DETERMINATION OF CARBOHYDRATES IN SUGARCANE BAGASSE PULP IN DIFFERENT TCF BLEACHING SEQUENCES

FARHAD ZEINALY,[*] AHMADREZA SARAEIAN,[**] KONSTANTIN GABOV[***] and PEDRO FARDIM[***]

[*]*Young Researchers and Elite Club, Karaj Branch, Islamic Azad University, Karaj, Iran*
[**]*Department of Pulp and Paper Industries, Faculty of Wood and Paper Engineering, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran*
[***]*Laboratory of Fiber and Cellulose Technology, Department of Chemical Engineering, Faculty of Technology, Åbo Akademi University, Turku, Finland*
✉ *Corresponding author: Ahmadreza Saraeyan, kimyachem@gmail.com*

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Changes in the carbohydrate content of cellulosic fibers during pulping and bleaching processes is a very important factor for evaluating every individual stage in pulp processing. The carbohydrate contents of raw depithed bagasse, soda pulp and bleached pulp, in each stage of two TCF sequences (OQPQP and OQPoQP), were determined by methanolysis and acid hydrolysis, and subsequently by gas-chromatography. Results showed that the bagasse hemicellulosic carbohydrates, representing about 24% of chemical components, were mostly composed of xyloses, glucose and L-arabinose sugars. The pulping process had the greatest effect on the amount of chemical components and their ratio, as the portion of all carbohydrates was increased. Also, the pulping process and all bleaching stages increased the cellulosic glucose content, while the hemicellulosic carbohydrates did not present significant changes in pulping and oxygen delignification stages. However, there was a significant reduction of hemicellulosic carbohydrates, especially in 5-carbon sugars, in the bleaching stages.

Keywords: bagasse, bleached pulp, carbohydrate, cellulose, hemicelluloses, lignin

INTRODUCTION

Wood is a natural composite material and a chemical complex of cellulose, lignin, hemicelluloses and extractives.[1] Cellulose is the framework substance, representing 40-50% of wood in the form of cellulose microfibrils, whereas hemicelluloses are the matrix substances present between cellulose microfibrils. Lignin, on the other hand, is the encrusting substance, solidifying the cell wall associated with the matrix substances.[2]

Hemicelluloses are chemically related to cellulose in that both are carbohydrates. Carbohydrates are chemical substances composed of carbon, hydrogen and oxygen, in which the last two elements are present in the same proportions as they are in water.[3] Separation of cellulose and hemicelluloses is based on their respective solubility in alkali; cellulose is not soluble in a 17.5% solution of caustic soda (NaOH), whereas hemicelluloses are.[4,5] The molecules of hemicelluloses are chain like, like those of cellulose, but the hemicelluloses polymerization degree is much smaller (on the average, about 150). Unlike cellulose, which is exclusively composed of glucose, hemicelluloses include a variety of monosaccharides, mainly comprising pentoses (β-D-xylose, α-L-arabinose), hexoses (β-D-glucose, β-D-mannose, α-D-galactose), uronic acids (β-D-glucuronic acid, α -D-galacturonic acid, α-D-4-O-methylglucuronic acid).[6]

Carbohydrates are fundamental components in cellulosic pulp production, especially in fully bleached pulps. Pulping and bleaching processes are generally used for the purification of cellulosic fibers, and, in these processes, the selective elimination of lignin occurs without significant loss or degradation of carbohydrates.[7]

During different pulping and bleaching processes, carbohydrate contents and ratios change. In the pulping process and in each stage of the bleaching sequence, different chemicals

and conditions are used. Therefore, the composition of lignocellulosic materials is affected in different ways.[8] Thus, an accurate analysis of carbohydrates in wood and pulp is important to monitor the process and product quality in the pulp and paper industry. In fully bleached pulp production, the carbohydrate type and amount are important factors, as they influence pulp properties and determine the final product type.

For carbohydrate analysis, fibers need to be first depolymerized to the constituent sugar units, which are then detected and quantified by subsequent analysis. Acid hydrolysis is the conventional method for depolymerization of carbohydrates in solid samples.[9,10] The main problem of this method is the degradation of labile acids, meaning that only neutral sugar units can be quantified.[11] Acid methanolysis is another method for depolymerization of hemicelluloses and pectins, which does not degrade uronic acids. Also, in this method, cellulose is not decomposed to glucose units, and thus the amount of glucoses is only related to hemicellulosic carbohydrates. Actually, cellulose chains are only slightly degraded by acidic methanolysis, meaning that most of the glucose formed is derived from non-cellulosic polysaccharides.[11,12] Therefore, the measurement of cellulosic glucose should be done by the acid hydrolysis method.

The quantitative determination of monosaccharides resulting from hydrolysis or methanolysis is usually performed by gas chromatography with flame ionization detection (GC-FID), gas chromatography with mass spectrometric detection (GC–MS), and high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD).[10,13]

In this research, the chemical composition of depithed bagasse in raw fiber form, unbleached soda pulp and bleached pulp, in each stage of two TCF bleaching sequences, was investigated. The carbohydrates analysis was performed by acid hydrolysis and acid methanolysis depolymerization methods and subsequently by quantitative determination by GC-FID.

EXPERIMENTAL

Materials

Raw depithed sugarcane bagasse fibers and unbleached soda bagasse pulp were obtained from Pars Paper Industries Company, which is located in the southwest of Iran. Bleached pulp samples were prepared by different TCF bleaching sequences: OQPQP and OQPoQP (Table 1). After each stage, a sample was collected, freeze-dried and ground.

Pulp bleaching

Bleaching sequences and stage conditions are indicated in Table 1. The oxygen pressure and pulp consistency, in all cases, were constant, respectively, 5 bar and 10%. Chelation stages were carried out with 0.3% DTPA at a controlled pH of 4-5.

Chemical composition analysis

Extractives and ash contents of raw bagasse and unbleached pulp were measured according to Tappi Standards Methods, respectively, T-204 and T-211.[14] For lignin measurements, both acid-insoluble and acid-soluble lignin, the Klason lignin method and subsequently UV-Vis analysis of the filtrate were applied, according to Tappi standards T-222 and UM-250.[14]

Table 1
Conditions of pulping and bleaching stages

Seq.	Stage	Time, min	Temp., ºC	H_2O_2, %	NaOH, %	Stage yield, %
UB	-	-	170	-	10	50.0
OQPQP	O_{90}	60	90	-	2.5	96.3
	O_{100}	60	100	-	2.5	94.8
	O_{110}	60	110	-	2.5	93.6
	$O_{110}Q_1$	60	70	-	-	99.8
	$O_{110}Q_1P_{1\text{-}120}$	120	90	2.0	2.0	98.3
	$O_{110}Q_1P_{1\text{-}180}$	180	90	2.0	2.0	98.0
	$O_{110}Q_1P_{1\text{-}180}Q_2$	60	70	-	-	99.9
	$O_{110}Q_1P_{1\text{-}180}Q_2P_2$	180	90	2.0	2.0	97.2
OQPoQP	$O_{110}Q_1Po_{2\%}$	60	110	2.0	2.0	96.5
	$O_{110}Q_1Po_{3\%}$	60	110	3.0	2.0	96.0
	$O_{110}Q_1Po_{2\%}Q_2$	60	70	-	-	99.9
	$O_{110}Q_1Po_{2\%}Q_2P$	180	90	2.0	2.0	98.6

Acid methanolysis

Acid methanolysis was performed according to the method explained by Sundberg *et al.*[11] About 10 mg of each freeze-dried sample was subjected to acid methanolysis, using 2 ml of 2 M HCl solution in anhydrous methanol for 5 hours at 100 ºC. After cooling to room temperature, all the samples were neutralized by adding 200 µl of pyridine. Three pear-shaped flasks were subjected to similar conditions after adding one ml of carbohydrate calibration solution (0.1 mg/ml MeOH) to each of them. Moreover, 4 ml of internal standard sorbitol (0.1 mg/ml MeOH) for pulp samples and 1 ml for calibration solutions were added. To eliminate fibers during the silylation, 1 ml of clear solution was transferred to a new test tube. Then, fiber-free solutions were evaporated under nitrogen gas at 50 ºC and were further dried in a vacuum oven for 20 minutes at 40 ºC. Samples were dissolved by adding 150 µl pyridine.

For silylation, after adding 150 µl HMDS (Hexa-Methyl Di-Silazane) and 70 µl TMCS (Tri-Methyl Chloro Silane) to the samples, they were kept overnight at room temperature. Small amounts of clear phase of silylation samples were transferred into GC-vials and inserted into a GC sampler.

Acid hydrolysis

Acid hydrolysis was carried out according to the method originally described by Saeman *et al.*[15] About 10 mg each of freeze-dried sample was weighed and placed in a test tube, to which 1 ml of 72% sulfuric acid was added afterwards. The tubes were kept in a 30 ºC water bath for 60 minutes, then the contents were diluted with 28 ml distilled water and put in an autoclave at 125 ºC for 60 minutes. After being cooled to room temperature, the samples were mixed with 1-2 droplets of bromocresol green indicator and some amount of $BaCO_3$ (the color of the clear phase of the samples turned blue). Additionally, 1 ml of internal standard sorbitol (5 mg/ml H_2O) was added to the samples and the test tubes were centrifuged for 10 minutes. One ml of clear solution and a few ml of pure acetone were transferred to a new test tube. Then, the clear solutions were evaporated under nitrogen gas at 50 ºC and were further dried in a vacuum oven for 20 minutes at 40 ºC. Samples were dissolved by adding 150 µl pyridine. The silylation procedure was the same as described above. Small amounts of clear phase of the silylation samples were transferred to GC-vials and inserted into the GC sampler.

RESULTS AND DISCUSSION

Bagasse is one of the most important non-wood fibers in pulp production. The chemical composition of bagasse has been reported by some researchers. As shown in Table 2, carbohydrates represent about 70% of this lignocellulosic material, and also the portion of pentosans exceeds 80% of hemicelluloses. In bagasse fibers, the main hemicellulose is composed of a backbone of xylose, branched with glucose and arabinose units[16,17,18] (Fig. 1).

Table 3 indicates the results of chemical composition assessment for raw depithed bagasse, unbleached soda pulp and bleached pulp samples in different TCF bleaching sequences, after each stage. Pentosans make up the main part of hemicellulosic carbohydrates in the bagasse and the produced pulps. The dominant 5-carbon sugar is xylose (Table 4). The ratio of sugars in bagasse hemicelluloses was about 79.1(Xyl.): 15.1(Glc.): 5.8(Ara.) (Table 4).

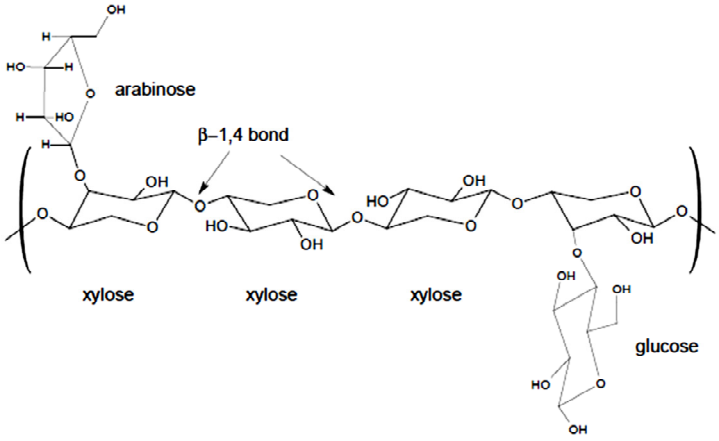

Figure 1: Simplified structure of bagasse main hemicellulose[17]

Table 2
Chemical composition of depithed sugarcane bagasse

Cellulose, %	Hemicelluloses, %	Pentosan, %	Lignin (acid insoluble), %	Ash, %	Extractives (alcohol: benzene), %	Reported by
44.09	23.89	19.98	21.42[a]	3.25	7.14	Recent study
40.50	33.20	13.70	27.70	4.80	3.80[b]	Hamzeh *et al*., (2013)[19]
47.40	-	-	20.35	1.74	3.15[c]	Samariha and Khakifirooz, (2011)[20]
55.75	-	-	20.50	1.85	3.25[c]	Hemmasi *et al*., (2011)[21]
42.34	28.60	23.90	21.70	2.10	1.85	Agnihotri *et al*., (2010)[22]
43.60	33.50	-	18.10	2.30	-	Sun *et al*., (2004)[16]
45.00	-	28.80	17.70	1.10	2.50	Sunján *et al*., (2001)[23]

[a] Total lignin content; [b] Soluble in Ethanol - dichloromethane mixture; [c] Soluble in alcohol - acetone mixture

Table 3
Chemical composition of depithed raw bagasse and pulp samples

Seq.	Stage	Cellulose, mg/g	Hemicellulose, mg/g	Pentosan, mg/g	Lignin, mg/g
RB	Raw bagasse[a]	440.9	238.9	199.8	214.2
UB	Soda pulp[b]	665.5	291.2	244.0	30.3
OQPQP	O_{90}	692.2	286.8	236.4	16.9
	O_{100}	694.2	287.6	236.6	13.8
	O_{110}	695.6	288.8	237.6	12.3
	$O_{110}Q_1$	706.8	282.2	234.9	11.6
	$O_{110}Q_1P_{1\text{-}120}$	710.4	284.3	237.1	6.0
	$O_{110}Q_1P_{1\text{-}180}$	712.8	281.1	234.2	5.8
	$O_{110}Q_1P_{1\text{-}180}Q_2$	719.1	275.1	227.5	5.7
	$O_{110}Q_1P_{1\text{-}180}Q_2P_2$	727.3	270.3	221.0	3.9
OQPoQP	$O_{110}Q_1Po_{2\%}$	716.4	281.2	232.0	4.8
	$O_{110}Q_1Po_{3\%}$	720.2	276.5	230.3	4.4
	$O_{110}Q_1Po_{2\%}Q_2$	719.7	278.0	229.6	4.6
	$O_{110}Q_1Po_{2\%}Q_2P$	730.2	268.5	218.9	3.6

[a] Ash: 32.5 mg/g; Extractives: 71.4 mg/g
[b] Ash: < 13.4 mg/g; Extractives: none

Table 4
Content of sugars and uronic acids in depithed raw bagasse and pulp samples

Seq.	Stage	Acid hydrolysis	Acid methanolysis									
		Glucose, mg/g	Glc, mg/g	Xyl, mg/g	Ara, mg/g	4-O-MeGlcA, mg/g	Gal, mg/g	GlcA, mg/g	GalA, mg/g	Man, mg/g	Rha, mg/g	Sum, mg/g
RB	Raw bagasse	476.3	35.4	186.1	13.7	1.5	1.6	0.3	0.0	0.3	0.0	238.9
UB	Soda pulp	707.5	42.0	227.2	16.8	2.4	2.0	0.4	0.0	0.4	0.0	291.2
OQPQP	O_{90}	738.6	46.4	220.6	15.8	2.0	2.0	0.0	0.0	0.0	0.0	286.8
	O_{100}	741.2	47.0	221.0	15.6	2.0	2.0	0.0	0.0	0.0	0.0	287.6
	O_{110}	742.8	47.2	222.0	15.6	2.0	2.0	0.0	0.0	0.0	0.0	288.8
	$O_{110}Q_1$	750.2	43.4	219.2	15.7	2.3	1.6	0.0	0.0	0.0	0.0	282.2
	$O_{110}Q_1P_{1\text{-}120}$	754.0	43.6	221.5	15.6	2.0	1.6	0.0	0.0	0.0	0.0	284.3
	$O_{110}Q_1P_{1\text{-}180}$	756.1	43.3	219.4	14.8	2.0	1.6	0.0	0.0	0.0	0.0	281.1
	$O_{110}Q_1P_{1\text{-}180}Q_2$	763.1	44.0	214.4	13.1	2.1	1.5	0.0	0.0	0.0	0.0	275.1
	$O_{110}Q_1P_{1\text{-}180}Q_2P_2$	773.1	45.7	211.6	9.4	2.0	1.5	0.0	0.0	0.0	0.0	270.3
OQPoQP	$O_{110}Q_1Po_{2\%}$	761.8	45.4	217.2	14.8	2.2	1.6	0.0	0.0	0.0	0.0	281.2
	$O_{110}Q_1Po_{3\%}$	764.3	44.1	215.7	14.6	2.1	1.6	0.0	0.0	0.0	0.0	276.5
	$O_{110}Q_1Po_{2\%}Q_2$	764.5	44.8	215.9	13.7	2.0	1.6	0.0	0.0	0.0	0.0	278.0
	$O_{110}Q_1Po_{2\%}Q_2P$	776.3	46.0	209.8	9.1	2.0	1.5	0.0	0.0	0.0	0.0	268.5

A yield loss occurred for each component at different degrees after the pulping process and bleaching stages, as this property is controlled by H-factor adjustment in pulping and by the selectivity factor in the bleaching process.[8] In the paper making industry, selective lignin removal is the main goal, but in the production of cellulose derivatives (dissolving pulp), the elimination of hemicelluloses is favorable.[24] In all chemical treatments (cooking or bleaching), the main yield loss was related to lignin removal (Table 3), but the carbohydrate amounts and their ratio also changed (Table 4, Fig. 2).

Carbohydrates and lignin together make up a major portion of the biomass samples. These constituents must be measured as part of a comprehensive biomass analysis. Carbohydrates can be structural or non-structural. Structural carbohydrates are bound in the matrix of the biomass, while non-structural carbohydrates can be removed using extraction or washing steps.[24]

In the acid methanolysis depolymerization, all dissolved glucose is related to the hemicelluloses, and cellulosic glucose does not play a significant role.[11,12] Therefore, the amount of cellulosic glucose can be determined by the subtraction of the amount of methanolysis glucose from its total amount measured by the acid hydrolysis method (Tables 3 and 4). As the results show, the total glucose amount of raw depithed bagasse fibers was 476.3 mg/g, out of which the cellulosic glucose was 440.9 mg/g.

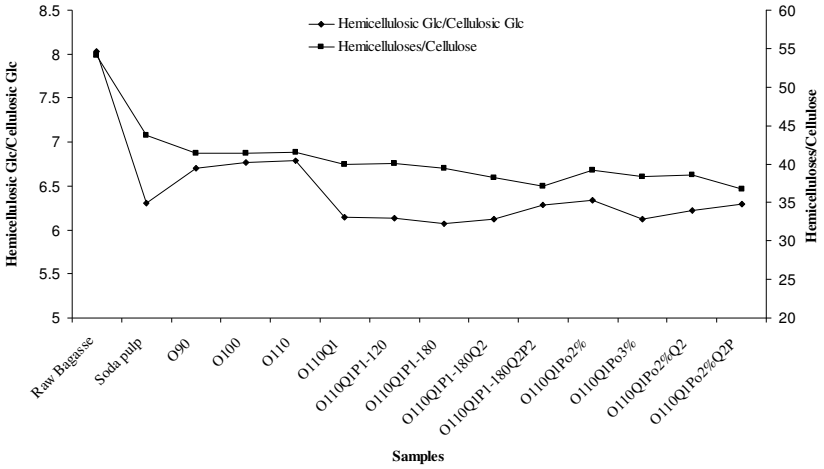

Figure 2: Relationship of hemicelluloses to cellulose and hemicellulosic glucose to cellulosic glucose in different stages

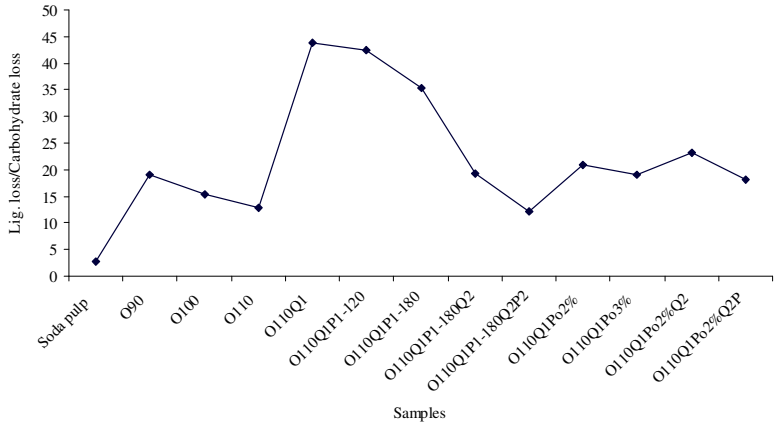

Figure 3: Relationship of lignin removal to carbohydrates loss in different stages

The cooking process caused a significant change in the amounts of chemical components and their ratio, as the glucose (cellulosic and non-cellulosic) represented more than 70% in the chemical composition of unbleached soda pulp. The cellulosic glucose was 665.5 mg/g and the amount of glucose related to hemicelluloses was only 42.0 mg/g (Tables 3 and 4). Factually, excluding the selective removal of lignin, the ratio between cellulose and hemicelluloses changed in the pulping process, and the portion of hemicelluloses was decreased by the partial hydrolysis of amorphous polymer chains. Figure 2 indicates the relationship between hemicellulosic glucose and cellulosic glucose amount, as well as that of total hemicelluloses and cellulose amount, in different samples. As shown in Figure 2, both ratios of Hem./Cel. and Hem.Glc./Cel.Glc. decreased by the soda pulping process. The Hem./Cel. ratio is mostly referred to as the ratio of 5-carbon saccharides to cellulosic glucose amount, because the predominant sugars of bagasse hemicelluloses are xylose and arabinose (Table 4).

The lignin content of soda pulp was of 3.03%, carbohydrates represented 95.67% of unbleached fibers, and also there was a slight amount of inorganic materials (1.34%). In the oxygen delignification treatment, the situation was the same as for the pulping process, and the carbohydrates content increased (Tables 3 and 4). In other words, the portion of carbohydrates was increased by the elimination of other components, mostly lignin. Results showed that the glucose amounts of both cellulose and hemicelluloses were increased by oxygen delignification, while the pentosan saccharides (xylose and arabinose) contents were decreased (Tables 3 and 4). As shown in Figure 2, the Hem.Glc./Cel.Glc. ratio rose, while Hem./Cel. ratio reduced. The instability of arabinose side-chains and xylose in the alkali environment of the oxygen delignification stage could be the reason of the decrease in pentosans. Also, another reason could be the depolymerization of some short and amorphous cellulosic chains in the methanolysis procedure, which caused an increase in the amount of hemicellulosic glucose.[13,26]

The higher resistance of long chain cellulose molecules in a hot alkaline environment, compared to amorphous and branched hemicelluloses, led to preventing cellulose from degradation in the oxygen delignification process. However, there was no significant degradation of hemicellulosic carbohydrates upon temperature rising from 90 ºC to 110 ºC in the oxygen delignification treatments (Table 4, Fig. 2). Also, the use of magnesium sulfate as a carbohydrate protector preserved the pulp yield and improved process selectivity.[27] In the oxygen delignification process, transition metals, such as manganese, copper, and iron, lead to creating hydroxyl radicals, which can attack carbohydrates with detrimental results. Thus, magnesium ion functions by precipitating as magnesium hydroxide, which absorbs transition metals or forms complexes with them, making them unavailable for creating hydroxyl radicals.[28] In oxygen delignification at 110 ºC, the loss of lignin was of 56.10%, which caused the pulp to have a lignin content of 12.3 mg/g. The cellulose amount rose up to 695.6 mg/g (an increase of 4.52%), the hemicellulosic sugars slightly decreased (0.82%), while hemicellulosic glucose rose up from 42.0 mg/g to 47.2 mg/g.

The ratio of lignin removal to carbohydrates loss in the pulping process was significantly lower than in the bleaching stages (Fig. 3). The reasons are the higher selectivity of the chemicals and conditions, which are usually used in bleaching sequences, and also that most of the unstable and extractable carbohydrates were removed in the pulping process.[8,24] In the oxygen delignification stage, this ratio was decreased by the rising reaction temperature, but the difference was little. In the bleaching stages, this ratio was higher than in the oxygen delignification stage and the ratio was decreased step by step, as it was 35.2 in the $P_{1\text{-}180}$ stage and 12.0 in the P_2 stage. Actually, when the lignin content was reduced by performing the bleaching stages, the lignin accessibility was decreased in the pulp, step by step, therefore bleaching chemicals would act on other components of fibers (carbohydrates) and so the selectivity of the stage was decreased (Fig. 3).

A little lignin reduction occurred in the Q_1 stage, but the cellulosic glucose amount had a significant expansion (Table 4). Hemicellulosic glucose and xylose had a decline, as shown in Figure 2, and both ratios (Hem.Glc./Cel.Glc. and Hem./Cel.) were decreased in the Q_1 stage. This can be explained by the higher degradation and dissolution of hydrophilic carbohydrates of hemicelluloses under the acidic conditions of the Q stage.[26] Also, the xylan chains were protected by the conversion of 4-O-methylglucuronic acid

groups to hexenuronic acid groups during a previous alkaline process. Thus, xylan hemicellulose had been more stable toward alkali and so the peeling reaction did not occurr.[29] As a result, the xylose did not dissolve very much in the next stage (Q_1).

Hexenuronic acid groups are formed by base catalyzed elimination of methanol from 4-O-methyl-D-glucurnoxylans, under alkaline conditions, but under mild acidic conditions, there is no formation of hexenuronic acid groups and deformation of 4-O-methylglucuronic acid groups.[29,30] Therefore, it could be expected that there would be an increase in 4-O-methylglucuronic acid groups in all Q stages.

There was an intense lignin loss of about 50% in the P_1 stage. Thus, the Lig.loss/Car.loss ratio reached the highest value; however, it was decreased when the reaction time was extended (Fig. 3). In the case of carbohydrates, there was an increase in all carbohydrates amounts, but most significantly in cellulose. Additionally, the carbohydrates ratio did not have significant change in P_1 stage by the time extension. Also, there was an important decrease in the amount of L-arabinose (Fig. 2, Table 4).

The situation in the Q_2 stage was the same as in Q_1, and so, the lignin and yield losses were slight. Both cellulosic and hemicellulosic glucose were increased, but the hemicellulosic glucose had greater expansion (Fig. 2, Table 4). In this stage, the amounts of xylose and L-arabinose were considerably decreased. The P_2 stage, just like P_1, led to a significant lignin removal (31.58%), but the Lig.loss/Car.loss ratio was lower than that of the P_1 stage (Fig. 3). Both 5-carbon sugars of hemicelluloses were decreased. L-arabinose loss was the most significant, but the hemicellulosic glucose exhibited some increase. Therefore, the Hem.Glc./Cel.Glc. ratio rose, while Hem./Cel. ratio reduced (Fig. 2). Hexenuronic acid groups are known as xylan chain protectors toward alkali peeling reaction, but these acidic groups are not stable under acidic conditions, because enol ethers are acid-labile.[29] In the acidic medium of the Q_2 stage, hexenuronic acid groups were hydrolyzed and removed. Therefore, the intense decline of 5-carbon saccharides might be related to the hydrolysis of hexenuronic acid groups in the previous stage (Q_2).

In the sequence of OQPoQP, the pulp was bleached by a similar process to that of the Q_1 stage. In the P_o stage, lignin elimination was performed to a greater degree, compared to the P_1 stage, but the carbohydrates degradation also increased, thus, the ratio of Lig.loss/Car.loss was lower than in the P_1 stage (Table 3, Fig. 3). Most carbohydrates loss occurred in xylose and L-arabinose, while the amounts of both cellulosic and hemicellulosic glucoses were significantly increased (Table 4). Also, the ratio of Hem.Glc./Cel.Glc. rose, but the Hem./Cell. ratio reduced (Fig. 2). Additional peroxide use in the P_o stage (3%) led to more changes in the ratios, but did not have a significant effect on lignin reduction (Tables 3 and 4).

Similarly to the previous sequence (OQPQP), in the Q_2 stage, there was some reduction in hemicellulosic carbohydrates, but the portion of cellulosic glucose was increased (Table 4). The reduction of hemicellulosic carbohydrates was intense in the P_2 stage, and the highest loss was related to 5-carbon sugars. The portion of cellulosic glucose was significantly expanded, therefore, the Hem./Cel. ratio was reduced, but the ratio of Hem.Glc./Cel.Glc. did not change (Fig. 2, Table 4). This result can be explained by the depolymerization of some short and amorphous cellulosic chains in the methanolysis analysis. Actually, a cleavage of the cellulose chains, during the bleaching process, produces molecules with a lower molecular weight and better solubility. In addition, the oxidation of the aldehyde end-group to the carbohydrate acid group (uronic acid) also increases solubility.[13,26]

CONCLUSION

The use of methanolysis and hydrolysis depolymerization methods was adequate for a quantitative determination of carbohydrates in lignocellulosic fibers. There was no degradation of uronic acids when applying the methanolysis method and no depolymerization of cellulose chains occurred in this system, therefore, the determination of both hemicellulosic and cellulosic glucoses was achieved by the hydrolysis method to measure the total glucose content.

The poor selectivity of the pulping process resulted in higher degradation of carbohydrates and changes in their ratios. Additional temperature increase in the oxygen delignification stage caused a little decrease in the Lig.loss/Car.loss ratio, but did not have any significant effect on the carbohydrates ratio. In the Q stages, there was an important decrease in hemicellulosic carbohydrates, especially in the 5-carbon sugars. Also, the reduction of 5-carbon

sugars was intense in the P and P_o stages, while in the P_2 and P_o stages, which presented higher yield losses, the hemicellulosic glucose content was increased. The reason was, probably, the depolymerization of some short and amorphous cellulosic chains in the methanolysis analysis, and also the reduction of cellulose viscosity led to an increase in the separation and dissolution of glucoses in the methanolysis system.

REFERENCES

[1] H. A. Core, W. A. Cote and A. C. Day, in "Wood Structure and Identification", 2nd ed., Syracuse University Press, New York, 1979, p. 182.

[2] D. N. S. Hon and N. Shiraishi, in "Wood and Cellulosic Chemistry", 2nd ed., Marcel Dekker Inc., Basel, Switzerland, 2001, p. 914.

[3] J. M. Berg, J. L. Tymoczko and L. Stryer, in "Biochemistry", 6th ed., W. H. Freeman & Co., New York, 2006, p. 1026.

[4] R. M. Rowell, R. A. Young and J. Rowell, in "Paper and Composites from Agro-Based Resources", CRC Press, 1997, p. 267.

[5] K. Wilson, E. Ringstrom and I. Hedlund, *Svensk Papperstidn*., **55**, 31 (1952).

[6] A. Ebringerova, Z. Hromadkova and T. Heinze, "Hemicellulose, Advances in Polymer Science", 2005, p. 67.

[7] F. Zeinaly, J. Shakhes and N. Zeinali, *Carbohyd. Polym*., **92**, 976 (2013).

[8] H. Sixta, in "Handbook of Pulp", 1st ed., Strauss GmbH, Morlenbach, Germany, 2006, p. 1352.

[9] B. L. Browning, in "Methods of Wood Chemistry", 1st ed., John Wiley & Sons., New York, 1967, p. 384.

[10] S. Willfor, A. Pranovich, T. Tamminen, J. Puls, C. Laine *et al*., *Ind. Crop. Prod*., **29**, 571 (2009).

[11] A. Sundberg, K. Sundberg, C. Lilland and B. Holmbom, *Nordic Pulp Pap. Res. J*., **11**, 216 (1996).

[12] B. Holrnbom and F. Orsa, in *Procs. 7th International Symposium of Wood Pulping Chemistry*, Beijing, 1993, pp. 810-817.

[13] M. Iakovlev, Doctoral dissertation, Aalto University Publication Series, Helsinki, Finland, 2011, p. 121.

[14] TAPPI (US Technical Association of Pulp and Paper Industry) Standard Testing Methods, 2006-2007.

[15] J. F. Saeman, W. E. Moore, R. L. Mitchel and M. A. Millet, *Tappi J*., **37**, 336 (1954).

[16] J. X. Sun, X. F. Sun, R. C. Sun and Y. Q. Su, *Carbohyd. Polym*., **56**, 195 (2004).

[17] S. N. Walford, in *Procs. The South African Sugar Technologists Association*, 2008, pp. 266-273.

[18] J. B. Shi, Q. L. Yang and L. Lin, *Polym. Degrad. Stabil*., **98**, 550 (2013).

[19] Y. Hamzeh, A. Ashori, Z. Khorasani, A. Abdulkhani and A. Abyaz, *Ind. Crop. Prod*., **43**, 365 (2013).

[20] A. Samariha and A. Khakifirooz, *BioResources*, **6**, 3313 (2011).

[21] A. H. Hemmasi, A. Samariha, A. Tabei, M. Nemati and A. Khakifirooz, *Am.-Eurasian J. Agric. Environ. Sci*., **11**, 478 (2011).

[22] S. Agnihotri, D. Dutt and C. H. Tyagi, *BioResources*, **5**, 1197 (2010).

[23] A. Sanjuan, J. Anzaldo, J. Vargas, J. Turrado and R. Patt, *Holz. Roh. Werkst*., **59**, 447 (2001).

[24] M. S. Jahan, S. Rawsan, D. A. N. Chowdhury and A. Al-Maruf, *BioResources*, **3**, 1359 (2008).

[25] A. Sluiter, B. Hames, R. Ruiz, C. Scarlata, J. Sluiter *et al*., in "Determination of Structural Carbohydrates and Lignin in Biomass", Laboratory Analytical Procedure (LAP), National Renewable Energy Laboratory (2011).

[26] H. U. Suess, in "Pulp Bleaching Today", 1st ed., Walter de Gruyter GmbH & Co. KG, Berlin/New York, 2010, p. 310.

[27] P. Bajpai, in "Developments in Environmental Management", 1st ed., Elsevier, Amsterdam, Netherlands, 2005, p. 277.

[28] J. C. F. Walker, in "Primary Wood Processing. Principles and Practice", 2nd ed., Springer, Dordrecht, 2006, p. 596.

[29] Z. H. Jiang, B.V. Lierop and R. Berry, *Tappi J*., **83**, 167 (2000).

[30] D. W. Clayton, *Svensk Papperstidn*., **66**, 115 (1993).