SHORT TERM SCIENTIFIC MISSION (STSM) – SCIENTIFIC REPORT

The STSM applicant submits this report for approval to the STSM coordinator

Action number: COST Action FP1405, Active and intelligent fibre-based packaging - innovation and market introduction (ActInPak)
STSM title: Sequential subcritical water extraction and acid hydrolysis for obtaining cellulose nanocrystals and xylans from rice husk to be used as reinforcing and active agents in food packaging materials
STSM start and end date: 01/09/2017 to 30/11/2017
Grantee name: Raquel Requena Peris

PURPOSE OF THE STSM/

Rice husks are lignocellulosic materials, with a hemicellulose fraction made up of substituted arabinoxylan, which breakdown leads to xylooligomers with food, medical, and pharmaceutical applications. Another way to valorise rice husk involves the exploitation of its high cellulose content for producing cellulose fibres and nanocrystals. Therefore, the main objective of the STSM at KTH has been the integral valorisation of the rice husk through two different processes. On the one hand, the traditional alkaline extraction followed by delignification and acid hydrolysis and, on the other hand, a sequential subcritical water extraction (SWE) followed by delignification and acid hydrolysis. In this way, it is possible to fractionate polymeric hemicelluloses (xylans) and, on the other hand, obtain cellulose nanocrystals (CNC) to be used as reinforcement materials. SWE is a promising green method for the isolation of hemicellulose fractions with preserved molecular functionalities and high molecular weight, unlike the traditional alkaline treatments for hemicellulose extraction from cereal and other plant biomasses. This method has proven to be effective for the extraction of diverse bioactive and nutraceutical compounds, including polysaccharides from plants and food matrices. These compounds could be incorporated into starch-polyesters films, thus obtaining antimicrobial and/or antioxidant food packaging. Regarding the reinforcement materials (CNC), they will be incorporated into starch-polyesters films in order to improve their properties in terms of stretchability and adhesion capacity.

The research group led by Dr. Francisco Vilaplana, Associate Professor and Principal Investigator at the Division of Glycoscience at KTH Biotechnology (Stockholm, Sweden), has broad experience on the biotechnological exploitation of bioactive polysaccharides from food by-products using environmentally friendly processes and their valorisation into functional materials. Moreover, his group is also experienced in the structural characterization and the chemical modification of polysaccharides. I have also benefited from the co-supervision of Dr. Rosana Moriana (KTH Fibre and Polymer Technology), who is a specialist on the preparation of cellulose nanostructures from a wide range plant lignocellulosic sources and the characterization of the macroscopic properties and the structure at the nano and macro dimensions. The combination of the excellent scientific competence and the research infrastructure at both KTH Glycoscience and KTH Fibre and Polymer Technology has guaranteed the successful implementation of the research plan for the stage.

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DESCRIPTION OF WORK CARRIED OUT DURING THE STSMS

The activities developed during the three months of the traineeship have been the following:

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- Bibliographic review on the fractionation in cascade of hemicelluloses and cellulose and on the obtaining of CNC, from rice husk and other cereals.

- Pretreatment of the rice husk in order to reduce the particle size, thus improving the process extraction. In this sense, the raw material was subjected to a milling process in a Wiley Mill with a particle size of 20 mesh.

- Sequential extraction of hemicelluloses from rice husk samples, previously milled, by SWE. The sequential extraction was carried out at 5, 15, 30 and 60 min to optimize the extraction time in terms of amount of xylan extracted. The SWE yield was calculated, and the extracts and the residue were freeze-dried for subsequent characterization.

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- Characterization of the composition and molecular structure of the fractions of hemicellulose at different times, by methanolysis followed by HPAEC-PAD and GC-MS, respectively.

- Implementation of the alkaline extractions and the successive delignification treatments from the milled rice husk to produce CNC. At this point, three consecutive alkaline extractions and five consecutive bleaching treatments were carried out. After each process, the residues were washed with water, filtered and dried at room temperature. Finally, the bleached materials were hydrolysed with sulfuric acid 64% and the CNC were purified by successive centrifugations and homogenized by sonication. The yield of each single stages of the process was calculated.

- Characterization of the composition and molecular structure of the alkaline extracts resulting from the successive alkaline treatments, through methanolysis followed by HPAEC-PAD and GC-MS, respectively.

- Implementation of successive delignification and acid hydrolysis reactions from rice husk residues resulting from SWE to produce CNC. Purification and homogenization of the CNC by centrifugation and sonication.

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Characterization of the different materials obtained along both processes. To this end, the following determinations were carried out:

- Shoxlet extraction of the rice husk to determine the total amount of extractives in water and ethanol.

- Klason lignin determination for each samples following the Tappi method T222 om-06

- Scanning electron microscopy (SEM) to observe the structure of follow the structure from macro- to nano-dimensions.

- Atomic force microscopy (AFM) to determine the size distribution of the CNCs. To this end, the samples were prepared using the layer-by-layer technique.

- Fourier Transform Infrared Spectroscopy (FTIR) with total attenuated reflection (ATR) to characterize morphologically the materials obtained after each treatment with respect to changes in their functional groups.

- X-ray diffraction analysis (XRD) to analyse the crystallinity of different materials, by calculating the crystallinity index, the size of the crystals and the proportion of crystalline inner chains.

- Thermogravimetric analysis (TGA) to determine, on the one hand, the thermal stability and on the other, the ash content of each material

DESCRIPTION OF THE MAIN RESULTS OBTAINED

The yield for the first alkaline treatment was different to the other ones, since in the first extraction the yield of the residue and the extract were around 56% and 16%, respectively, whereas the second and the third one had residue and extract yields around 95% and 5%, respectively. These differences can be explained by the complete removal of the extractives during the first treatment. The extracts were dialyzed with 3.5 KDa membrane in order to remove the remaining reagent, previously to the freeze-dry step, which could explain that the yields for the first alkaline treatment do not reach 100%. On the other hand, the yield of the SWE in terms of extract obtained after 60 min was considerably higher (27%). It should be noticed that the environmental effect of both, SWE and alkaline extraction are quite different, being the first one more environmental-friendly.

After 5 min, the extracts of the SWE contains mainly glucose (>90%), probably due to the presence of starch residues coming from the rice husking process. But as time progresses, the xylan content in the extracts increases as a result of the extraction of hemicelluloses, reaching a xylan content of 80% after 60 min. Likewise, the xylan content was higher in the second and the third alkaline extractions compared to the first one. However, the yields of xylan extracted were always lower than in the extracts coming from the SWE compared to alkaline treatment.

As the chemical composition analysis show, after the alkali treatments, most of extractives and ashes were removed, as well as some hemicelluloses. Likewise, after the SWE, the hemicellulose content also decreased to an even greater extent respect to the raw material, but the Klason lignin remained constant. During the bleaching treatment most of the lignin was removed, thus opening the cell wall and liberating the cellulosic fibres as shown the SEM micrographs. However, the surface of the bleached materials coming from the SWE residues showed the presence of some fibres that keep together due to the lower efficiency of the SWE to remove the amorphous components, which could difficult the CNC obtention.

The thermal stability analysis also validated the extraction of the amorphous components during the alkaline and bleaching treatments since the main degradation peak showed smaller shoulders on the DTG curve. Likewise, this change was less noticeable in the SWE residue due to the milder conditions of this extraction process. The materials resulting from the different treatments degraded within a narrower temperature range and showed better thermal stability than the raw material. The hydrolysis reaction gave rise to more thermosensitive samples, being the CNCs coming from the traditional approach more thermostable than those coming from the SWE.

Regarding the FTIR analysis, the alkaline and the bleached samples showed a higher peak in the region related to the OH vibrations, due to an increase in the hydrogen bond strength, because of the removal of the amorphous components. Moreover, the peaks related to the hemicellulose and the lignin were disappearing along the conversion from macro- to nano-dimension. XRD analysis also demonstrated the progressive removal of the amorphous compounds along the conversion from macro- to nano-dimension, since the crystallinity increased progressively, which was more noticeable for the traditional approach.

FUTURE COLLABORATIONS (if applicable)

The obtained experimental outcomes will certainly result in a joint publication about the cascade valorisation of the rice husk into valuable polysaccharides, both bioactive hemicelluloses and cellulose nanocrystals. Some of the target journals for this publication considered at this stage include Bioresource Technology, ACS Sustainable Chemistry and Engineering, and Green Chemistry. Moreover, this research stage opens the possibility for further collaborations between the sending group at UPV (Spain) and the host groups at KTH (Sweden) in the valorization of biomass byproducts from agricultural streams. There is a huge potential in exploring the biomass resources from the Nordic and the Mediterranean regions and the complementary characterization infrastructure and competences in both groups. KTH can provide valuable biochemical and biotechnological understanding of the polysaccharides in plant biomass, together with a biorefinery approach on recovering the different fractions from lignocellulosic biomass, whereas UPV has a wide experience in the preparation and characterization of active packaging materials. This will hopefully result in joint project applications at the national (e.g. Spanish and Swedish research calls) and the EU level (e.g. Horizon 2020, Biobased Industries), and future exchange of personnel between the two institutions.

The yield for the first alkaline treatment was different to the other ones, since in the first treatment the yield of the residue and the extract was around 56% and 16%, respectively, whereas the second and the third one had residue and extract yields around 95% and 5%, respectively (Table 1). These differences can be explained by the complete removal of the extractives during the first treatment. The extracts were dialyzed with 3.5 KDa membrane in order to remove the remaining reagent, previously to the freeze-dry step, which could explain that the yields for the first alkaline treatment do not reach 100%. On the other hand, the yield of the SWE in terms of extract obtained after 60 min was considerably higher (27%) compared to the alkaline extraction (Table 2). It should be noticed that the environmental effect of both, SWE and alkaline extraction, are quite different, being the first one more environmental-friendly. While, SWE conditions are water at 160 ºC for 60 min, the alkaline extraction conditions are three consecutive extractions with NaOH (4.5% w/v) at 80 ºC for 2h each one.

Table 1. Gravimetric yield (wt%) for both, the residues and the extracts, obtained after each treatment calculated on a dry weight basis.

Alkali treatment	**Residue yield (%)**	**Extract yield (%)**
1st	56±4	16±5
2nd	93±7	5.2±1.3
3rd	94±2	2.3±0.1

Table 2. Gravimetric yield (wt%) for the extracts obtained after each treatment calculated on a dry weight basis.

SWE Time (min)	**Extract yield (%)**
5	10.5±0.7
15	3.8±0.1
30	4.2±0.8
60	7±0.5

Regarding the bleaching treatments, the yield in terms of residue was higher for the traditional approach (59%) compared to the alternative method (50%) (Table 3), since the staring material coming from the alkaline treatments had less amorphous components due to the higher effectivity of the alkaline treatment removing these components, compared to the SWE.

Table 3. Gravimetric yield (wt%) obtained after each treatment calculated on a dry weight basis.

Approach	**Treatment**	**Residue yield (%)**
Traditional	Alkaline	48±2
	Bleaching	59±3
Alternative	SWE	69.2± 0.8
	Bleaching	50±2

The visual aspect of the samples after the different treatments (Figure 1) also demonstrates that the more aggressive conditions in the alkaline treatment led to residues with less amorphous components. For this reason, the bleaching treatment was more effective on the alkaline residue, thus obtaining more bleached materials (with less lignin content).

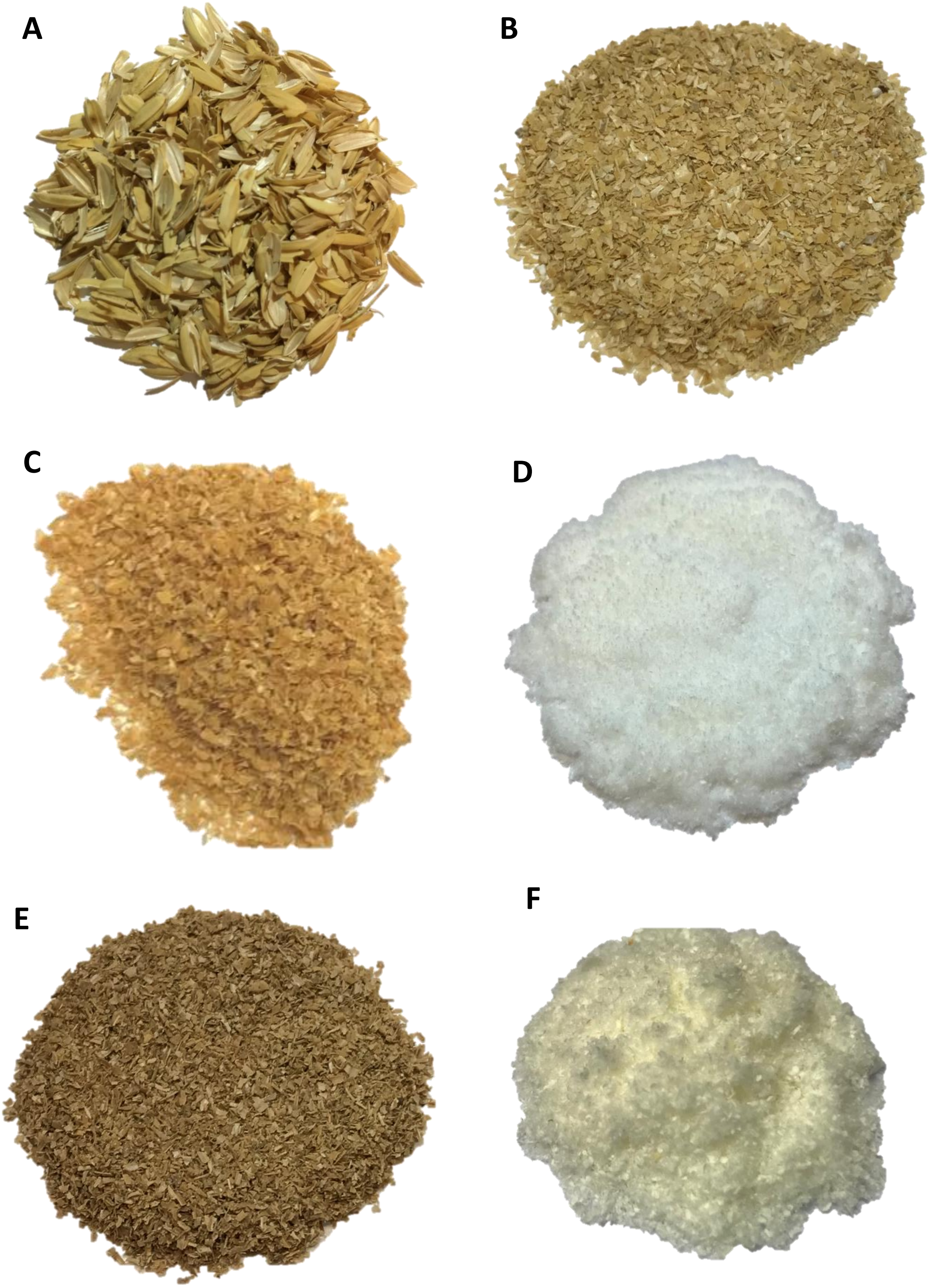

Figure 1. Visual aspect of the samples after the different treatments: A: rice husk; B: 20 mesh rice husk; C: alkaline residue; D: bleached alkaline residue; E: SWE residue; F: bleached SWE residue.

As has been commented above, after the alkaline and bleaching treatment, most of the amorphous components are removed, opening the cell wall and liberating the cellulosic fibres (Figure 2). However, the Klason lignin content remains constant in the SWE residues (Table 4), and as a result, the surface of these bleached materials shows the presence of some fibres that keep together.

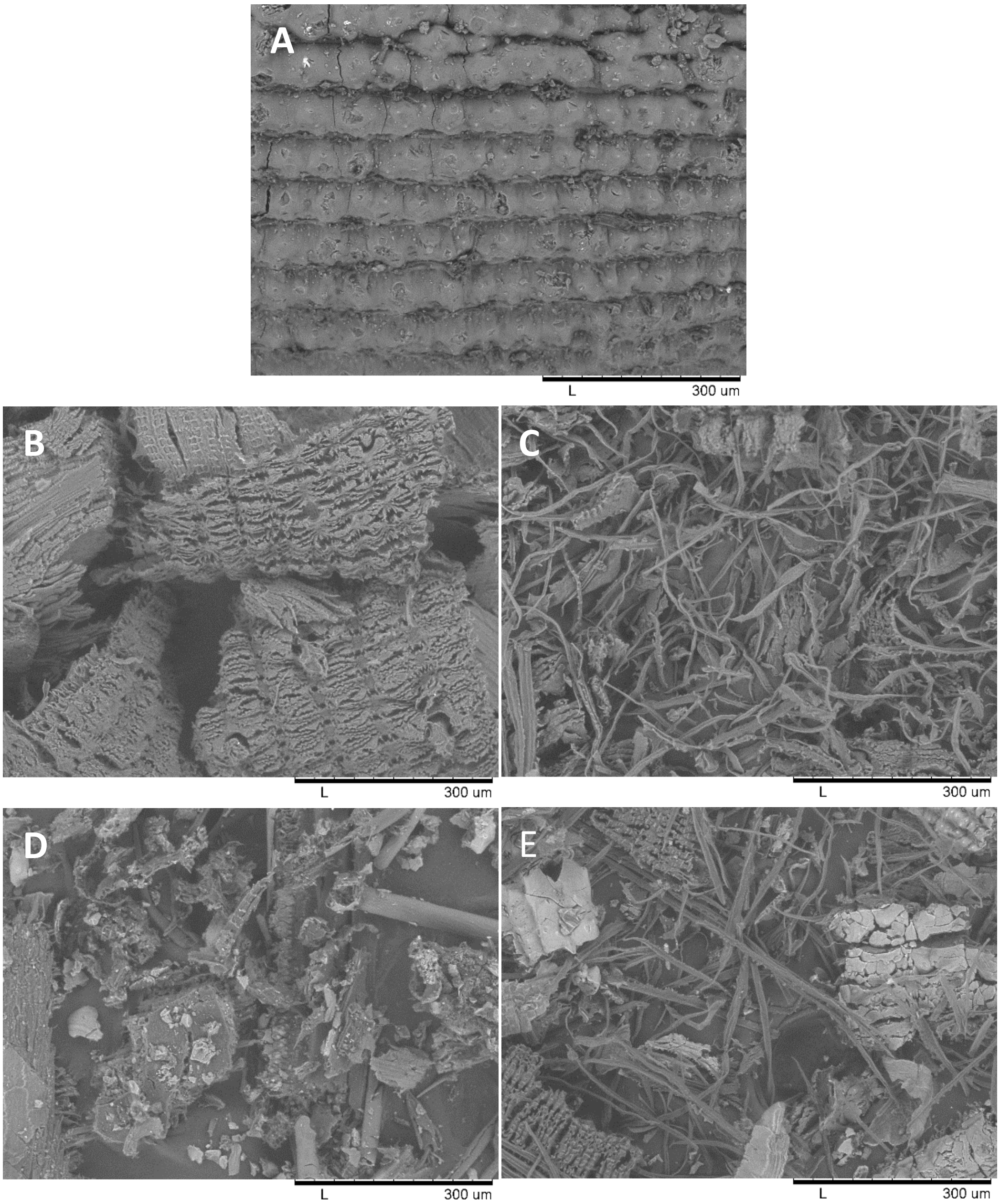

Figure 2. SEM micrographs for raw (A), alkaline (B) and bleached rice husk (C); SWE (D) and bleached SWE residue (E).

After 5 min, the extracts of the SWE contains mainly glucose (>90%), probably due to the presence of starch residues coming from the rice husking process. But as time progresses, the xylan content in the extracts increases progressively as a result of the extraction of hemicelluloses, reaching a xylan content of 80% after 60 min (Figure 3). Likewise, the xylan content was higher in the second and the third alkaline extractions compared to the first one (Figure 4). However, the amount of xylan extracted was always lower than in the extracts coming from the SWE.

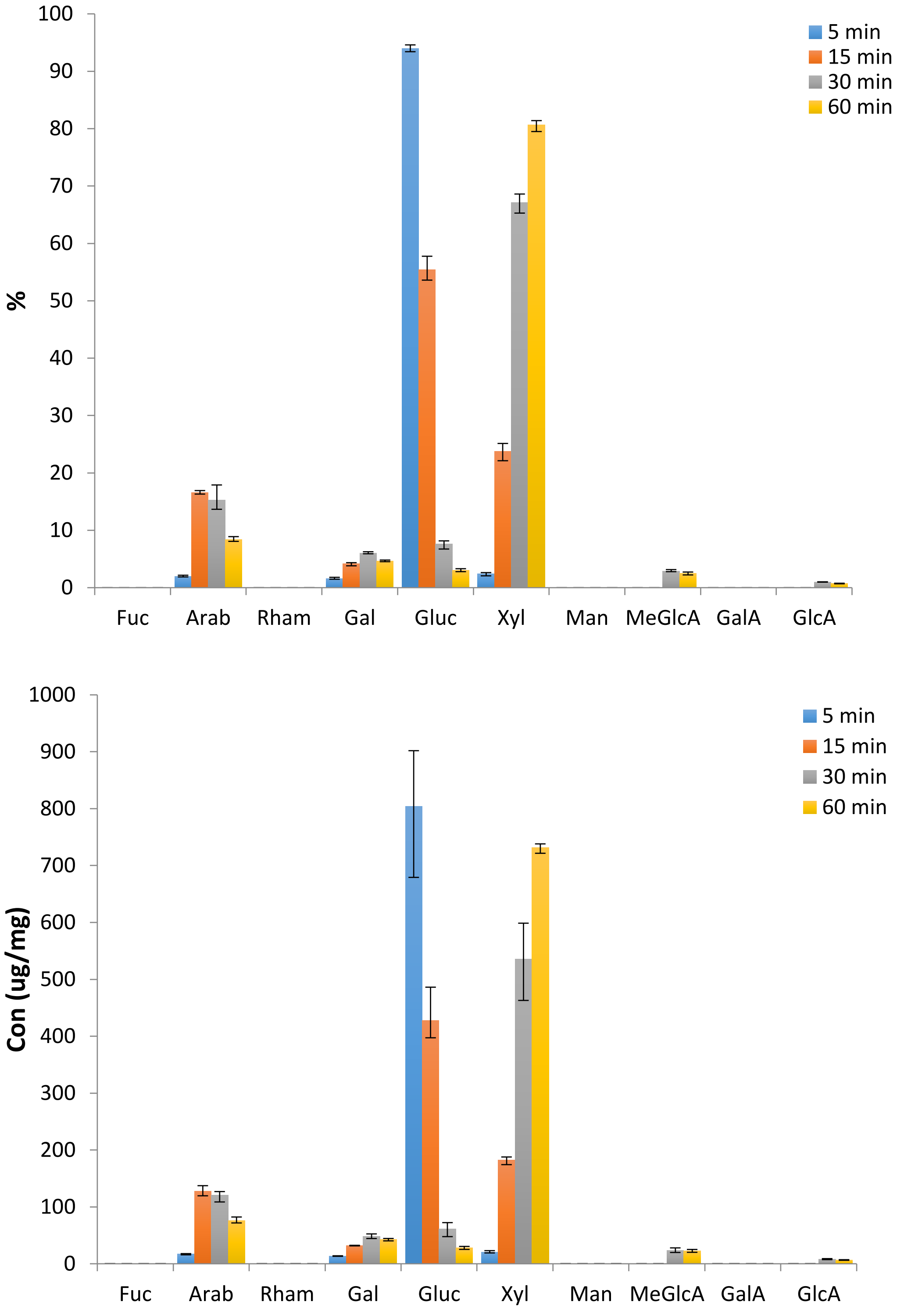

Figure 3. Monosaccharide composition of the extracts after different times of SWE.

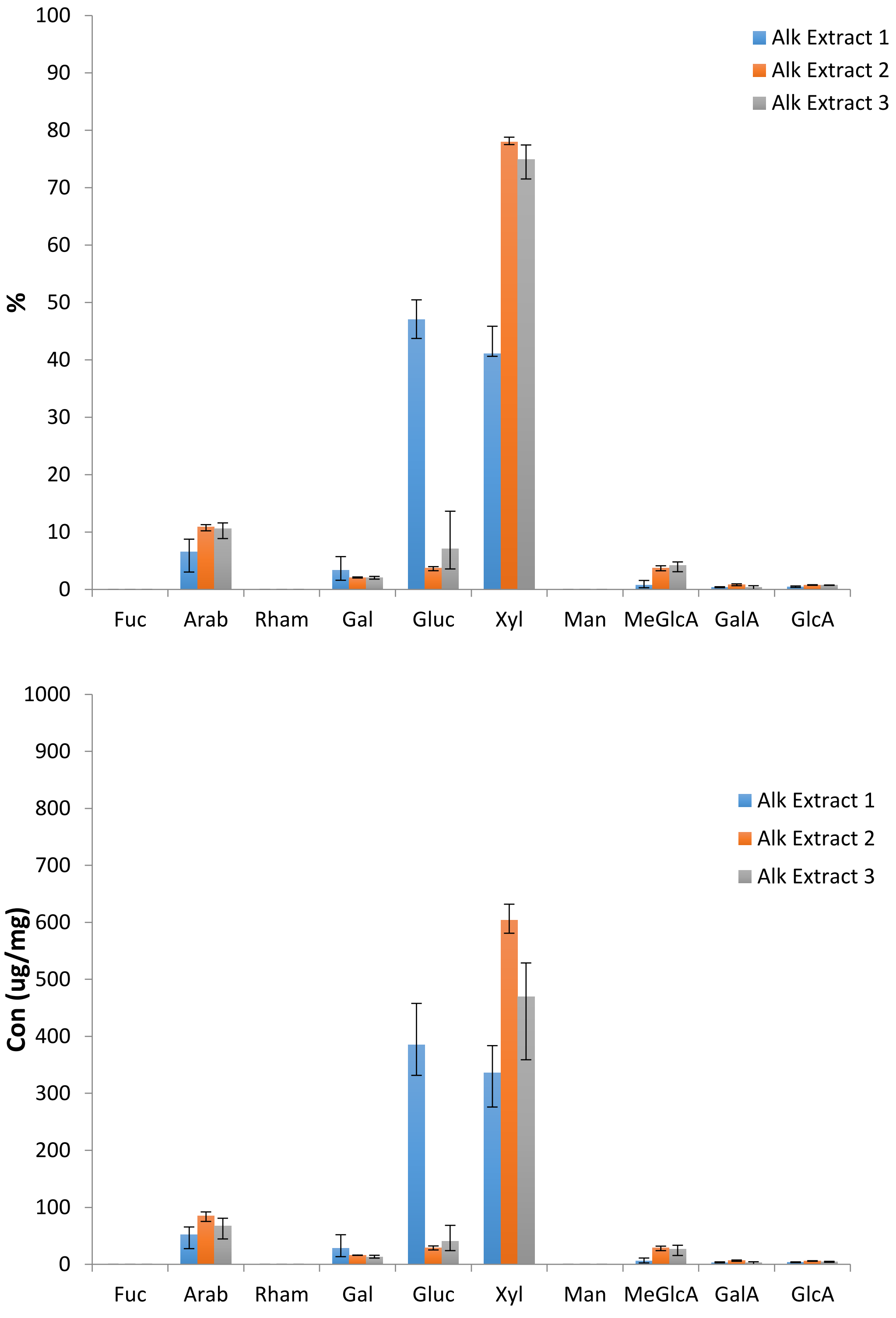

Figure 4. Monosaccharide composition of the extracts after each consecutive alkaline treatment

As the chemical composition analysis showed, after the alkali treatments most of extractives and ashes were removed, as well as some hemicelluloses. Likewise, after the SWE, the hemicellulose content also decreased, but the Klason lignin remained constant (Table 4), due to the milder conditions of this extraction process.

Table 4. Chemical composition (in %wt) after the different steps of the isolation of cellulose nanocrystals from rice husk

	Original	Traditional approach			Alternative		
		Alkaline	Bleaching	Hydrolysis	SWE	Bleaching	Hydrolysis
Fucose	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Arabinose	1.82±0.13	2.16±0.03	1.35±0.05	n.d	0.44±0.03	0.41±0.07	n.d
Rhamnose	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Galactose	0.91±0.16	0.69±0.02	0.20±0.03	n.d	n.d	n.d	n.d
Glucose	35.09±0.38	59.82±2.21	73.52±0.10	94.11±5.34	41.33±0.80	109±19	62±6
Xylose	16.66±1.37	12.20±0.15	17.00±0.36	1.04±0.11	11.13±0.21	15±3	n.d
Mannose	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Total carbohydrates	54.48±1.27	74.87±2.41	92.01±0.20	95.14±5.45	52.91±0.79	62±6	99±2
Klason lignin	33.81	20.46	8.95	n.d	39.52	12.77	n.d
Ash	17.0±0.2	5.8±1.2	3.5±0.2	n.d	17.4±0.7	16.6±0.1	0.4±0.2
Extractives	5.46±0.01	N/A	N/A	N/A	N/A	N/A	N/A

n.d: not detected

N/A: non applicable

Two main mass loss steps at higher and lower temperatures are noticed in the TG and DTG curves of the different samples, excluding the CNC samples. The mass loss step (<3%) at lower temperature (25-150°C) is attributed to the evaporation of the absorbed water, whereas the main step (>55%) at temperatures between 180 and 550 °C is assigned to the thermal degratation of the samples (Figure 5). The thermal stability analysis also validated the extraction of the amorphous components during the alkaline and bleaching treatments since the main degradation peak showed smaller shoulders on the DTG curve. Likewise, this change was less noticeable in the SWE residue due to the milder conditions of this extraction process. The materials resulting from the different treatments degraded within a narrower temperature range and showed better thermal stability than the raw material (Table 5). The hydrolysis reaction gave rise to more thermosensitive samples, being the CNCs coming from the traditional approach more thermostable than those coming from the SWE. Both kind of CNCs exhibited a degradation pattern where three overlapping steps can be distinguished: the first one at lower temperature associated with the sulfate groups that catalyze the degydration process of cellulose; the second one related to the breakdown of the more accesible region in the crystal interior; and the last one at higher temperature associated with the less accesible crystal interior of the CNCs.

Table 5. Thermogravimetric parameters of the rice husk and their alkaline, bleached and hydrolyzed samples.

Sample	[25-150] °C		[180-550] °C			Residue
	Mass loss (%)	T_{max} (°C)	Onset (°C)	Mass loss (%)	T_{max} (°C)	Mass (%)
RH	2.77±0.04	70.3±0.9	252.3±1.3	55.0±0.4	345.4±0.8	32.6±0.2
Alkaline	3.01±0.05	67.2±2.1	274.6±0.5	63.6±1.3	330.8±0.1	23.8±1.7
Alk-Bleached	2.86±0.09	60.5±4.2	303.0±0.3	74.7±0.2	346.8±0.1	15.9±0.2
Alk-CNC	n.d	n.d	223.1±3.2	14.2±2.8	*	83.8±3.3
SWE	2.13±0.10	59.3±0.4	318.3±0.3	59.9±0.3	363.8±0.5	28.9±0.4
SWE-Bleached	2.63±0.01	55.03±0.6	301.8±1.3	63.5±0.4	344.4±0.1	27.9±0.6
SWE-CNC	n.d	n.d	173.9±2.4	8.3±0.4	*	89.9±0.5

*

Sample	T_{peak1} (°C)	T_{peak2} (°C)	T_{peak3} (°C)
Alk-CNC	270.9±6.4	314.9±6.4	415.7±4.9
SWE-CNC	216.4±0.8	353.8±2.3	420.7±0.7

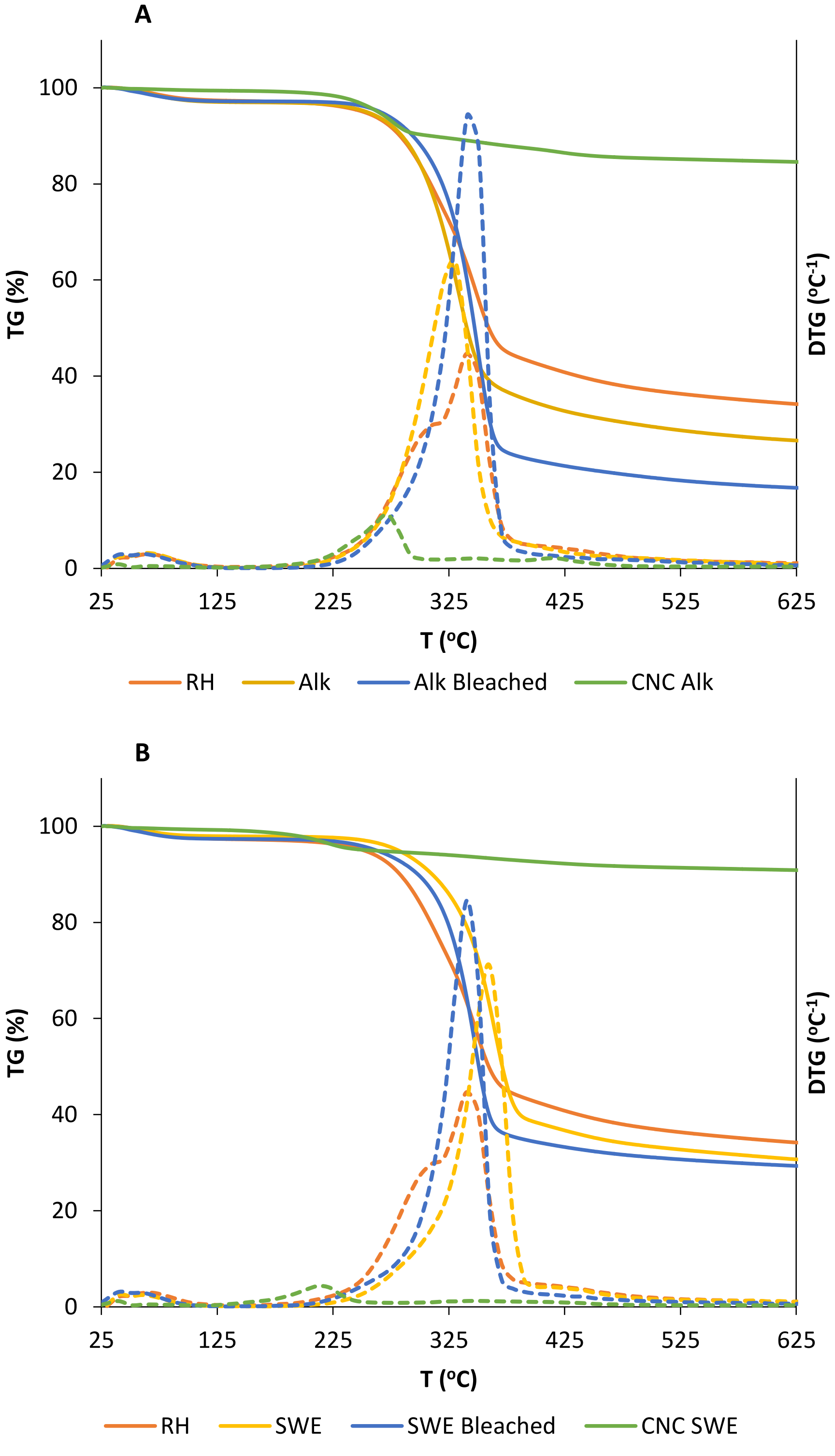

Figure 5. Thermogravimetric (TG) and derivative curves (DTG) for the rice husk and the different materials resulting from the alkaline extraction (A) and the SWE (B).

Regarding the FTIR analysis, the alkaline and the bleached samples showed a higher peak in the region related to the OH vibrations, due to an increase in the hydrogen bond strength, because of the removal of the amorphous components (Figure 6). This chemical structure change was not noticed after the SWE, due to the presence of some remaining amorphous components, since the SWE conditions are milder than in the alkaline treatment. In the same way, the peaks related to the hemicellulose and the lignin (1738-1590, 1509 and 1250 cm-1) are disappearing along the conversion from macro- to nano-dimension. XRD analysis also demonstrated the progressive removal of the amorphous compounds along the conversion from macro- to nano-dimension, since the crystallinity increased progressively, which was more noticeable for the traditional approach.

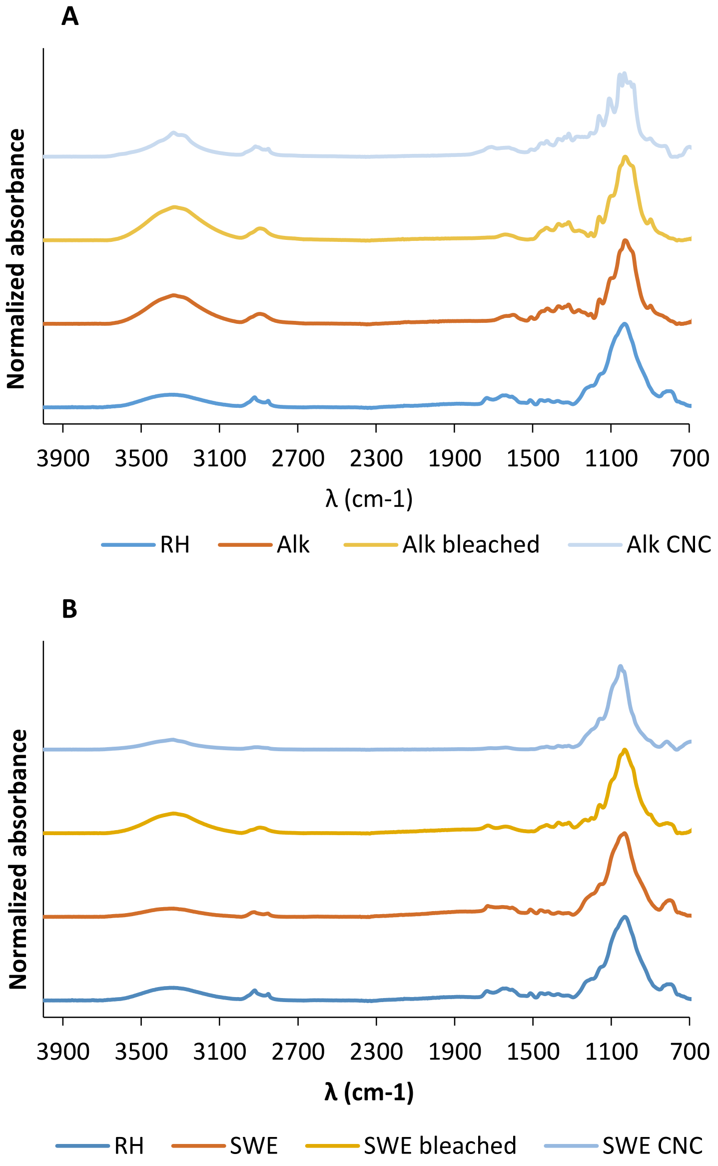

Figure 6. FTIR spectra for the rice husk and the different materials resulting from the alkaline extraction (A) and the SWE (B).