European Cooperation in Science & Technology

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SEQUENTIAL SUBCRITICAL WATER EXTRACTION FOR RICE HUSK VALORIZATION, OBTAINING BIOACTIVE XYLANS AND CELLULOSE NANOCRYSTALS

SHORT TERM SCIENTIFIC MISSION (STSM)

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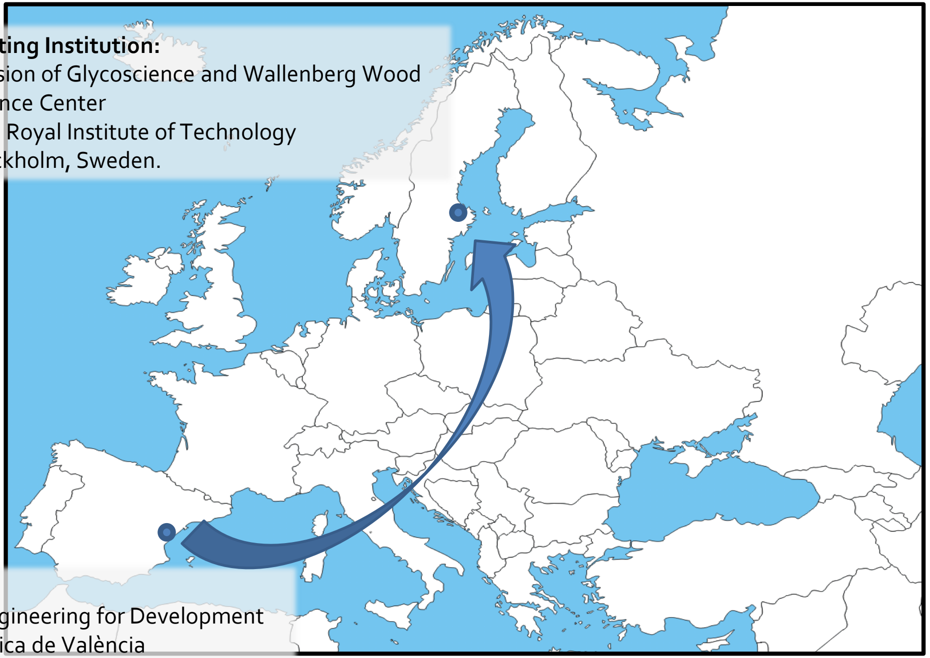
THE INSTITUTIONS

Duration:
01/09/2017 – 30/11/2017

Hosting Institution:
Division of Glycoscience and Wallenberg Wood Science Center
KTH Royal Institute of Technology
Stockholm, Sweden.



Home institution:
Institute of Food Engineering for Development
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Valencia, Spain

JUSTIFICATION

Rice husks (RH)

By-product from the food industry

- ✓ Hemicellulose fraction (20-30%) made up of substituted **arabinoxylan (AX)** with potential food, medical, and pharmaceutical applications.
- ✓ High cellulose content (30-40%) for producing **cellulose nanocrystals (CNCs).**

AX: Bioactive compounds

CNC: Reinforcement materials

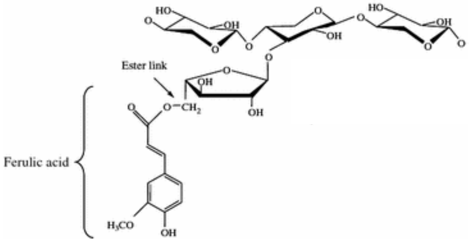
Food Packaging Materials

Integral valorization

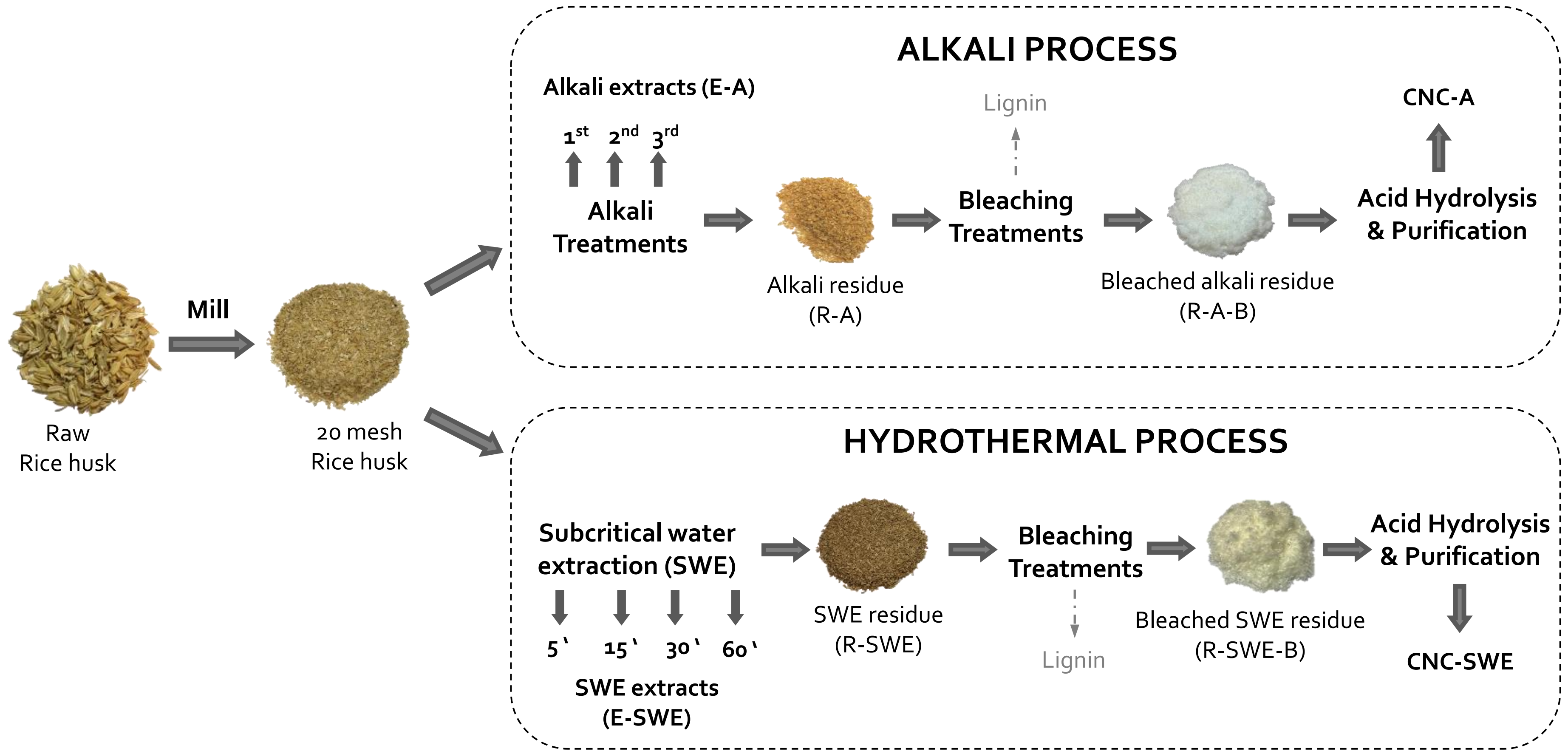
1) **Sequential subcritical water extraction (SWE)**
2) **Alkali extraction**

- ✓ Environmental friendly

- ✓ Preserve the molecular functionalities of the isolated hemicellulose fractions



EXPERIMENTAL DESIGN



WORKING PLAN

Pretreatment: Wiley Mill

Lower particle size improves the extraction
Particle size: 20 mesh.

ISOLATION OF THE HEMICELLULOSES

A) Subcritical water extraction

Sequential fractionation of hemicelluloses at different times: 5, 15, 30 and 60 min.
160 ºC, deionised water
Dionex™ ASE™ 350

B) Alkali extraction

4 wt% in NaOH (4.5 w/v)
80 ºC; 2h
3 different treatments
Dialysis of the resulting extracts

ISOLATION OF THE CNCS

Bleaching

4 wt% in 1water:1buffer acetate: 1aqueous chlorite (1.7%)
5 different treatments at 80 ºC; 4h

Acid Hydrolysis

4 wt% in 65 wt% sulphuric acid
45 ºc; 40 min.

CNC purification

1. **Successive centrifugations:** until constant supernatant pH; 25000 g; 20 min
2. **Dialysis**: purified water 1 week
3. **Sonication:** 10 min; 7,125 W/ml
4. **Centrifugation:** remove higher particles

Freeze-dry extracts and residues

WORKING PLAN

CHARACTERIZATION OF THE RESIDUES

Chemical composition analyses

Soxhlet extraction
NREL's LAP

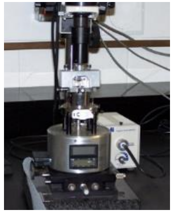
Klason lignin.
Tappi method T222 om-06

Ash content
TGA

Monosaccharide composition
Acid hydrolysis and HPAEC-PAD

Scanning Electron Microscopy (SEM)

Atomic Force Microscopy (AFM)



Fourier Transform Infrared Spectrometry (FTIR)

X-Ray Diffraction Analysis (XRD)

Thermogravimetric Analysis (TGA)

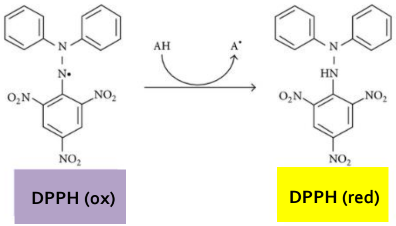
WORKING PLAN

CHARACTERIZATION OF THE EXTRACTS

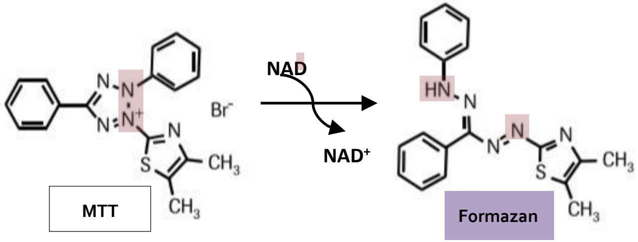
Monosaccharide composition
Acid hydrolysis and HPAEC-PAD

Molar mass distribution
Size-Exclusion Chromatography

Antioxidant activity. DPPH scavenging activity



Antibacterial activity. MTT assay



RESULTS. Morphological changes of the residues

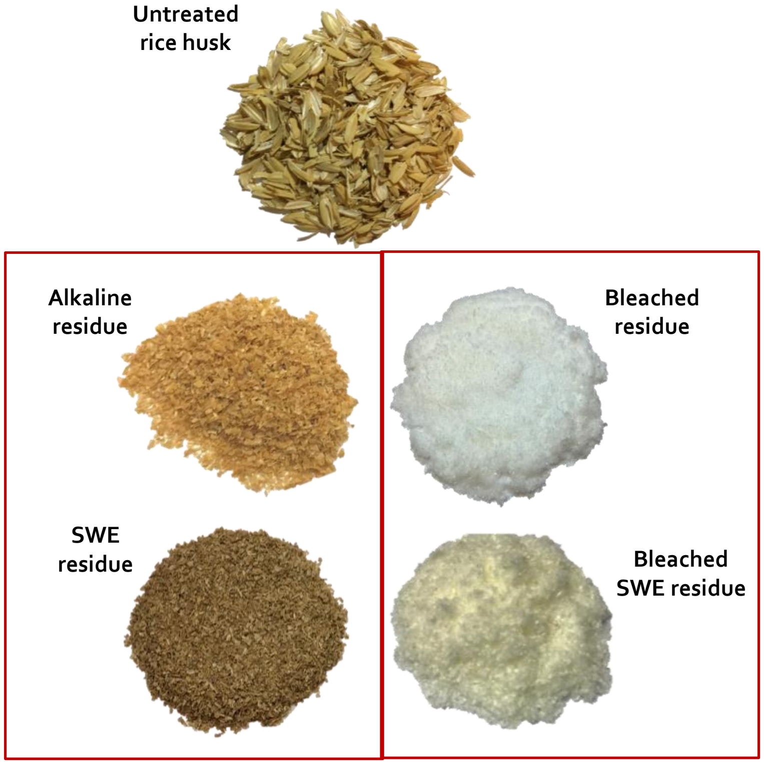


Figure 1. Visual aspect of the samples after the different treatments

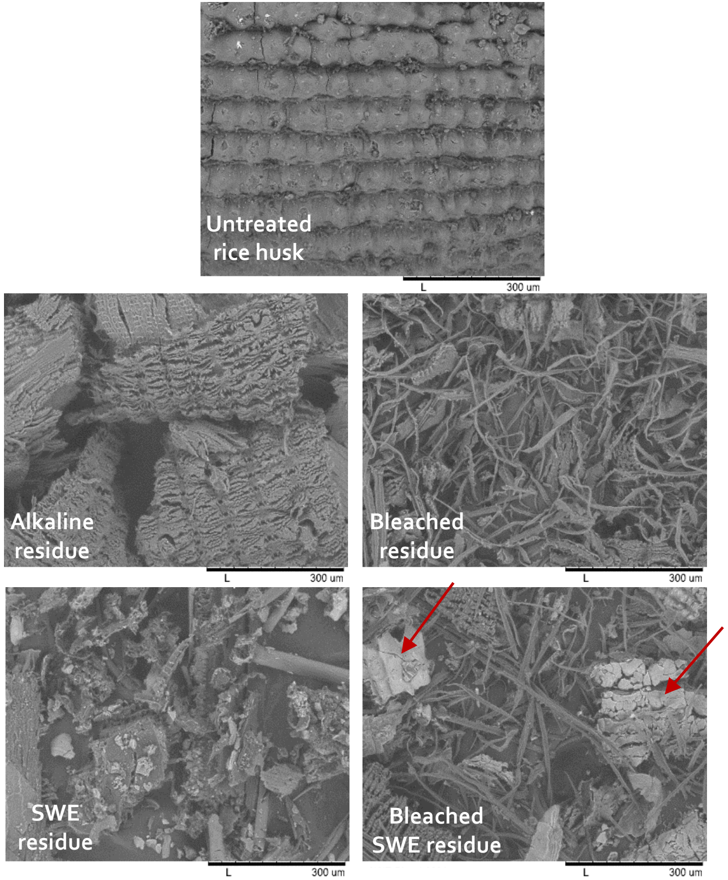


Figure 2. SEM of the samples after the different treatments

RESULTS. Chemical composition of the residues

Table 1. Yield and chemical composition (in %wt) after the different steps of the isolation of cellulose nanocrystals from rice husk

	Rice Husk	Alkali process			Hydrothermal process		
		Alkaline	Bleaching	Hydrolysis	SWE	Bleaching	Hydrolysis
Yield[a]	100	54.4±0.1	65±2		69±1	58±1	
Fuc	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Ara	1.8±0.1	2.16±0.03	1.35±0.05	<0.1	0.4±0.1	0.4±0.1	<0.1
Rha	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Gal	0.9±0.2	0.69±0.02	0.20±0.03	<0.1	<0.1	<0.1	<0.1
Glc	35.1±0.4	60±2	73.5±0.1	96±5	41±1	60±2	95±6
Xyl	17±1	12.2±0.2	17.0±0.4	1.0±0.1	11.1±0.2	15±3	<0.1
Man	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
GalA	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
GlcA	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Total carbohydrates	**55±1**	**75±2**	**92.0±0.2**	**96±5**	**53±1**	**60±2**	**95±6**
Cellulose	35.1±0.4	60±2	73.5±0.1	96±5	41±1	60±2	95±6
Hemicellulose	19±2	15.0±0.2	18.4±0.3	1.0±0.1	11.6±0.2	15±3	<0.1
Klason lignin	33.8	20.5	9.0	N/A	39.5	25.0	N/A
Ash	17.0±0.2	6±1	3.5±0.2	n.d	17±1	16.6±0.1	0.4±0.2
Extractives	5.46±0.01	-	-	-	-	-	-

[a]The gravimetric yields for each treatment were calculated based on the total dry weight (100%) of the previous treatment
n.d: not detected; N/A: non applicable

RESULTS. Chemical structure of the residues (FTIR)

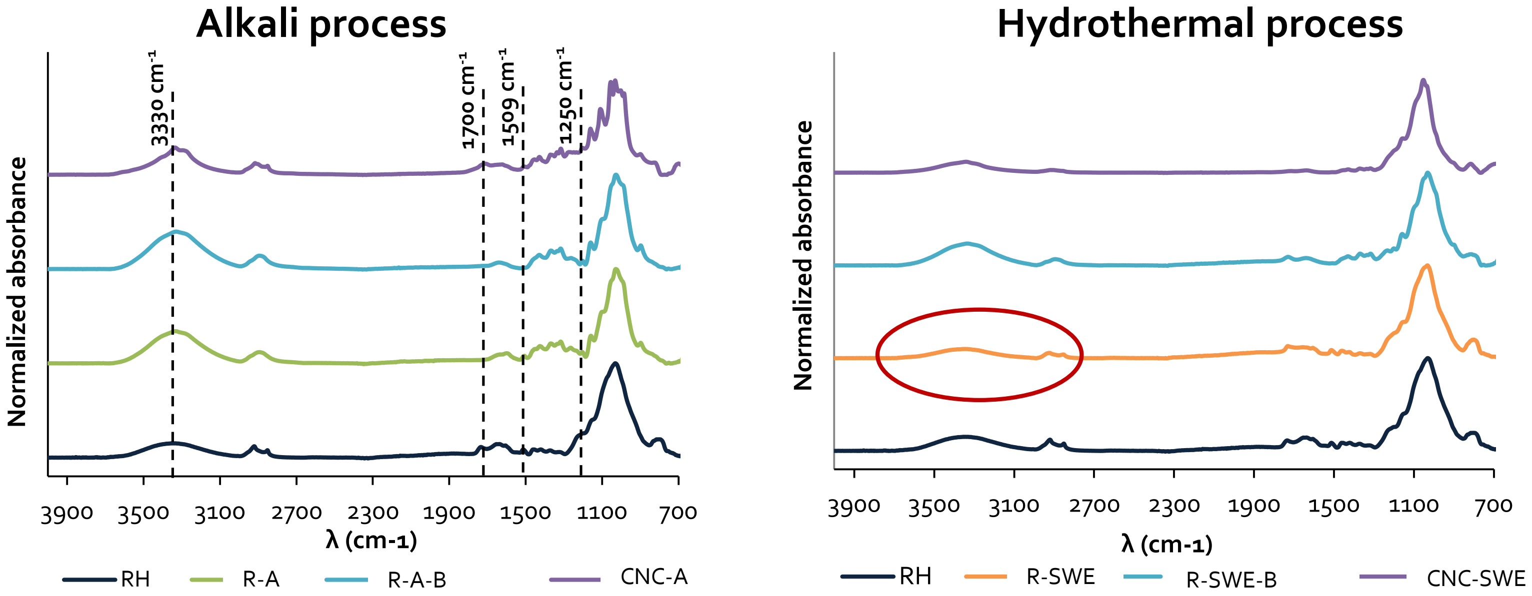


Figure 3. FTIR spectra for the different materials obtained throughout both processes

RESULTS. Crystallinity of the residues (XRD)

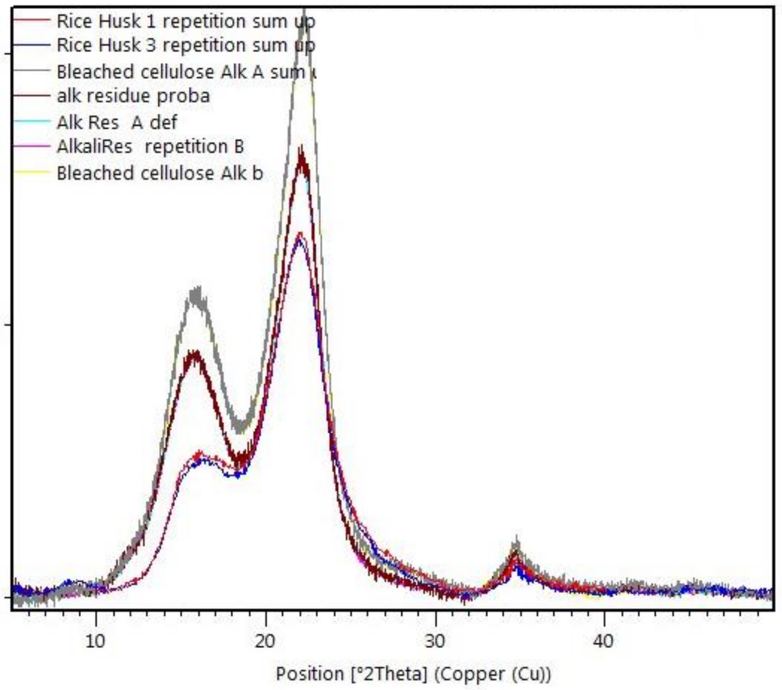
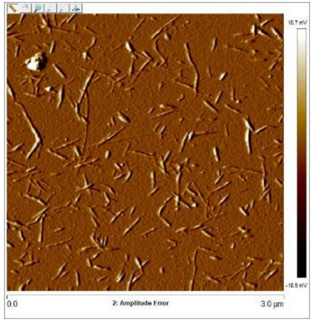


Figure 4. XRD patter of the residues along the conversion from macro to nano dimensions

Table 2. Crystallinity index (CrI) after each step of both CNC isolation processes

	CrI (%)
RH	40.1 ± 0.5
R-A	71.3 ± 0.8
R-A-B	72.3 ± 0.7
CNC-A	80.± 0.9
R-SWE	50.0 ± 2.1
R-SWE-B	58.4 ± 1.8
CNC-SWE	53.0

RESULTS. Morphology of the CNCs (AFM)



Aspect ratio (L/D)

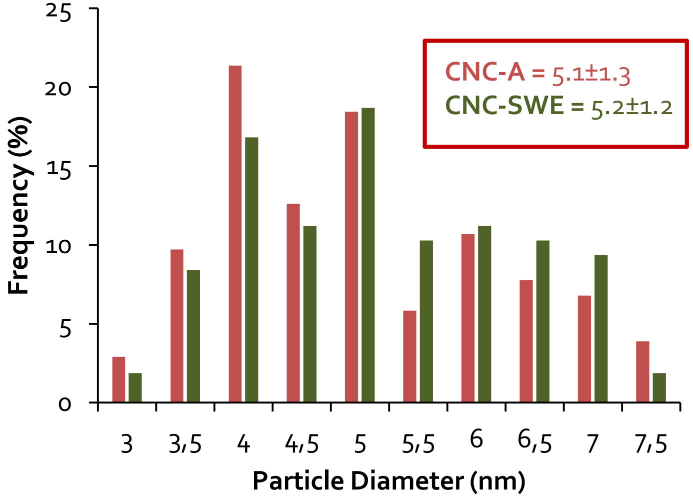
CNC-A = 30-70
CNC-SWE = 35-75

→

>10
Good reinforcing material

L/D = 10-20
Collazo-Bigliardi et al., 2018
Johar et al., 2012

Particle Diameter (D)



Particle Length (L)

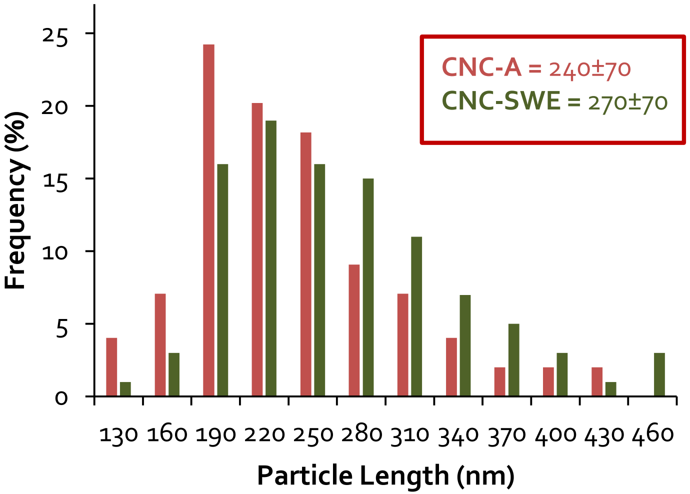


Figure 5. AFM analysis of the CNCs isolated from rice husk through the alkaline process and the hydrothermal process: particle diameter and length. Averaged diameter and length calculated from 100 individual CNC particles using AFM

RESULTS. Thermal behaviour of the residues (TGA)

Table 3. Thermogravimetric parameters of the rice husk and their alkaline, bleached and hydrolyzed samples.

Sample	[25-150] ºC		[180-550] ºC		
	Mass loss (%)	T_{max} (ºC)	T_{onset} (ºC)	Mass loss (%)	T_{max} (ºC)
RH	2.77±0.04	70.3±0.9	252.3±1.3	55.0±0.4	345.4±0.8
R-A	3.01±0.05	67.2±2.1	274.6±0.5 ↑	63.6±1.3	330.8±0.1
R-A-B	2.86±0.09	60.5±4.2	303.0±0.3	74.7±0.2	346.8±0.1
CNC-A	n.d	n.d	223.1±3.2 ↓	14.2±2.8	271±6/315±6/416±5
R-SWE	2.13±0.10	59.3±0.4	318.3±0.3 ↑	59.9±0.3	363.8±0.5
R-SWE-B	2.63±0.01	55.03±0.6	301.8±1.3	63.5±0.4	344.4±0.1
CNC-SWE	n.d	n.d	173.9±2.4 ↓	8.3±0.4	216±1/354±2/421±1

Degradation patter of the CNCs → 3 overlapping steps:
1st at lower temperature: sulphate groups that catalyse the dehydration process of cellulose
2nd breakdown of the more accessible region in the crystal interior
3rd at higher temperature: less accessible crystal interior of the CNCs

RESULTS. Monosaccharide composition of the extracts

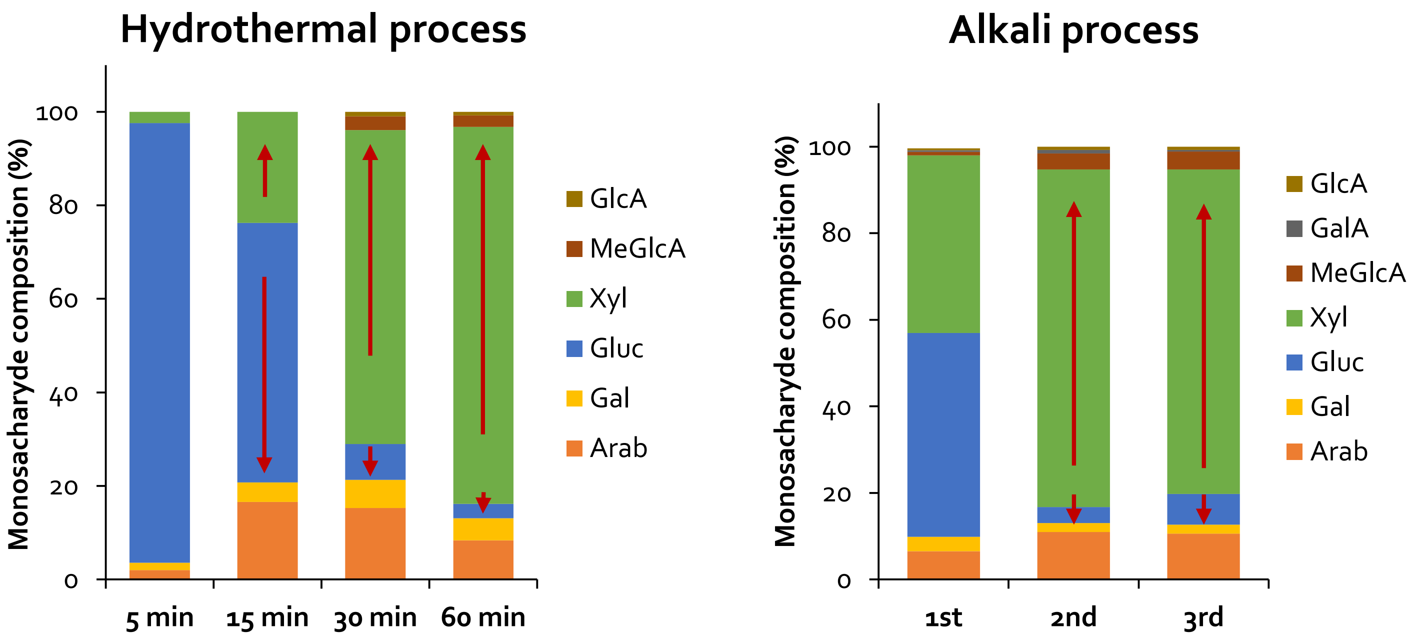


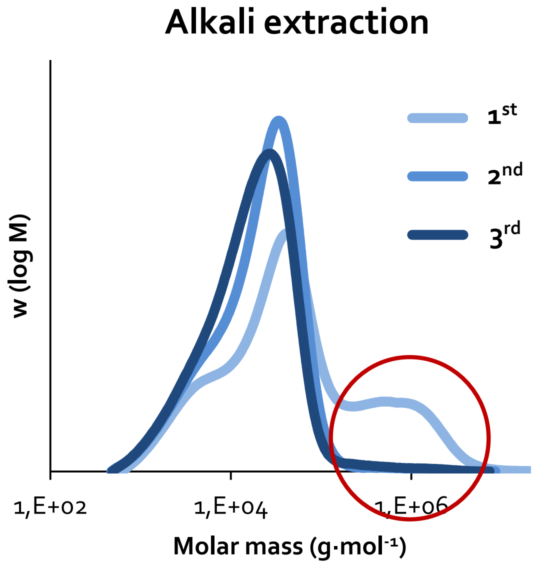
Figure 6. Monosaccharide composition of the extracts after different times of SWE and after each consecutive alkaline extraction.

RESULTS. Monosaccharide composition of the extracts

Table 4. Monosaccharide composition (in %wt) of the rice husk extracts resulting from the three consecutive alkaline extractions and the sequential fractionation by subcritical water extraction.

	Alkaline process			Hydrothermal process			
	1st	2nd	3rd	5 min	15 min	30 min	60 min
Fuc	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Ara	5.2±2.1	8.5±0.9	6.7±2.0	1.7±0.1	12.8±0.9	12.1±1.0	7.6±0.5
Rha	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Gal	2.8±2.1	1.6±0.1	1.3±0.3	<0.1	3.2±0.1	4.8±0.4	4.2±0.2
Glu	38.5±6.5	2.9±0.4	4.0±2.4	80.4±11.4	42.8±5.0	6.1±1.3	2.8±0.3
Xyl	33.6±5.5	60.4±2.6	47.0±9.6	2.1±0.2	18.3±0.7	53.6±6.8	73.2±0.9
Man	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
MeGlcA	0.6±0.2	2.9±0.4	2.7±1.0	<0.1	<0.1	2.3±0.4	2.2±0.3
GalA	0.3±0.1	0.6±0.1	0.3±0.1	<0.1	<0.1	<0.1	<0.1
GlcA	0.4±0.1	0.6±0.1	0.5±0.1	<0.1	<0.1	0.8±0.1	0.7±0.1
Xylan content (%)[a]	40±5	72±4	57±13	3.8±0.3	31±2	69±7	83.7±1.4
Total carbohydrates	81.3±10.2	77.5±3.9	62.4±10.6	85.5±11.6	77.0±6.3	79.7±8.9	90.7±1.7

RESULTS. Molar mass distributions of the extracts



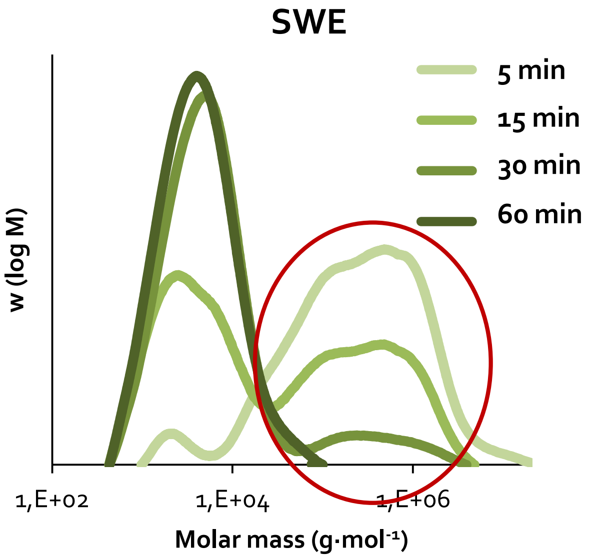
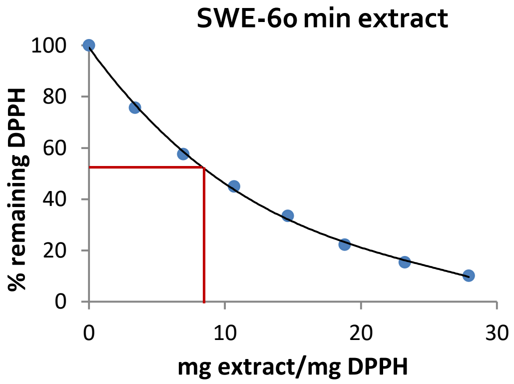


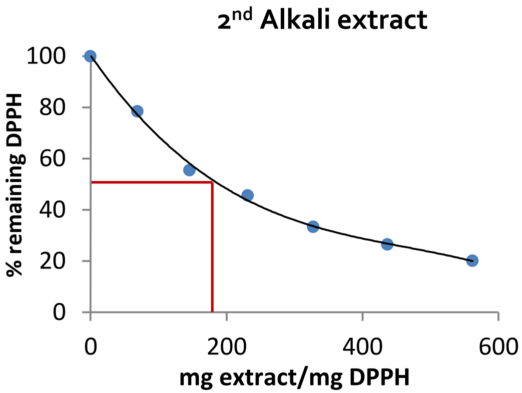
Table 5. Number-average molar mass (Mn) and weight-average molar mass (Mw)

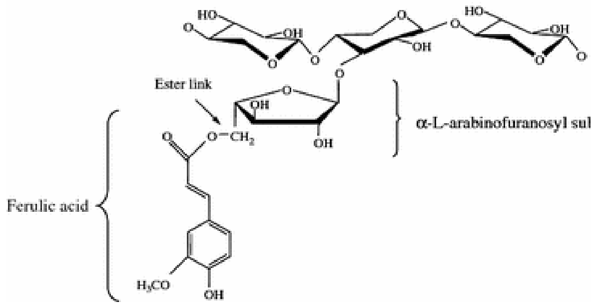
	Mn	Mw
E-A-1	12150	271700
E-A-2	8784	35970
E-A-3	8128	35230
E-SWE-5min	36810	691700
E-SWE-15min	4291	250600
E-SWE-30min	3254	59990
E-SWE-60min	2705	6499

Figure 7. Molar mass distributions of the rice husk extracts resulting from (A) the three consecutive alkaline extractions and (B) the sequential fractionation by subcritical water extraction

Antioxidant activity







EC50 = 9.6±0.6 mg/mg

EC50 = 170±21 mg/mg → 18-fold lower antioxidant activity

p-coumaric acid → EC50 = 0.2 mg/mg
ferulic acid → EC50 = 20.8 mg/mg
(Brand-Williams et al., 1995)

Actividad antimicrobiana

SWE-60 min extract

E. Coli → MIC = 95 ± 2 mg/mL

L. Innocua → MIC = 55 ± 2 mg/mL

2nd Alkali extract

No antimicrobial activity

APPLICATIONS

Arabinoxylans:
Food packaging materials
Food fomulations

Extend food shelflife
Improving food quality

CNCs:
Reinforcing materials

Improving mechanical properties
of packaging materials

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