COMPARISON BETWEEN UV SPECTROPHOTOMETRY AND ANION EXCHANGE CHROMATOGRAPHY TECHNIQUES FOR QUANTIFICATION OF HEXENURONIC ACID (HEXA) IN UNBLEACHED KRAFT PULPS OF *EUCALYPTUS GLOBULUS*

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ABSTRACT

Two different methodologies were optimized for hexenuronic acid (HexA) quantification in *E. globulus* kraft pulps. In one, pulps were hydrolyzed with mercuric chloride/sodium acetate and quantified by ultraviolet spectrophotometry (UV technique); in the other one, the hydrolysis was performed with sulfuric acid and the degradation products of HexA quantified by anion exchange chromatography (AEC technique). In order to determine the optimal conditions for maximum removal of HexA from pulps by acid hydrolysis, the variables time, pH, and temperature were studied. The influences of these three variables were determined by means of a response surface methodology (RSM). The optimized conditions for the hydrolysis by the UV technique were: 45 min, pH 5.7 and 80°C. While, for hydrolysis using the AEC technique, the optimized conditions were: 3.1 h, pH 0.7 and 97°C. Both AEC and UV methods presented good reproducibility with 2.1% and 1.4% relative standard deviation (RSD), respectively. However, HexA values measured by the AEC technique method were 11-30% lower than those measured using the UV technique. For additional information, five selected pulps were analyzed by other methods, known as KTH and HUT. The results obtained by the four methods employed showed significant variations in the HexA quantification. The highest HexA values were obtained using the UV technique. The KTH, AEC and HUT methods provided HexA amounts in 7-16%, 17-24% and 24-30% lower than the values obtained by UV method, respectively, for the same set of pulps. The UV technique is more susceptible to be interfered with pulp hydrolysis byproducts, attributed mainly to lignin solubilization, while the AEC technique allows direct measurement of HexA hydrolysis products without interference from other compounds.

Keywords: *Eucalyptus globulus*, kraft pulping, hexenuronic acids, UV spectrophotometry, anion exchange chromatography.

INTRODUCTION

Hemicelluloses are one of the three main components of lignocellulosic materials, where cellulose and lignin are the other two. Their amount and structure were reviewed by Ebringerová et al.¹. The delignification of wood during chemical pulping processes is also accompanied by the simultaneous dissolution and degradation of carbohydrates, especially hemicelluloses². During kraft pulping, 4-*O*-methyl- α -D-glucuronic acid (MeGlcA) groups present in xylan chains of the hemicelluloses are partially converted into hexenuronic acid (HexA) depending on the cooking conditions, such as hydroxyl ion concentration, pulping time and temperature³⁻⁷. HexA formation is more pronounced in hardwoods than in softwoods due to the large amount of acetyl-4-*O*-methylglucuronoxylan in the hemicellulose composition of hardwood species⁸.

HexA quantification is important in alkaline pulping processes because it interferes in the method when determining residual lignin in unbleached pulps (known as kappa number) and may contribute to an erroneous overestimation of lignin content that could affect bleaching chemicals calculation and consumption^{7,9,10}. Due to its double bound functionality, HexA react with and consume electrophilic bleaching chemicals, such as chlorine dioxide, ozone and peracids. HexA also reduces the pulp's brightness stability and can cause yellowing of the paper^{5,7,11-13}.

Several analytical methods for quantification of HexA in softwood and hardwood kraft pulps have been proposed. Vuorinen et al.⁷ proposed a method for HexA measurement in kraft pulps, referred as the HUT method, based on the selective hydrolysis of HexA with sodium formate buffer, followed by UV spectrophotometric detection at 245 nm. Although, this is a rapid method, it can present interference from substances such as the acid-soluble fraction of residual lignin that can also present UV absorption at the selected wavelength¹⁴. Other methods used for HexA quantification involve pulp hydrolysis with cellulolytic and hemicellulolytic enzyme mix, followed by chromatographic and/or spectroscopic analysis of the hydrolysates, using anion exchange chromatography coupled with amperometric pulse detection¹⁵, capillary zone electrophoresis (CZE)¹⁶ and nuclear magnetic resonance (also referred as VTT method)¹⁷. The enzymatic method is very time consuming (at least two days) due to the low enzymatic hydrolysis rate of pulps. Gellerstedt & Li¹⁰ developed a method to determine HexA by HPLC using a combination of hydrolysis with mercury acetate, oxidation with periodate, and reaction with thiobarbituric acid to form a colored structure (TBA- β -formyl pyruvic acid adduct), which could be determined by chromatographic separation (named as

KTH method). The hydrolysis of HexA by the KTH method is very efficient; however, it requires several experimental steps before the quantification of the hydrolysis products.

Another method developed to determine HexA in kraft pulps was based on selective hydrolysis of the pulp with sulfuric acid to degrade HexA to 2-furancarboxylic acid (FA) and 5-formyl-2-furancarboxylic acid (FFA) followed by the quantification of the products by anion exchange chromatography (AEC)¹⁴. The detection of degradation products of HexA hydrolysis using the AEC technique was compared with detection with CZE (on the same pulp hydrolysate), finding a good agreement between both techniques with respect to the similarity of the quantification results. Chai et al.⁹ also described a fast and simple method to determine HexA amount in kraft pulps, in which pulp hydrolysis is performed with mercuric chloride and sodium acetate solution and the quantification of products performed by UV spectrophotometry at two wavelengths (260 and 290 nm). The HexA content was calculated by an equation that includes correction factors for the spectral contribution of lignin and eliminates possible measurement errors due to spectral background shift in absorption experiments.

The effect of HexA groups and the benefit of their removal from unbleached pulps depend on their content in the pulp as well as on the bleaching sequence used. Reasonable, reliable, and simple methods to quantify HexA in kraft pulps are needed.

The aim of this work was to compare and to optimize two quantification methods for HexA in *E. globulus* kraft pulps. One is based in the hydrolysis of the pulps with mercuric chloride/sodium acetate and quantification of the products by UV spectrophotometry, and the other is based in the hydrolysis with sulfuric acid and quantification of the products by anion exchange chromatography. The reproducibility of both methods was also evaluated. In order to compare, some samples were also analyzed by KTH and HUT methods.

EXPERIMENTAL

Kraft pulping

Kraft pulping was performed by cooking industrial-size wood chips (2.5 x 1.5 x 0.2 cm) from 10-12 years old *E. globulus* provided by a local pulp mill. Wood chips were well mixed to create a single uniform sample, air-dried and stored in dry conditions before use. The samples were cooked in a 1000 mL Parr reactor (Parr Instrument Co., Moline, IL, USA) loaded with 50 g of wood chips (dry basis) and a liquor:wood ratio of 5:1 (v/w). Cooking liquor composition was 12%, 15% or 20% active alkali (AA) and 25% of sulfidity (both calculated

on dry wood basis and expressed as Na₂O equivalents). The reactor was heated at 1.6°C min⁻¹ and pulping experiments were performed at two different temperatures, 155°C and 165°C. Cooking time was 15, 30, 60, 120, 180, 240 or 300 min to produce pulps with different amounts of residual lignin and HexA. A total of 135 kraft pulps were prepared as a result of the combination of the different pulping conditions used (3 AA x 2 temperatures x 7 cooking time, in triplicate, plus 9 additional pulps produced as a fourth repetition of some of the conditions). After cooking, pulps were disintegrated in a TAPPI laboratory blender, thoroughly washed with tap water, and centrifuged. Total pulp yield was determined based on the weight of the pulp divided by wood chips weight (both on dry basis) multiplied by 100%.

Quantification of HexA

UV-technique

HexA content in kraft pulps was quantified based in the dual-wave UV spectrophotometry analysis published by Chai et al.⁹ Approximately 50 mg of air-dried pulp with known moisture content was weighed and placed in a 20 mL vial containing 10 mL of the hydrolysis solution (0.6% of mercuric chloride and 0.7% of sodium acetate). The experimental conditions were 45 min of reaction time, pH 5.7 and 80°C. These conditions were selected following a full factorial design (Table 1) with the aim to obtain the maximum hydrolysis of HexA. After each reaction, the solution was cooled to room temperature, filtered through a nitrocellulose filter (0.22 µm pore), and the UV absorption of the solution was measured at two wavelengths, 260 nm and 290 nm. The products of the acidic degradation of HexA are 2-furancarboxylic acid (FA) and 5-formyl-2-furancarboxylic acid (FFA). The fresh hydrolysis solution was used as a blank for the UV absorption measurements. The HexA content was calculated using the dual-wavelength equation (Eq. 1) as proposed by Chai et al.⁹, where C_{HexA} is the concentration of HexA in pulp (mmol kg⁻¹ of pulp); 0.287 is the calibration factor for the dual-wavelength method; A_{260} and A_{290} are the measured absorption intensities at 260 nm and 290 nm, respectively; 1.2 is the ratio of lignin absorption at the two wavelengths; V is the volume of the hydrolysis solution (mL) and w is the oven-dried weight of the pulp sample (g.)

$$C_{HexA} = 0.287 \frac{(A_{260} - 1.2A_{290})V}{w} \quad (\text{Eq. 1})$$

AEC technique

This method was firstly proposed by Jiang et al.¹⁴ Approximately 100 mg of air-dried pulp with known moisture content was weighed and placed in a 20 mL vial containing 10 mL of a diluted sulphuric acid solution. The hydrolysis was carried out at the optimum experimental conditions at 97°C, pH 0.7 and 3.1 h determined by a full factorial design (Table 1) for the maximum hydrolysis of HexA. The hydrolysate was filtered through a nitrocellulose filter (0.22 µm pore), and diluted 10 times with 4 mM sodium hydroxide. The solution was analyzed by AEC with suppressed conductivity detection. Hydrolysis products (FA and FFA) were separated on an AS11 4 mm analytical column with an AG11 4 mm guard column (Dionex), using 4 mM sodium hydroxide as eluent delivered at 0.7 mL min⁻¹. The HexA content was calculated using calibration curves for standards of two hydrolysis products of the HexA degradation at concentrations of 1 to 4 mg L⁻¹.

Quantification of hexenuronic acids by HUT and KTH methods

Other methods found in the literature were also used to quantify the HexA in some selected pulps in order to compare the results obtained by the previous optimized methodologies. These methods were named HUT, proposed by Vuorinen et al.⁷, and KTH, proposed by Gellerstedt & Li.¹⁰ In the first, approximately 330 mg of air-dried pulp (with known moisture content) was treated with 10 mL of 10 mM sodium formate buffer solution at 110°C by 1 h, followed by UV spectrophotometric detection at 245 nm (molar absorptivity = 10.8x10³ L mol⁻¹cm⁻¹). The KTH method was based on the hydrolysis of 250 mg of pulp in a test tube containing 7.5 mL of water and 7.5 mL of 70 mM mercuric acetate solution. The reaction is carried out during 20 min at room temperature and filtrated. Then, 300 µL of the filtrate were acidified with 60 µL of concentrated HCl and immediately oxidized with 0.5 mL of periodate solution (0.1 mM sodium metaperiodate in 2.8 M phosphoric acid) during 20 min at room temperature. The resulting solution was treated with 2 mL of arseniate solution (4% sodium arseniate in 0.5 M hydrochloric acid) until the brown color disappeared. Thiobarbituric acid (0.3% in water) was added to the test tube to a total volume of 10 mL. The tube was heated at 50°C in a water bath for 170 min resulting in the TBA-β-formyl pyruvic acid adduct. An aliquot of 100 µL of the red-colored adduct formed was separated in a Licrocart

250-4 (RP-18) column using 0.5% of trifluoroacetic acid aqueous solution as mobile phase for 5 min, followed by a linear gradient of methanol-water mixtures containing 0.5% of trifluoroacetic acid (from 0 to 100% methanol in 14 min) at a flow rate was 0.8 mL min⁻¹. The TBA-adduct was monitored at 549 nm and quantified through a calibration curve using ammonium 3-deoxy-2-keto-octonate as standard. The standard (1.0 mg ± 0.1) was dissolved in 1 mL of water, and 20, 40, 80, and 160 µL aliquots were taken and dissolved in 1 mL of 35 mM mercuric acetate. All solutions were subsequently treated with periodate, arsenite, and TBA as described above and analyzed for the TBA-3-formyl pyruvic acid adduct.

Response surface methodology

In order to determine the conditions to obtain maximum HexA hydrolysis from the pulps, the variables pH, time and temperature were studied. To determine these conditions, one single pulp with high HexA content was used (49 mmol kg⁻¹ pulp based in UV determination). The influences of these three variables were determined using response surface methodology (RSM).¹⁸ This model is based on a central composite circumscribed design made of a factorial design and star points. The variable values were coded and normalized in unitary values: -1 was defined as the lowest value of a variable and +1 was defined as the highest value. From the extreme variable values, the central point (coded 0) was set and assayed in triplicate. Four star points distributed at a distance of 1.68 from the central point were included. The complete experimental design for the reaction conditions of the two methods optimized for HexA quantification is presented in Table 1.

A second-order function that best describes the system behavior was determined by a multiple lineal regression method (MLR). The statistical validation was performed by a one-way ANOVA test with 95% confidence level. The optimal conditions values were determined based on the response surface calculated using the SIMPLEX method.¹⁸ All the calculations were performed with the software Modde 7.0.0.1 (Umetrics, USA).

Table 1. Optimization experimental design for HexA hydrolysis of kraft pulp.

Run	UV			AEC		
	T (°C)	pH	Time (min)	T (°C)	Time (h)	pH
1	65 (-1)	4 (-1)	25 (-1)	80 (-1)	2 (-1)	1 (-1)
2	95 (1)	4 (-1)	25 (-1)	110 (1)	2 (-1)	1 (-1)
3	65 (-1)	6 (1)	25 (-1)	80 (-1)	4 (1)	1 (-1)
4	95 (1)	6 (1)	25 (-1)	110 (1)	4 (1)	1 (-1)
5	65 (-1)	4 (-1)	65 (0)	80 (-1)	2 (-1)	2 (1)
6	95 (1)	4 (-1)	65 (0)	110 (1)	2 (-1)	2 (1)
7	65 (-1)	6 (1)	65 (0)	80 (-1)	4 (1)	2 (1)
8	95 (1)	6 (1)	65 (0)	110 (1)	4 (1)	2 (1)
9	55 (-1.68)	5 (0)	45 (0)	70 (-1.68)	3 (0)	1.5 (0)
10	105 (1.68)	5 (0)	45 (0)	120 (1.68)	3 (0)	1.5 (0)
11	80 (0)	3.3 (-1.68)	45 (0)	95 (0)	1.3 (-1.68)	1.5 (0)
12	80 (0)	6.7 (1.68)	45 (0)	95 (0)	4.7 (1.68)	1.5 (0)
13	80 (0)	5 (0)	11 (-1.68)	95 (0)	3 (0)	0.7 (-1.68)
14	80 (0)	5 (0)	79 (1.68)	95 (0)	3 (0)	2.3 (1.68)
15	80 (0)	5 (0)	45 (0)	95 (0)	3 (0)	1.5 (0)
16	80 (0)	5 (0)	45 (0)	95 (0)	3 (0)	1.5 (0)
17	80 (0)	5 (0)	45 (0)	95 (0)	3 (0)	1.5 (0)

RESULTS AND DISCUSSION

The chemical composition of the *E. globulus* wood chips used in this work was: 49.6% glucan, 16.1% xylan, 26.3% lignin, 5.3% MeGlcA and 1.2% extractives. The wood chips were cooked in a batch kraft process using different pulping conditions with the aim to produce pulps with different degrees of delignification and amounts of hexenuronic acids. Table 2 presents the range of values for the pulp characteristics evaluated.

HexA compounds containing enol ether or unsaturated carboxylic acid groups that are unstable in mild acidic solutions and generate distinct degradation products according to the hydrolysis conditions used (Figure 1)^{7,10,14}.

Table 2. Pulp yield and chemical composition ranges of *E. globulus* kraft pulps.

	Lignin (%)	Kappa number	Glucan (%)	Xylan (%)	MeGlcA (mmol/kg)	Pulp yield (%)
Minimum content	1.4	8.5	64	12.0	220	46
Maximum content	18.0	62.4	77	18.0	360	70
Average std	0.3	0.6	0.7	0.3	10.3	1.2

Figure 1. Products generated from the mild acid hydrolysis of HexA present in kraft pulps.

Quantification of HexA by UV-technique

This methodology utilizes mercuric chloride and sodium acetate as hydrolysis solution. Mercuric chloride can selectively hydrolyze hexenuronic acid groups from pulp (Gellerstedt & Li, 1996). Additionally, since mercuric chloride is stable and has a relatively low absorptivity in the UV range especially at wavelengths above 250 nm, direct UV absorption spectroscopy can be applied for HexA determination⁹. To evaluate hydrolysis conditions, screening by means of factorial design was developed, varying the temperature, time and pH of reaction.

From the experimental design data (Table 1) and its corresponding HexA concentration determined by using the equation (Eq. 1) proposed by Chai et al.⁹, whose results are presented in Table 3, a quadratic polynomial was determined (Eq. 2) and validated by the ANOVA test.

$$Y = 7.58 \pm 0.35 - 0.04 \pm 0.16X_1 + 0.83 \pm 0.16X_2 + 0.36 \pm 0.16X_3 - 0.35 \pm 0.18X_1^2 - 0.55 \pm 0.18X_2^2 - 0.33 \pm 0.18X_3^2 \quad (\text{Eq. 2})$$

where X_1 is the temperature ($^{\circ}\text{C}$), X_2 is the pH, X_3 is the reaction time (min) and Y is the HexA content (mmol kg^{-1} pulp). The coefficients have been normalized in order to compare the relative importance of the model's variables. The error values corresponded to a 95% confidence level. The response surface for the polynomial (plot inside the dominium limited for the experimental planning) is shown in Figure 2.

The linear terms for pH and reaction time have positive coefficients, meaning that the response incremented with the increase in these variables. The temperature did not contribute as a linear term. Quadratic terms for all variables are negative, indicating the presence of maximum values for each variable. Considering the confidence intervals, the interaction between variables did not have a significant effect on the HexA hydrolysis. The variable pH is the main factor for HexA hydrolysis according to the polynomial. The hydrolysis

solution seems be more effective near the neutral range.

Based in the experimental conditions used in the experimental design, the polynomial was used to predict the amount of HexA. The responses were close to the experimental values (Table 3) with a correlation coefficient (r^2) = 0.95. These values together with the ANOVA test provided a statistical validation of the model.

The optimum values of the variables for hydrolysis of the HexA were determined by the SIMPLEX method using the maximum values of the response surface¹⁸. The predicted values were: 45 min, pH 5.7 and 80°C . According to the polynomial, the response was $51.3 \pm 1.7 \text{ mmol kg}^{-1}$ pulp for the pulp employed in the experimental design. The experimental HexA hydrolysis for this pulp obtained at these conditions was $50.6 \pm 0.3 \text{ mmol kg}^{-1}$ pulp, agreeing with the predicted value.

Quantification of HexA by AEC-technique

For HexA determination by AEC, pulps were hydrolyzed with sulfuric acid solution because other hydrolysis solutions such as formic or trifluoroacetic acid causes interference during the detection of the degradation products of HexA by chromatography¹⁴. The hydrolysis solution used in the UV technique cannot be employed because chloride also presents a peak signal superimposed with the peaks of the hydrolysis products of the HexA degradation. Thus, kraft pulps were hydrolyzed with sulfuric acid and the hydrolyzate was analyzed by AEC under reproducible and validated chromatographic conditions. The compounds FA and FFA were separated with great efficiency with retention times of 7.68 ± 0.07 min and 9.77 ± 0.14 , respectively. The relative standard deviation (RSD) for several analyses done in triplicate was smaller than 2% and the quantification limit was 0.40 ppm for both compounds in the chromatographic conditions used.

In order to determine the optimal conditions of HexA hydrolysis, the response surface methodology was also used. In the experimental design, the temperature, reaction time and pH were varied. The same pulp used for UV quantification was used in AEC quantification of HexA. From the experimental design data (Table 1) and its corresponding HexA concentration (Table 3), a quadratic polynomial was determined (Eq. 3) and validated by the ANOVA test.

$$Y = 3.64 \pm 0.18 + 0.49 \pm 0.11X_1 - 0.30 \pm 0.11X_2 + 0.19 \pm 0.11X_3 - 0.93 \pm 0.12X_1^2 - 0.32 \pm 0.12X_2^2 \quad (\text{Eq. 3})$$

where X_1 is the temperature ($^{\circ}\text{C}$), X_2 is the pH, X_3 is the reaction time and Y is the HexA content (mmol kg^{-1} pulp). The coefficients have been normalized in order to compare the relative importance of the model's variables. The error values corresponded to a 95% confidence level.

The linear terms for temperature and hydrolysis time with positive coefficients indicated that the response increases with increases in temperature and time. The pH shows a negative coefficient, meaning that the response decreases with the increase in pH. Only the quadratic term for temperature and time are important, indicating the presence of a maximum value for the variable. Considering the confidence intervals, the variable temperature is the main factor for HexA hydrolysis by this procedure. The polynomial predicted responses that were close to the experimental values (Table 3) with a correlation coefficient (r^2) = 0.97. These values together with the ANOVA test provided a statistical validation for the model. The response surface polynomial is shown in Figure 3.

The optimum values of the variables for HexA hydrolysis were determined by the SIMPLEX method from the maximum values of the response surface. The predicted values were 3.1 h, pH 0.7 and 97°C . According to the polynomial, the response was $35.5 \pm 1.6 \text{ mmol kg}^{-1}$ pulp. The experimental HexA hydrolysis obtained at these conditions was $36.5 \pm 0.2 \text{ mmol kg}^{-1}$ pulp for the pulp employed in the experimental design, agreeing with the predicted values from the polynomial.

HexA measured by the AEC method were 22% lower than those measured by the UV method. This variation in the determination may be due to possible interference of other compounds in the pulp hydrolyzate that can also absorb UV light at the same wavelengths used to measure hydrolysis products. On the other hand, the AEC technique allows direct quantification of the HexA hydrolysis products, reducing interference and increasing the accuracy of HexA quantification. Under the applied conditions, approximately the 95% of HexA groups are hydrolyzed for both techniques in. That was determined by applying at least three successive hydrolysis for the same pulp in the optimal conditions and measuring the amount of HexA formed in each one. The use of same hydrolysis for both methods is difficult, because for HexA determination

by AEC technique, chloride in the hydrolysis solutions affects the hydrolysis products' chromatographic signal and in the UV technique, the sulfuric acid solution could facilitate the lignin hydrolysis affecting quantification of HexA.

Table 3. Experimental and calculated HexA (mmol kg⁻¹ pulp) content values by the response polynomial.

UV		AEC	
Experimental HexA	Calculated HexA	Experimental HexA	Calculated HexA
32.3	33.9	15.0	16.9
34.6	33.3	27.1	25.2
47.4	44.7	18.6	20.2
44.6	44.1	28.4	28.5
39.0	38.6	13.3	12.3
37.5	37.9	22.0	20.6
50.7	49.4	16.8	15.6
46.0	48.8	24.8	23.9
42.0	43.4	2.8	1.7
43.5	42.5	13.6	15.6
30.5	30.1	19.5	20.5
47.4	50.2	26.2	26.1
37.7	39.3	36.2	35.0
48.3	47.2	24.9	27.2
50.1	49.3	30.3	31.1
49.5	49.3	31.5	31.1
48.5	49.3	31.9	31.1

Figure 2. Surface response for the hydrolysis of HexA in *E. globulus* kraft pulps determined by UV spectrophotometric method.

Figure 3. Surface response for the hydrolysis of HexA in *E. globulus* kraft pulps determined by anion exchange chromatographic method.

Quantification of HexA in kraft pulps by spectrophotometric and chromatographic optimized methods

With the optimum hydrolysis conditions determined for each methodology, all the 135 kraft pulps produced were hydrolyzed and analyzed by both techniques. In this work, we observed that the values of HexA in pulps by AEC technique were lower than the values obtained by UV (Figure 4). It is important to clarify that HexA quantification by UV method was obtained with the equation developed by Chai et al.⁹, which has a factor used to correct for lignin interference. This equation was developed using a pulp characterized firstly by the KTH method and then used in the calibration for UV, which indicates that the accuracy of the UV present method is greatly dependent on the accuracy of the original value for the standard pulp obtained by the KTH method.

Figure 4. Relationship between HexA content determined by anion exchange chromatographic method and UV spectrophotometric in kraft pulps of *E. globulus*.

The reproducibility of UV and AEC techniques was evaluated in three groups of experiments, each one carried out in triplicate or quadruplicate for a selected kraft pulp using a confidence limit of 95%. Very good reproducibility was found for both techniques at $p < 0.05$. RSD was 2.1% and 1.4% for AEC and UV, respectively, indicating the high precision and good reproducibility of the methods (Table 4). Dhalman et al.¹⁶ using enzymatic hydrolysis and subsequent capillary electrophoresis, obtained RSD between 2.8% and 5.1%. Chai et al.⁹ and Jiang et al.¹⁴ found RSD near to 2.0% based on five replicate determinations of the entire analytical process including sampling, acid hydrolysis and the analysis, indicating the high precision of their methods. Thus, we can confirm that the precision offered by the UV and AEC techniques is very close to other methods reported in the literature¹⁶.

Table 4. Reproducibility of UV and AEC techniques.

Experiment No.	HexA content means (mmol kg pulp ⁻¹)	N	SD	Variance	F	p-level
AEC-1	35.84	4	0.49	0.24	0.36	0.71
AEC-2	35.45	4	0.94	0.88		
AEC-3	35.45	3	0.71	0.50		
Average	35.59	11	0.69	0.48		
UV-1	50.76	4	0.95	0.89	0.81	0.48
UV-2	50.64	4	0.33	0.11		
UV-3	50.09	3	0.76	0.57		
Average	50.53	11	0.71	0.50		
Methods	HexA (mmol kg pulp ⁻¹)			Confid. +95%	Variance	RSD
	Average	Minimum	Maximum			
AEC	35.6	34.2	36.4	0.47	0.69	1.94
UV	50.5	49.3	51.6	0.48	0.71	1.40

HexA data measured by the AEC technique were 11-30% lower than those measured by UV technique. In order to compare, five pulps were analyzed by other HexA determination techniques, known as KTH and HUT. When comparing the data obtained by the four methods employed, these reported variations in HexA measurements. The highest HexA values were obtained with the UV technique; while the KTH, AEC and HUT methods provided HexA amounts that were 7-16%, 17-24% and 24-30% lower for the same set of pulps, respectively (Table 5). Similar results have been reported by Tenkanen et al.¹⁹ when comparing HexA determination by the KTH, VTT and HUT methods in unbleached and bleached softwood kraft pulps. Different results were obtained with the three methods. The highest values were obtained by KTH method, while the values obtained by the VTT and HUT methods were 5-10% and 14-25 % lower than those obtained by KTH, respectively. No method for the analysis of HexA was clearly superior to the others according to the authors. Because reproducibility of all methods is relatively good, the results from any of the methods could result in a systematic error in the range of 10–30%^{9,19}. However, in the quantification of HexA by the UV technique, a systematic error propagation from the KTH method could affect the results. The use of AEC allows direct measurement of HexA hydrolysis products avoiding this problem.

Table 5 shows that samples B, C and D with kappa number between 9 and 20 can be determined for all methods employed. However, sample E with kappa number 58, due to the high lignin content, presented problems in the determination the HexA content using the HUT method. The lignin dissolved from pulps during the acid hydrolysis in this method interferes with HexA quantification¹⁴, which could be a disadvantage for this technique since samples with high kappa number can not be determined by HUT method. On the other hand, the AEC technique could not be used in HexA determination in ECF bleached kraft pulp (sample A) because the presence of some residual chloride in the pulp affects the hydrolysis products' chromatographic signal. The main characteristics and comparison of AEC, UV, KTH and HUT methods regarding time to carry procedures, reproducibility, hydrolysis performance a quantification are showed in Table 6.

Table 5. Comparison between methods in the determination HexA in kraft pulps.

Kraft pulp	Kappa number	HexA (mmol kg pulp ⁻¹)			
		UV	AEC	KTH	HUT
A*	---	17.7±0.2	-----	15.1±0.1	13.4±0.2
B	12.7±0.5	62.4±0.6	48.7±0.2	52.8±0.6	43.9±0.4
C	9.2±0.1	25.9±0.4	21.6±0.1	24.2±0.1	20.5±0.3
D	18.9±0.7	44.5±0.3	33.7±0.4	40.5±0.6	42.1±0.4
E	58.3±0.9	14.7±0.4	11.4±0.1	12.4±0.2	30.3±0.7

* Bleached kraft pulp (ECF)

Table 6. Main characteristics of HexA quantification by different analytical methods.

	UV	AEC	KTH	HUT
Hydrolysis time	45 min	180 min	210 min	60 min
Analysis time	< 5 min	~ 25 min	~ 25 min	< 5 min
Reproducibility	Good	Good	Good *	Good *
Hydrolysis efficiency of HexA	≥ 95%	≥ 95%	ND	Incomplete **
Quantification of hydrolysis products	Indirect ***	Direct	Direct	Indirect ***

* According to the literature (not evaluated in this work); ** According to the literature (not evaluated in this work); *** Possible interference of other compounds during UV analysis; ND...not determined.

CONCLUSIONS

The reproducibility of UV and AEC techniques used for HexA quantification in kraft pulps of *E. globulus* was good. As indicated, a good agreement was observed between UV and AEC techniques with the others

existent quantification techniques (HUT and KTH). It is difficult to establish which technique is the most accurate since reproducibility is good for both methods. In our opinion the use of chromatographic techniques which allows direct measurement of HexA hydrolysis products is more indicated and possibly the more reliable.

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