commercially important polymers in product manufacturing it is of crucial importance to establish a viable extraction method for hemicelluloses that also allow for the recovery of lignin, cellulose and other valuable organic compound found in biomass. In addition, it is just as important to provide hemicellulose derivatives for practical applications.

1.2. Objectives

The main aim of this study was to extract arabinoxylan from oat and wheat bran using a protocol for the recovery of arabinoxylan from barley husk outlined by Nylander *et al*.[4] as well as to examine the structure, composition and yield of the recovered fractions. Due to the structural variations between arabinoxylans derived from different plant species and cultivars, additional investigations were made into the possibility of hydroxypropyl methylate arabinoxylan derived from both oat and wheat bran. Attention was also paid to the scalability of the modification processes of arabinoxylan by investigating how the empty reaction space in a small autoclave (minicalve) reactor influenced the degree of conjugated hydroxypropyl and methyl groups.

2. THEORY

2.1. Hemicellulose

Hemicelluloses are branched heterogenous polysaccharides found in plant cell walls. They are composed of many different monosaccharides that can be divided into four different polysaccharide classes: xylans, mannans, β-glucans and xyloglucans.[2, 5, 9] The quantity and structure of isolated hemicelluloses shows significant differences between those isolated from cereal grains such as barley, oat and wheat and those isolated from wood, as well as between cultivars. Plant-source hemicelluloses such as those from cereals are usually more structurally diverse and complex than those derived from wood.[3, 5, 6]

Much of the lignin in the cell wall is primarily covalently linked to hemicelluloses, forming what is generally known as lignin-carbohydrate complexes (LCC). Despite the low occurrences of these linkages, their breakage resistance poses a fundamental problem in the isolation of hemicelluloses.[7, 10]

2.1.1. Xylan polysaccharides

As with all hemicelluloses, the amount and structure of xylan-type polysaccharides differs depending on its source of isolation, varying from 20-30 % in hardwood and up to 50 % for grasses and cereals. Xylans comprise of a linear β-(1→4)-D-xylopyranose backbone, branched by short carbohydrate chains. These carbohydrate side chains are found on the main chain in the O-2 or O-3 position or both, usually represented by D-glucuronic acid, its 4-O-methyl derivate and/or by L-arabinofuranose units, see Figure 1.[6, 9, 11]

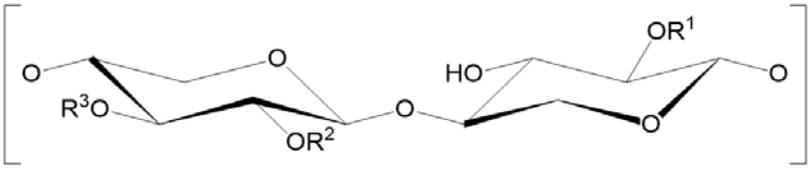

Figure 1. Structure of some xylan-type hemicelluloses. Xylan: $R^1 = R^2 = R^3 = H$; Glucuronoxylan: R^1 = D-glucuronic acid, $R^2 = R^3 = H$; Arabinoxylan: $R^1 = H$; $R^2 = H$, R^3 = L-arabinofuranose; R^2 = L-arabinofuranose, $R^3 = H$ or $R^2 = R^3$= L-arabinofuranose [11]

2.1.2. Arabinoxylan

Arabinoxylan (AX) is a xylan-type hemicellulose with L-arabinofuranose substituents occupying the O-2 and/or O-3 position of the xylan backbone, see Figure 1. AX represent the main hemicellulose in the cell walls of cereal grains which predominantly constitute of two hemicellulose polymers: AX and β-glucan (in minor amounts).

The extraction of AX is most effectively performed under alkali conditions due to its efficient ability to separate AX from other cell wall components. Alkali treatment cleaves the covalent ester linkages between lignin and AX, furthermore it causes swelling of cellulose breaking the hydrogen bonds that link it to AX.[1, 12] The choice of extraction method along with the starting materials plant species and cultivars all will determine the AX yield, its structure and thereby its attributes. Generally, rye and wheat are rich in AX, while oat and barley show elevated β-glucan content.[6, 7, 9]

2.1.3. Hydroxypropyl methylation of AX

Hydroxypropyl methylation of cellulose is a well-established cellulose derivative that has found use in construction, cosmetic, food and pharmaceutical application.[13] One application of HPMC is as water-soluble film in medical capsules. This is a product area where HPMAX may supplement cellulose. The hydroxypropyl methylation of arabinoxylan is achieved by initially treating it with a sodium hydroxide and then allowing it to react with propylene oxide and iodomethane, see Figure 2.

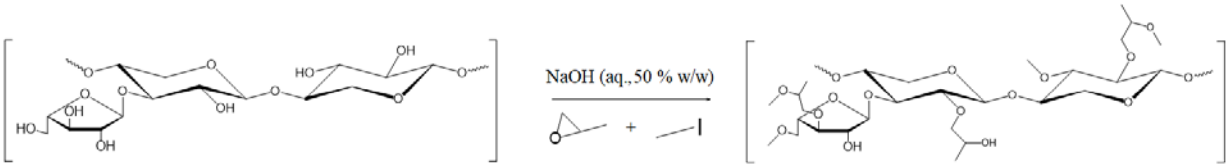

Figure 2. Schematic picture of how hydroxypropyl and methyl groups attach to the arabinoxylan chain.

3. EXPERIMENTAL

3.1. Materials

3.1.1. Chemicals

The oat and wheat brans were received as a generous gift from Lantmännen. Sodium chlorite ($NaClO_2$), sodium dithionite ($Na_2S_2O_4$), hydrochloric acid (HCl), iodomethane (CH_3I), isopropyl acetate ($C_5H_{10}O_2$) and enzyme (α-amylase) were all purchased from Sigma-Aldrich Corporation. Propylene oxide (C_3H_6O) was obtained from Acron organics, ethanol (C_6H_6O) from Solveco chemicals, diethyl ether ($(C_2H_5)_2O$) from VWR international and sodium hydroxide from Fisher chemicals.

3.1.2. Apparaturs

Centrifuge, Heraeus megafuge 40.
FT-IR Spectrometer, PerkinElmer.
Ion chromatograph, Dionex ICS-3000.
UV-Vis Spectrophotometer, Varian Cary 4000.
Büchiglasuster Miniclave (small autoclave), 50 mL.

3.2. Methods

3.2.1. Extraction of arabinoxylan

The extractions of arabinoxylan from oat and wheat bran were performed according to a protocol originally made for arabinoxylan extraction from barley husk by Nylander *et al*.[4]

Initially 500 g of each batch oat/wheat bran was pre-treated with 0.05 M HCl (aq.) and stirred vigorously for 20 hours at room temperature in order to remove extractive substances. The solution was then filtered and the solid phase was delignified in a solution with $NaClO_2$ (aq., pH 3.2) for two hours, occasionally stirred, at 80 °C. Subsequently, the solution was filtered and the solid phase was suspended in a solution of 1 M NaOH (aq.) and $Na_2S_2O_4$ (aq.) for 20 hours, continuously stirred, at room temperature. The solution was then incrementally neutralized by stepwise addition of dilute HCl (aq.) and centrifuged (4300 rpm, 15 min) to separate precipitated cellulose. The supernatant was recovered and enzymatically purified with an enzyme-to-oat/wheat ratio of 0.48 μL/g at 60 °C overnight. Finally, the enzymatically purified supernatant was precipitated in 1.2 volume parts ethanol (95 %). The precipitate was recovered by centrifugation (4300 rpm, 15 min) and dried at room temperature.

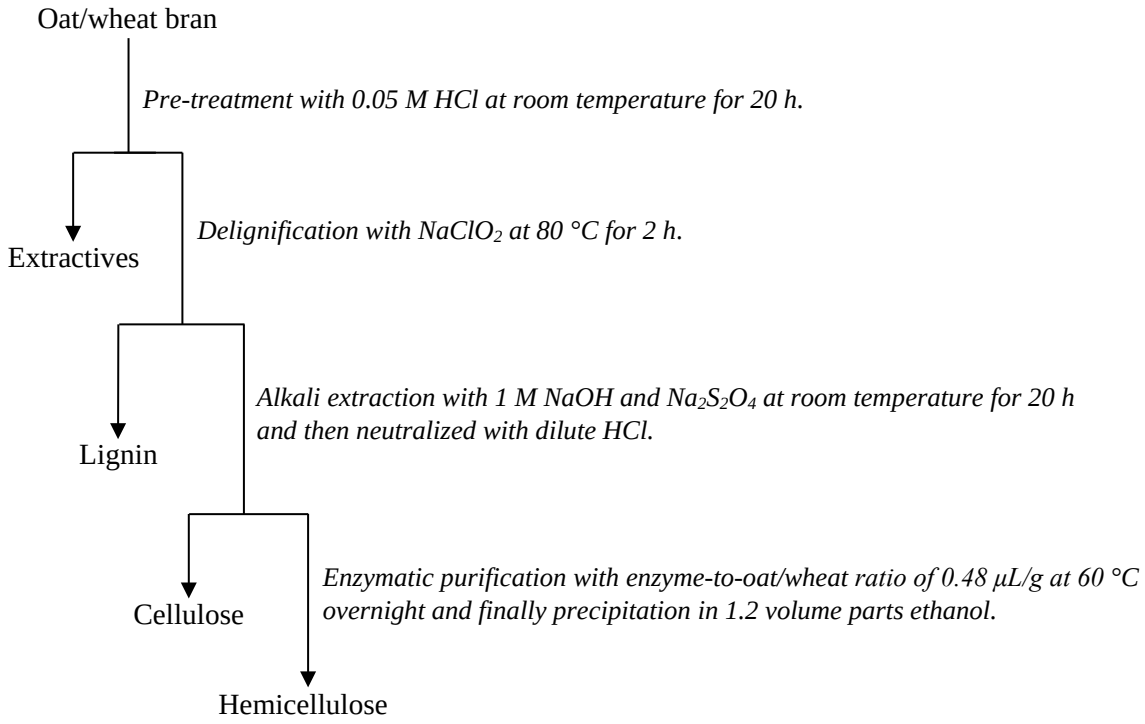

Figure 3. Schematic flow chart representing the extraction process of hemicellulose.

3.2.2. Chemical modification of AX

The initial hydroxypropyl methylation of AX synthesis experiments were carried out in closed vials. Samples of 1 g AX were first pre-activated by dissolving them in 4.7 mL H_2O for 20 min at 60 °C, followed by addition of 1.46 g NaOH (aq., 50 % w/w) and heating at 60 °C for 20 min. Next 4.7 mL iodomethane and 5.3 mL propylene oxide were added to the samples before they were subjected to heat for 80 min at 60°C, followed by another 80 min at 90 °C. Soon after the reaction mixtures were neutralized with concentrated HCl (aq.). The neutralized reaction mixtures were then mixed with 1 volume part ethanol and isopropyl acetate before being precipitated by the addition of diethyl ether. The supernatants were decantated and the remaining samples dried in a desiccator and stored at room temperature.

The final synthesis experiments were pre-activated by addition of H_2O and NaOH simultaneously and heated at 60 °C for 40 min. Onwards the experiments were carried out under the same conditions with respect to time and heat but were performed in a miniclave reactor and the entire process were stepwise scaled up from the initial vial component amount size, to two times the initial vial component amount size and finally three times the initial vial component size. Degreasing the amount of empty space in the reactor with each scale up, see Table 1.

Table 1. Amount of chemical substance for each synthesis of HPMAX as well as the degree of empty space in each reactor.

	[g]			[mL]			
	AXO	AXW	NaOH[1]	H_2O	MeI[2]	Propylene oxide	ERS[3]
Closed vials							
HPMAXW	–	1.00	1.46	4.70	4.70	5.30	n.a.
HPMAXO	1.00	–	1.46	4.70	4.70	5.30	n.a.
Miniclave reactors							
HPMAXWI	–	1.00	1.46	4.70	4.70	5.30	33.84
HPMAXWII	–	2.00	2.92	9.40	9.40	10.60	17.68
HPMAXWIII*	–	3.00	4.38	14.10	14.10	15.90	1.52
HPMAXOI	1.00	–	1.46	4.70	4.70	5.30	33.84
HPMAXOII	2.00	–	2.92	9.40	9.40	10.60	17.68
HPMAXOIII*	3.00	–	4.38	14.10	14.10	15.90	1.52

[1] *50% (w/w) NaOH solution.*
[2] *Abbreviation of Iodomethane.*
[3] *Abbreviation for Empty Reactor Space.*
** Showed a pressure increase of approximately 1.0 bar.*

3.2.3. Characterization methods

3.2.3.1. Carbohydrate analysis

Data about the chemical composition of the carbohydrates of oat and wheat brans as well as of isolated oat and wheat arabinoxylans were obtained with a technique known as high-performance anion-exchange (HPAE) chromatography. HPAE chromatography exploit the weakly acid nature of carbohydrates by exposing the carbohydrates to an alkaline environment causing them to partially ionized. The carbohydrates are then separated by anion-exchange columns and detected with pulsed electrochemical detection.[14]

The sample preparation procedure for the ion chromatograph were made by addition of H_2SO_4 (aq. 72 % w/w) to 200 mg of dry sample. The sample mixtures were impregnated in a desiccator for 15 min, thereafter heated for 60 min at 30 °C, followed by addition of 84 mL of deionized water. Next, the diluted sample mixtures were hydrolysed for 60 min in an autoclave at 125 °C and succeeded by vacuum filtration. The filters were rescued and the amount of acid insoluble substances worked out gravimetrically. The filtrates were diluted to 100 mL with deionized water, 5 mL of each dilute filtrate were diluted a second time to 50 mL with deionized water. Finally, the second diluted filtrates were filtrated into 1.5 mL plastic vials, which were sealed. The measurements were accomplished using hydroxide-, and sodium acetate-based eluents.

The measurment of acid soulable lignin was made with a UV-Vis spectrophotometer between 200-400 nm (appendix 2).

3.2.3.2. FT-IR spectroscopy analysis

The information about the chemical structures of the isolated arabinoxylans and the hydroxypropyl methylated arabinoxylans were obtained by subjecting them to Fourier-transform infrared (FT-IR) spectroscopy analysis. The HPMAXs were then compared to their respective arabinoxylan to determine if substitution had taken place.

Preparation of the FT-IR sample were made by grinding 2 mg of dry material together with 200 mg of potassium bromide (KBr) into a fine powder. The powder was then pressed by a force of 8 tons under vacuum for 1 min to form a transparent pellet. The measurements were recorded

within the infrared region of 4000 cm^{-1} to 400 cm^{-1} using 20 scans per pellet sample. Three pellets were prepared for each material and their FT-IR spectra analysed with the SIMCA multivariate data analysis software.

3.2.3.3. Clouding point analysis

The clouding behaviour also known as phase behaviour of the different HPMAXs were recorded using an ultraviolet-visible (UV-Vis) spectrophotometer.

Samples for the clouding point determination were preparade by dissolving 100 mg HMPAX sample in 10 g milli-Q water. Glass cuvettes were filled with 1.5 mL of each dilute sample and sealed. The measurements were made in a temperature interval of 40-85 °C at 600 nm with a stepwise temperature increase of 1.0 °C/min. The solution was heated and cooled within the temperature interval four times to ensure that correct clouding point values were obtained. The clouding point was assigned to the temperature at which first sign of solution clouding was displayed.

4. RESULTS AND DISCUSSION

4.1. Evaluation of chemical composition

The carbohydrate composition of oat and wheat bran is very similar, see Table 2. Wheat bran is richer in carbohydrates (87.2 %) and has less solids (12.8 %) compared to oat bran (81.8 %) and (18.2 %). The same can be said about their respective AX fractions, AXO and AXW have similar carbohydrate composition but there is a larger solid content in AXO. The cellulose yield is very much the same in both brans but there is a significant difference in AX yield. Although the AX yield is significantly larger from oat bran it also has a higher solid content compared to wheat bran which should be taken into account when comparing the two. However, the higher AX yield from oat bran may not just be due to higher solid content and other contaminates but rather it indicates that the extraction method used it much more effective in isolating AX from oat bran than wheat bran.

The obtained arabinose/xylose ratio differ significantly from other literature ratios. In this study a 1:4 arabinose/xylose ratio was obtained whereas the literature values are close to a 1:1 arabinose/xylose ratio.[15-17]

Table 2. Chemical composition of carbohydrates and solids of oat and wheat bran and their respective isolated AX fraction.

	Oat	Wheat	AXO	AXW
Carbohydrate and solids (% of dry organic material)	*81.8*	*87.2*	*83.5*	*88.7*
Arabinose	4.3	4.6	14.2	15.5
Galactose	0.7	0.7	3.9	3.8
Glucose	55.1	58.1	1.1	1.8
Xylose	21.4	23.5	64.3	67.6
Mannose	0.3	0.3	–	–
Solids (% of dry organic material)	*18.2*	*12.8*	*16.5*	*11.3*
Acid insoluble	15.9	10.5	14.1	8.4
Acid-soluble lignin	2.3	2.3	2.4	2.9
Yield arabinoxylan (%)	20.2	13.6	–	–
Yield cellulose (%)	34.4	33.4	–	–

4.2. Evaluation of FT-IR spectra

4.2.1. Spectra of isolated arabinoxylans

The FT-IR spectra of the isolated arabinoxylans AXO and AXW are illustrated in Figure 4. The broad absorption peaks seen at around 3436 cm^{-1} are ascribed to the stretching of -OH groups, and the weak band at 2925 cm^{-1} are C-H stretching vibrations. The large peak at 1632 cm^{-1} is due to bending mode of the remaining water. Bending vibrations of C-H and C-O are indicated at 1465, 1418 and 1385 cm^{-1} respectively. The 1200-1000 cm^{-1} region is dominated by ring vibrations and C-OH side group stretching vibrations as well as C-O-C glycosidic bond vibrations.[18, 19] The presence of arabinofuranose side chains are characterised by the low intensity shoulders at 1158 and 989 cm^{-1}. These shoulders are more prominent in AXO than in AXW but nonetheless present (appendix 1). The small band at 896 cm^{-1} is due to the C1 group frequency and arise from the β-glycosidic linkade between the sugar units of the arabinoxylan backbone.[18] Depending on whether the arabinofuranose units occupy the O-2 and/or O-3 position of the xylan backbone, the shoulder bands shows spectral shape variations. Higher substitution in the O3 position give a lower intensity peaks at 1158 and 896 cm^{-1} as well as a loss in peak multiplicity between 1120-1000 cm^{-1} compared to a substitution at the O2 or O2 and O3 position. [11, 18]

Differences between the two spectrums can be seen in the finger print region (1500-500 cm^{-1}). AXW have significantly larger peaks at 1262 cm^{-1} and 1117 cm^{-1} compared to AWO. The former is related to the ring breathing and C-O stretching vibration of lignin and xylan, and the latter arise from their C-O-C stretch and bending vibrations.[20] The loss in peak multiplicity between 1120-1000 cm^{-1} of AXW compared to AXO suggests that it has a higher degree of substitution at the O3 position.

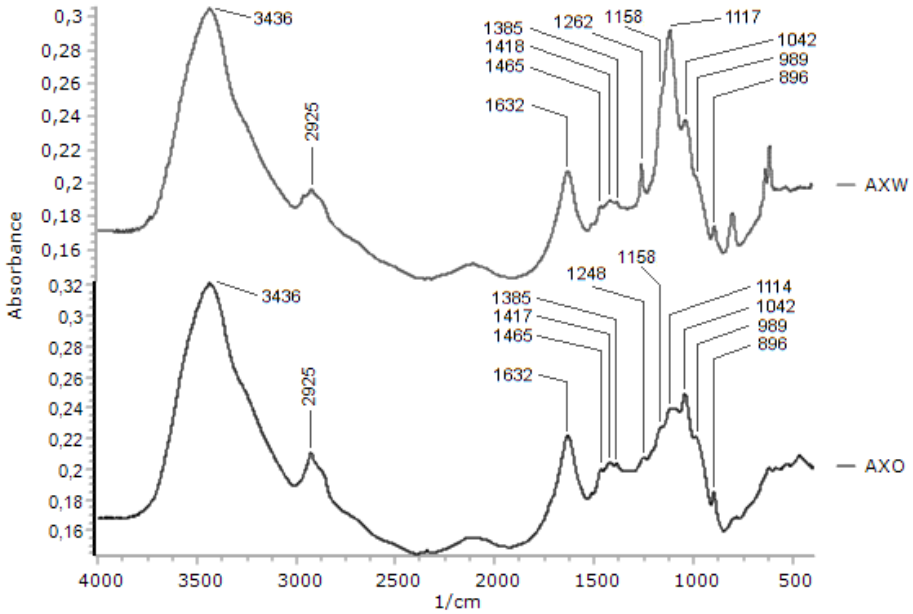

Figure 4. Spectrum of isolated arabinoxylan from oat (AXO) and wheat (AXW) in the infrared region of 4000-400 cm^{-1}. The peaks highlighted show signs of chemical group as well as typical signs of arabinoxylan.

The standard normal variate (SNV) pre-treated principal component analysis (PCA) plot of the triplet FT-IR spectra of each AX fraction can be seen in Figure 5. AX from wheat bran show a more dens data point distribution compared to AX from oat bran. This indicate that there are smaller variations within the AXW sample compare to AXO. Further, the distribution of variation between AXW and AXO indicates that the sample are different to one another which can also be seen in Figure 4.

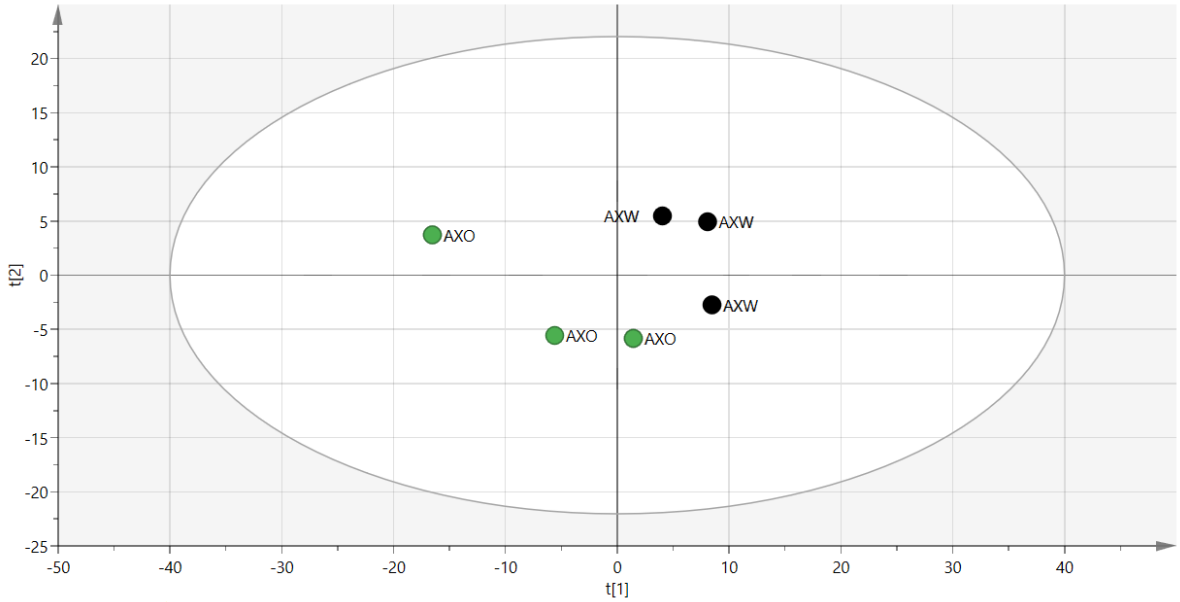

Figure 5. PCA plot of the SNV pre-treated FT-IR spectra of arabinoxylan from oat (AXO) and wheat bran (AXW).

4.2.2. Spectra of hydroxypropyl methylated arabinoxylan

Figure 6 show a comparison of the FT-IR spectra of the isolated arabinoxylans AXO and AXW and their respective hydroxypropyl methyl derivates HPMAXO and HPMAXW. Prominent differences can be seen in the spectral band 3020-2770 cm^{-1} which is assigned to the C-H stretching vibrations of the methyl group as well as in the spectral peaks at 1463, 1416 and 1381 cm^{-1} which are associated with the bending vibrations of C-H and C-O respectively. The larger peaks of the derivatives (HPMAXO and HPMAXW) at these regions compared to their precursor compounds (AXO and AXW) are indicative of successfully conjugated hydroxypropyl and methyl groups.[21]

Significant spectral differences between HPMAXO and HPMAXW were hard to identify, except for one signal, namely that at 1718 cm^{-1} ascribed to C=O stretching. Suggesting that the pre-activation of AX resulted in oxidation of its glycosidic linkages and hydroxyl groups – more so in AXO than in AXW.[22]

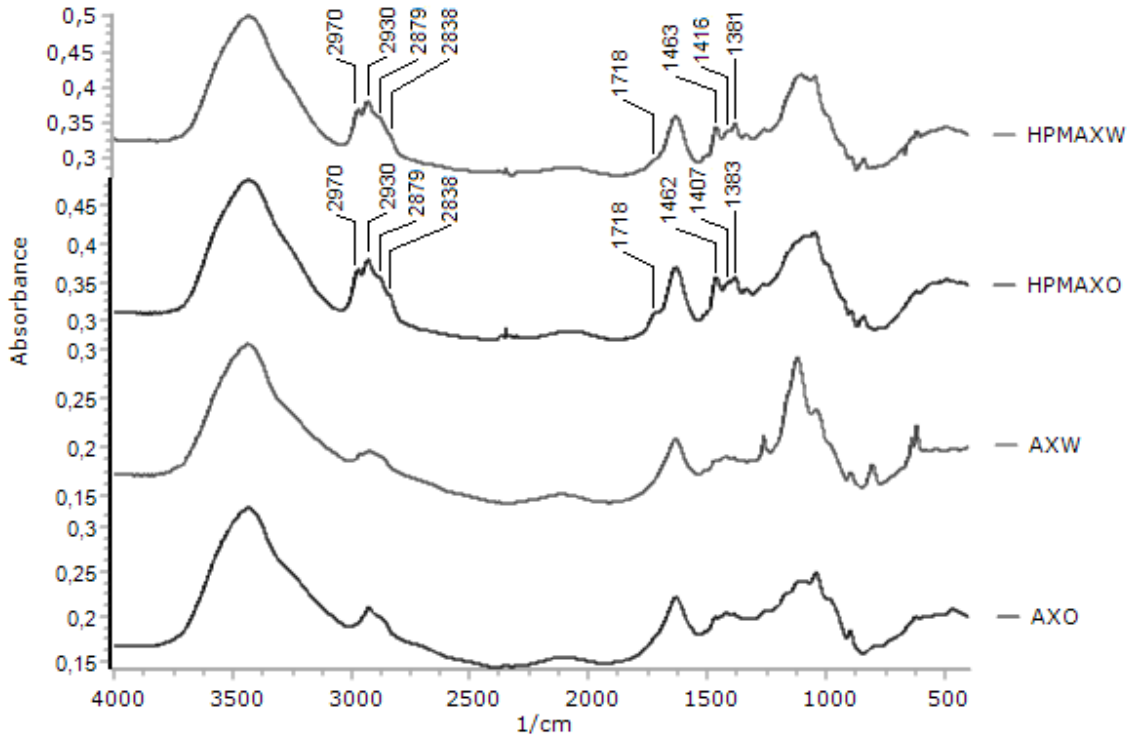

Figure 6. Spectrum of isolated arabinoxylan from oat (AXO), wheat (AXW) and their respective hydroxypropyl derivatives (HPMAXO and HPMAXW) in the infrared region of 4000-400 cm-1. The peaks highlighted show typical signs of conjugated hydroxypropyl groups.

Figure 7 show the SNV pre-treated PCA plot of the triplet FT-IR spectra of hydroxypropyl methylated AX from oat and wheat bran. HPMAXW show a slightly more dens data point distribution compared to HPMAXO indicating that there are less variations within the HPMAXW sample compare to HPMAXO. The distribution of variation between HPMAXW and HPMAXO suggest that the samples are different to one another which cannot be seen clearly in their FT-IR spectra, Figure 6.

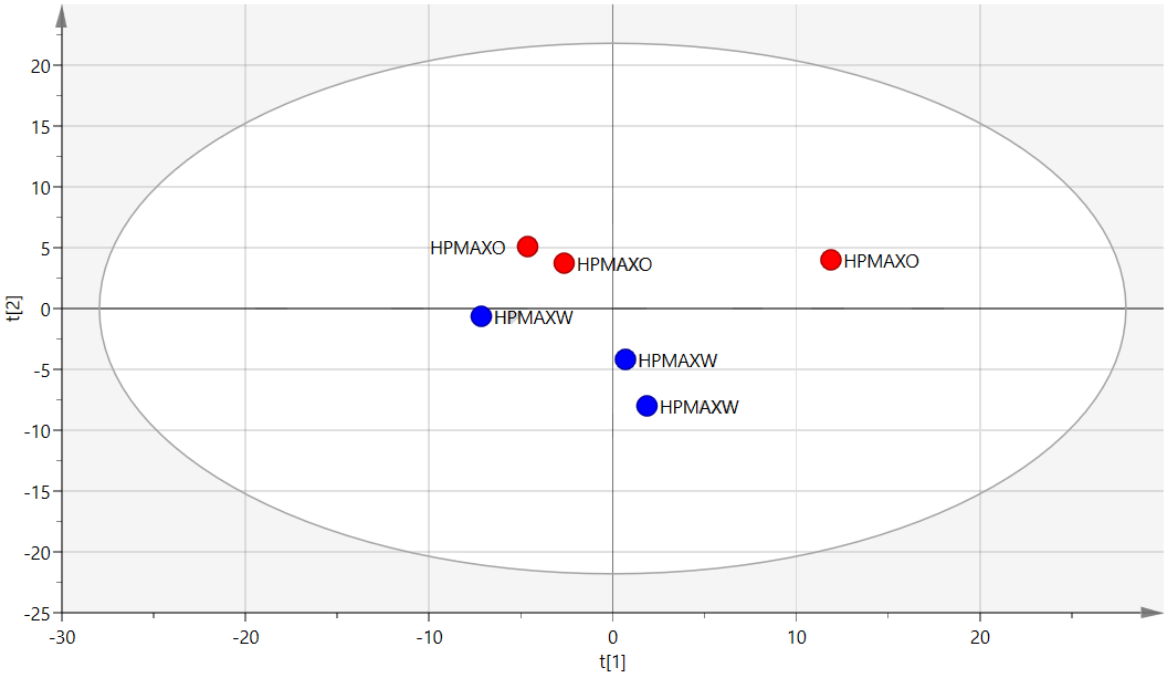

Figure 7. PCA plot of the SNV pre-treated FT-IR spectra of hydroxypropyl methylated arabinoxylan from oat (HPMAXO) and wheat bran (HPMAXW).

4.2.3. Spectra of scaled up hydroxypropyl methylated arabinoxylan

The FT-IR spectra of the scaled up HPMAXs (HPMAXOI-HPMAXOIII and HPMAXWI-HPMAXWIII) show no significant differences, Figure 8. However, minor differences in peak proportion can be seen in the regions characterised by conjugated hydroxypropyl and methyl groups (3020-2770, 1463, 1416 and 1381 cm^{-1}), indicating that less empty reaction space give rise to increased hydroxypropyl methyl substitution.

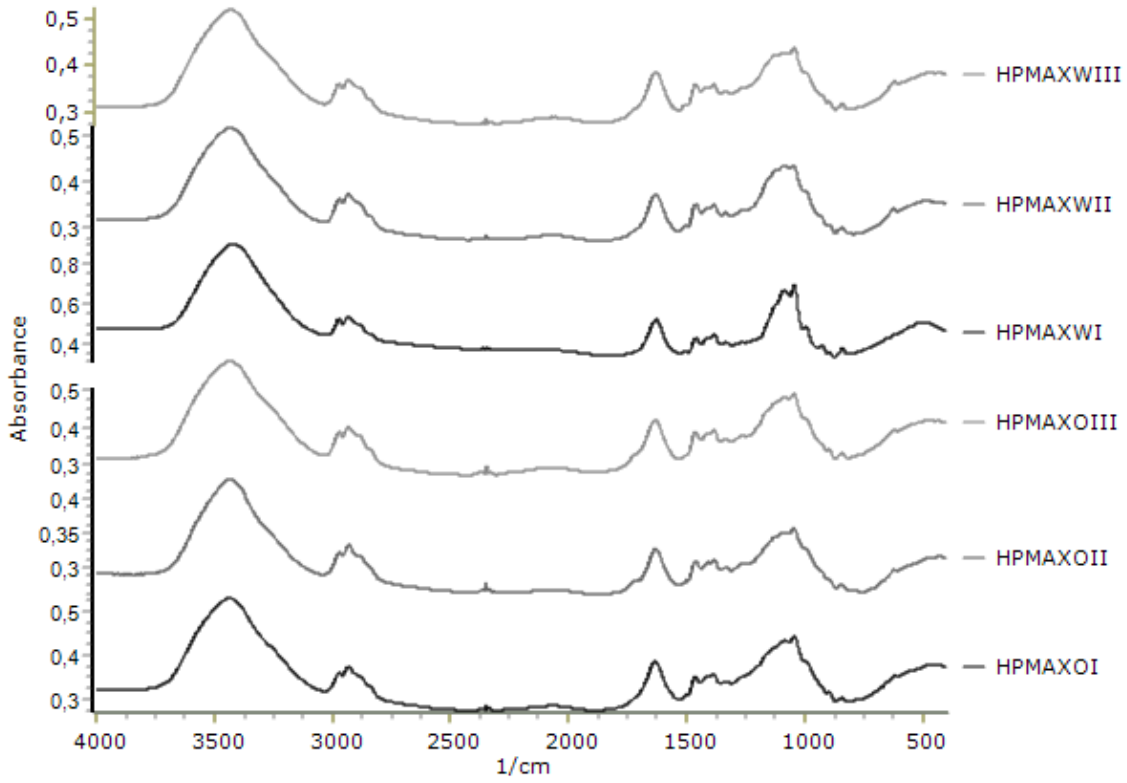

Figure 8. Spectrum of scaled up HPMAX from oat (HPMAXOI-HPMAXOIII) and wheat (HPMAXWI-HPMAXWIII).

The SNV pre-treated PCA plot of the triplet FT-IR spectra of the scaled up hydroxypropyl methylated AX from oat and wheat bran can be seen in Figure 9-10. The data point distribution pattern is hard to assess but the figures suggest that there are significant differences both within the same sample and between different scale up batches. Further, the data plots suggest that the empty reactor space influence both the degree of substitution between scale up batches as well as the bond substituent pattern within the same batch.

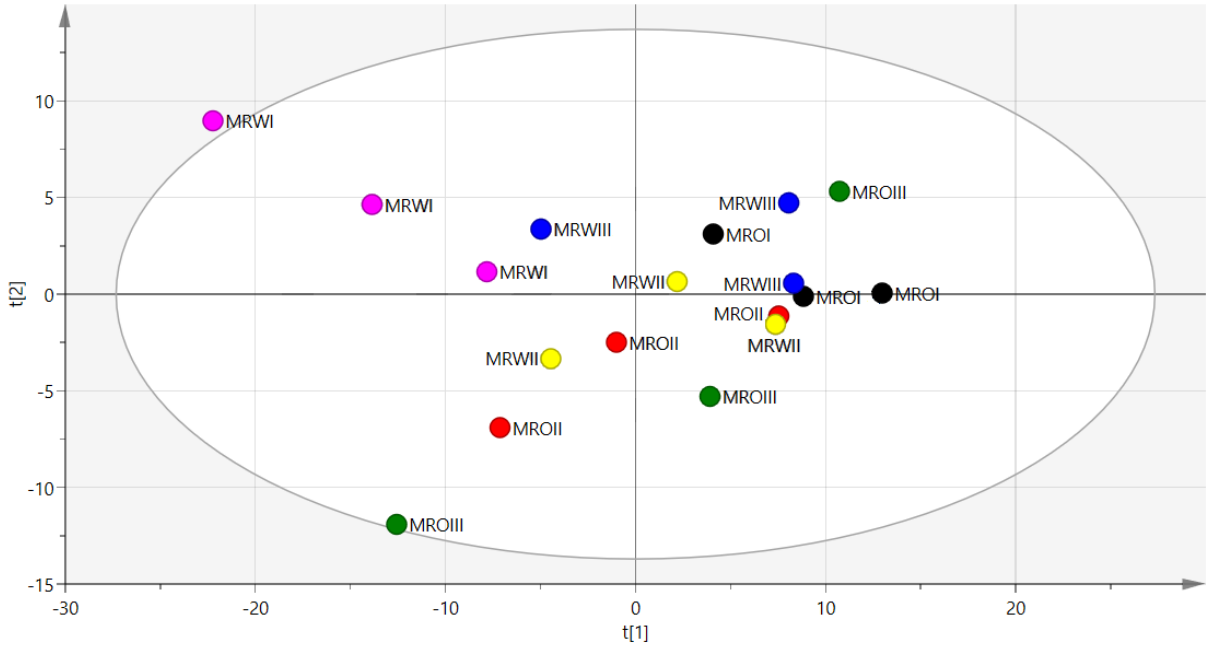

Figure 9. PCA plot of the SNV pre-treated FT-IR spectra of scaled up hydroxypropyl methylated arabinoxylan from oat and wheat bran with t[1] and t[2] axis graph. MRW(I-III): hydroxypropyl methylated AX from wheat bran made in a miniclave reactor; MRO(I-III): hydroxypropyl methylated AX from oat bran made in a minicalve reactor.

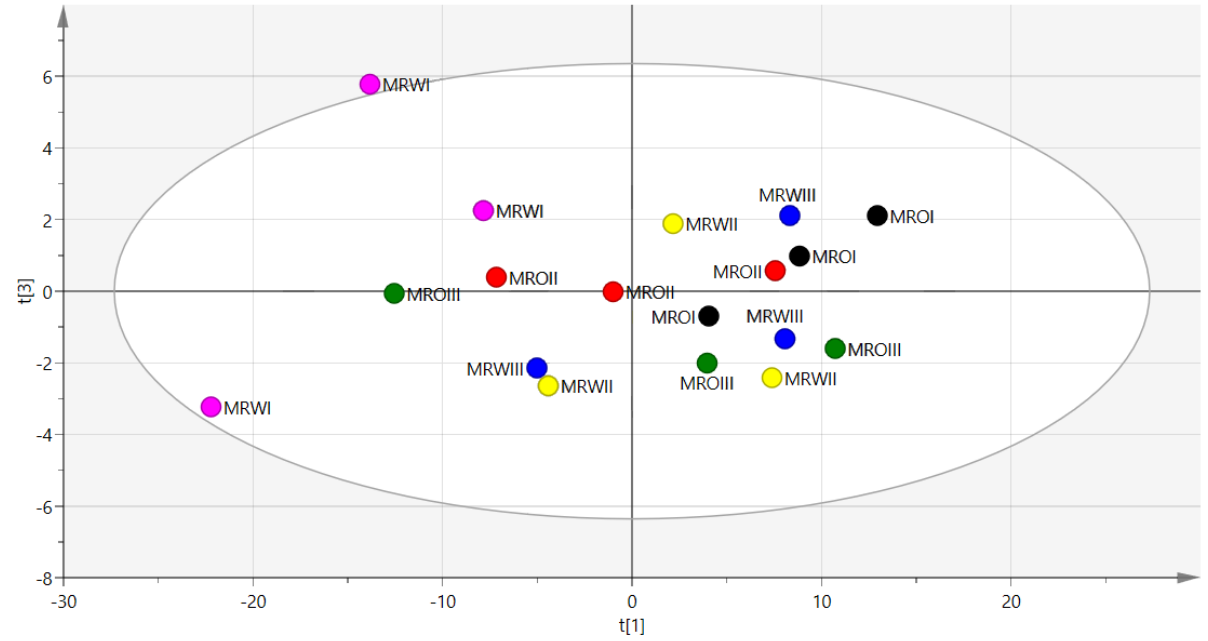

Figure 10. PCA plot of the SNV pre-treated FT-IR spectra of scaled up hydroxypropyl methylated arabinoxylan from oat and wheat bran with t[1] and t[3] axis graph. MRW(I-III): hydroxypropyl methylated arabinoxylan from wheat bran made in a miniclave reactor; MRO(I-III): hydroxypropyl methylated arabinoxylan from oat bran made in a minicalve reactor.

4.3. Evaluation of clouding behaviour

There are no significant differences in clouding point temperature between miniclave reactor scale size (II) and (III) but the (I) samples have a very high clouding point temperature. This suggest that the degree of substitution of hydrophobic groups is higher in (II) and (III) batches and that their distribution patterns are more homogenous compared to the (I) batches. In addition, this further indicate that the degree of substitution increases as the empty reactor space decrease. Further, comparing the hydroxypropyl methylated arabinoxylans made in vials (HPMAXO and HPMAXW) to the ones done in miniclave reactors its demonstrated that the vial HPMAXs have a steep curve decline whereas the miniclave HPMAXs have large absorbance fluctuations as temperature increase. Moreover, the vial HPMAXs have the lowest clouding temperature, this suggest that the vial HPMAXs have the most homogenous substituent pattern of them all.[23]

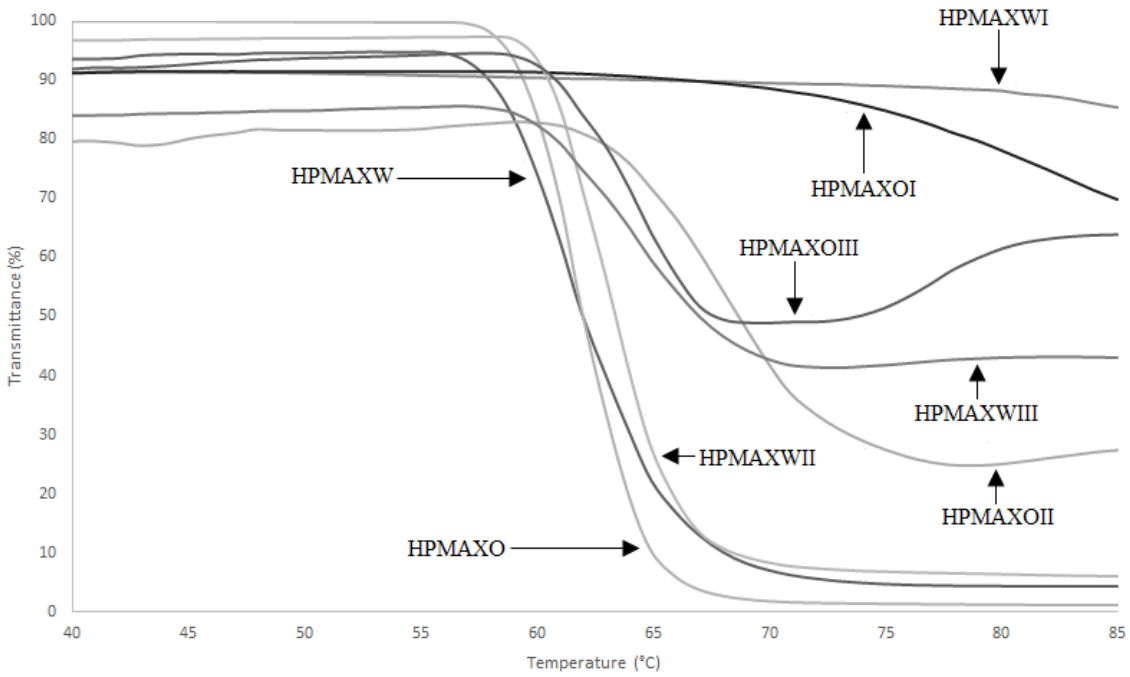

Figure 11. Show the clouding behaviour of all the HPMAX batches.

5. CONCLUSIONS

The result showed successful arabinoxylan isolation from oat and wheat bran with the extraction method used. However, the arabinose/xylose ratio was lower than literature ratios. Arabinoxylan yield was shown to be significantly larger from oat bran compared to wheat bran and the FT-IR result demonstrated that there are structural differences between the two arabinoxylans.

It was shown that both arabinoxylans were successfully hydroxypropyl methylated and that they had similar phase behaviours. Further, it was demonstrated that as the empty reaction space decreased the degree of conjugated hydroxypropyl methyl increase, indicating that the empty reaction space does influence the degree conjugated hydroxypropyl and methyl groups.

6. FUTURE WORK

A natural continuation of the study is to further examine the effectiveness of the modification and extraction method used on more plant-sources. Further, it would be of interest to see if the chemical usage in the extraction procedure can be decreased or if the extraction method can be completely replaced with a method that requires less chemicals.

Regarding the modified arabinoxylan, it would be interesting to see how well HPMAX stands in comparison to HPMC in different product applications. And since the hydroxypropyl methylation of arabinoxylan was shown to be successful the next step would be to further examine if other well-established cellulose derivatives can be replicated with arabinoxylan.

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Appendix 1

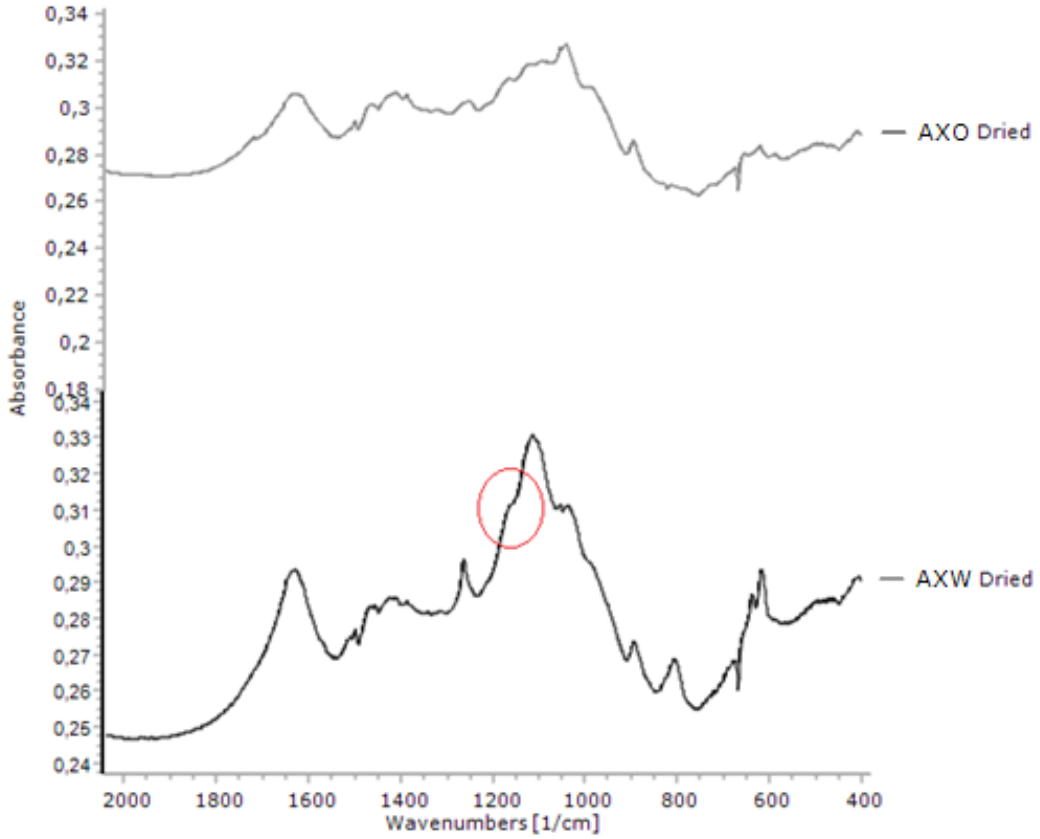

Figure A.1. Spectra show a closer look of dried AXO and AXW between 2000-400 cm^{-1}. The highlighted low intensity shoulders of AXW at 1158 cm^{-1} is here much more prominent.

Appendix 2

Measurements were made between 200-400 nm. The amount of acid soulable lignin were determined with Beer's law (A = εcl). Absorbance (A) of interest was at 205 nm, path length (l) was 1 cm, litterature value for the absorptivity (ε) was 110 (L g^{-1} cm^{-1}).[i] This way the concentrations of the solutions were determined.

Acid-soluble lignin (g/l) = c = $\frac{A}{l \times \varepsilon}$

Acid-soluble lignin (%) = $\frac{c \times V \times 100}{W}$

where W = dried weight sample, 0.2 g and V = total volume of filtrate, 1 L.

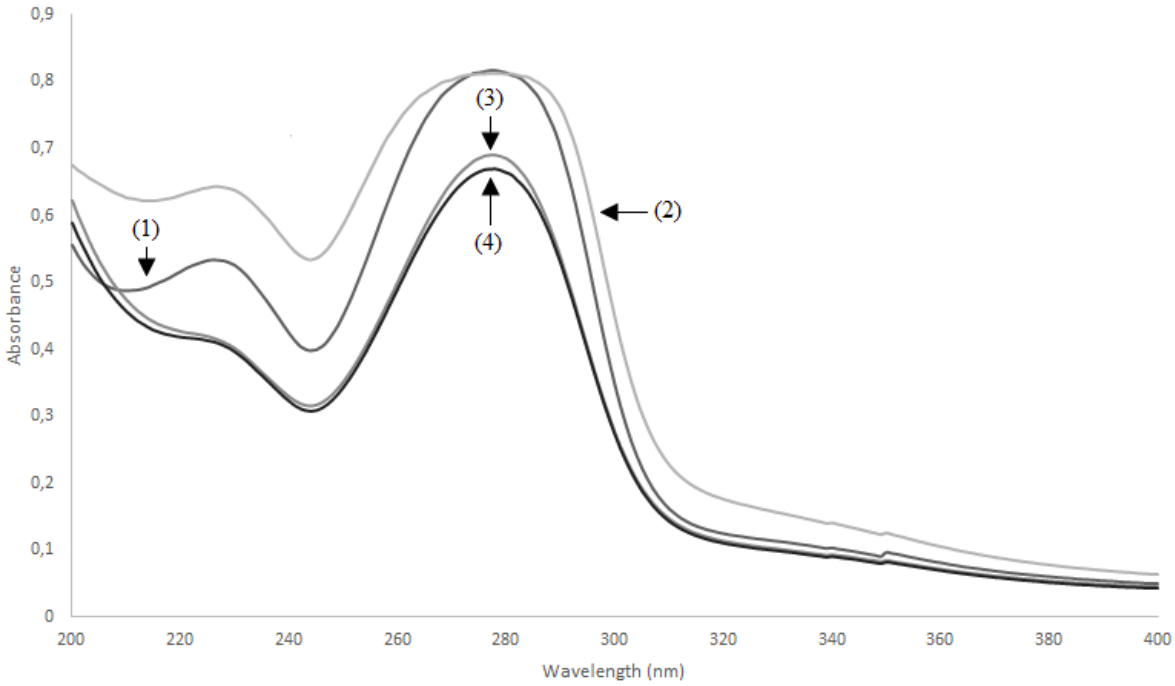

Figure A.2. Show UV-Vis absorption spectra of: (1) Wheat (2) AXW (3) AXO and (4) Oat between 200-400 nm.

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