CHEMICAL CHARACTERIZATION OF LIGNIN FROM ANNUAL PLANT GROWING IN NORTH OF ITALY

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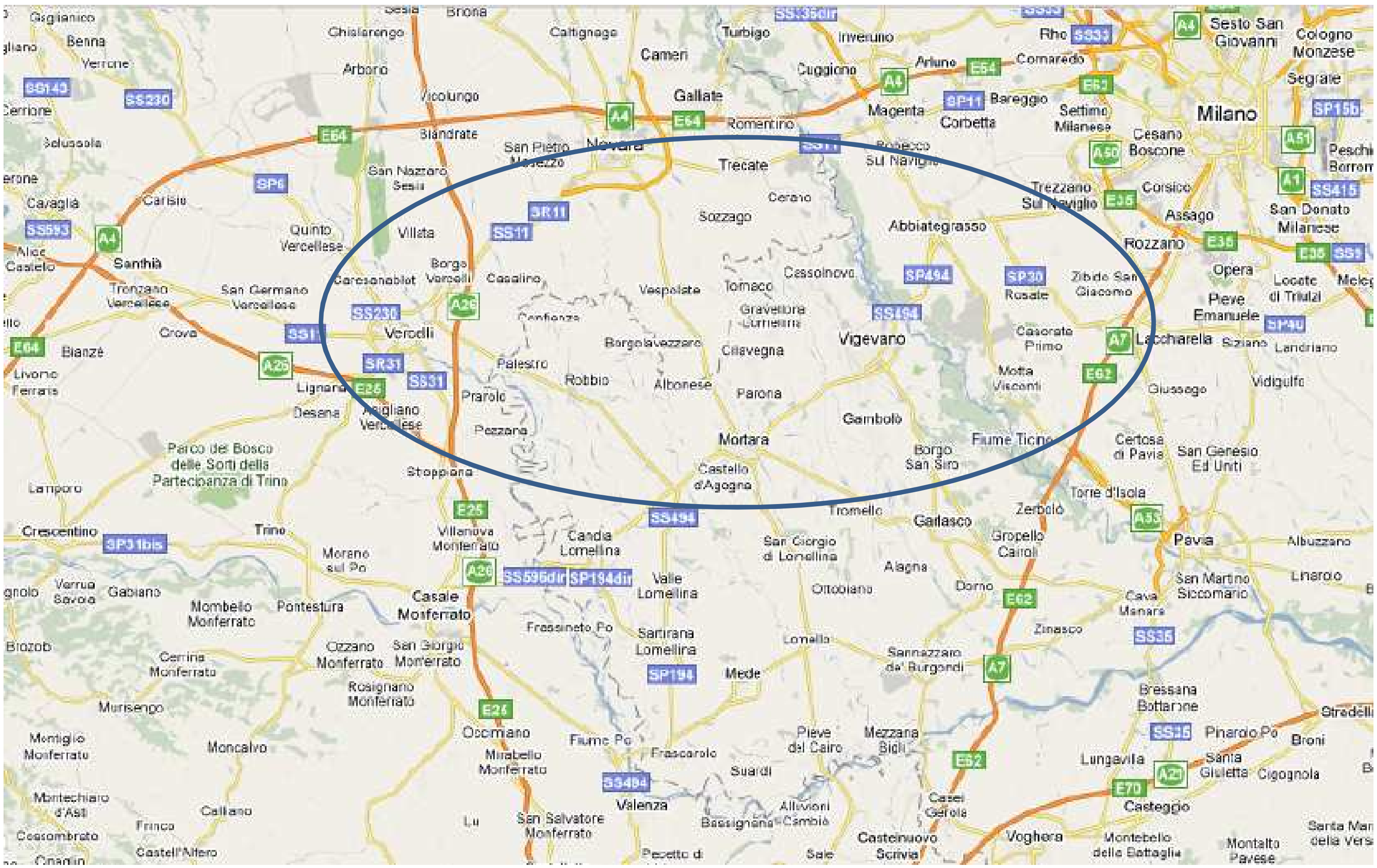
WHERE ARE CULTIVATED IN ITALY RICE AND GIANT CANE



WHAT IS RICE HUSK ?

• Rice is one of the most cultivated crops in the world with a global production of about 680 million tons/year). Italy produces approximately 1.4 million tons/year of rice, with the 90% of this production concentrated in the Northern Italy;

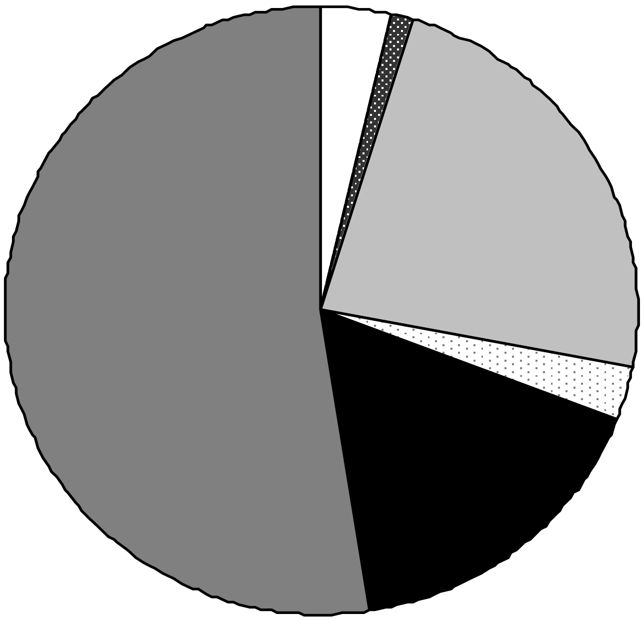
• Rice husk, the outer cover of rice grain, is among the principal processing side-products of the rice milling industry and accounts for about 20% by weight of rice;

• Rice husk does not possess a remarkable commercial interest and its price is very low (30-40 €/ton in Italy;

• Because of the elevated ashes and lignin content, rice husk is not appropriate as animal feed raw material;

• Rice husk can be burnt under controlled conditions to obtain a large amount of silica.

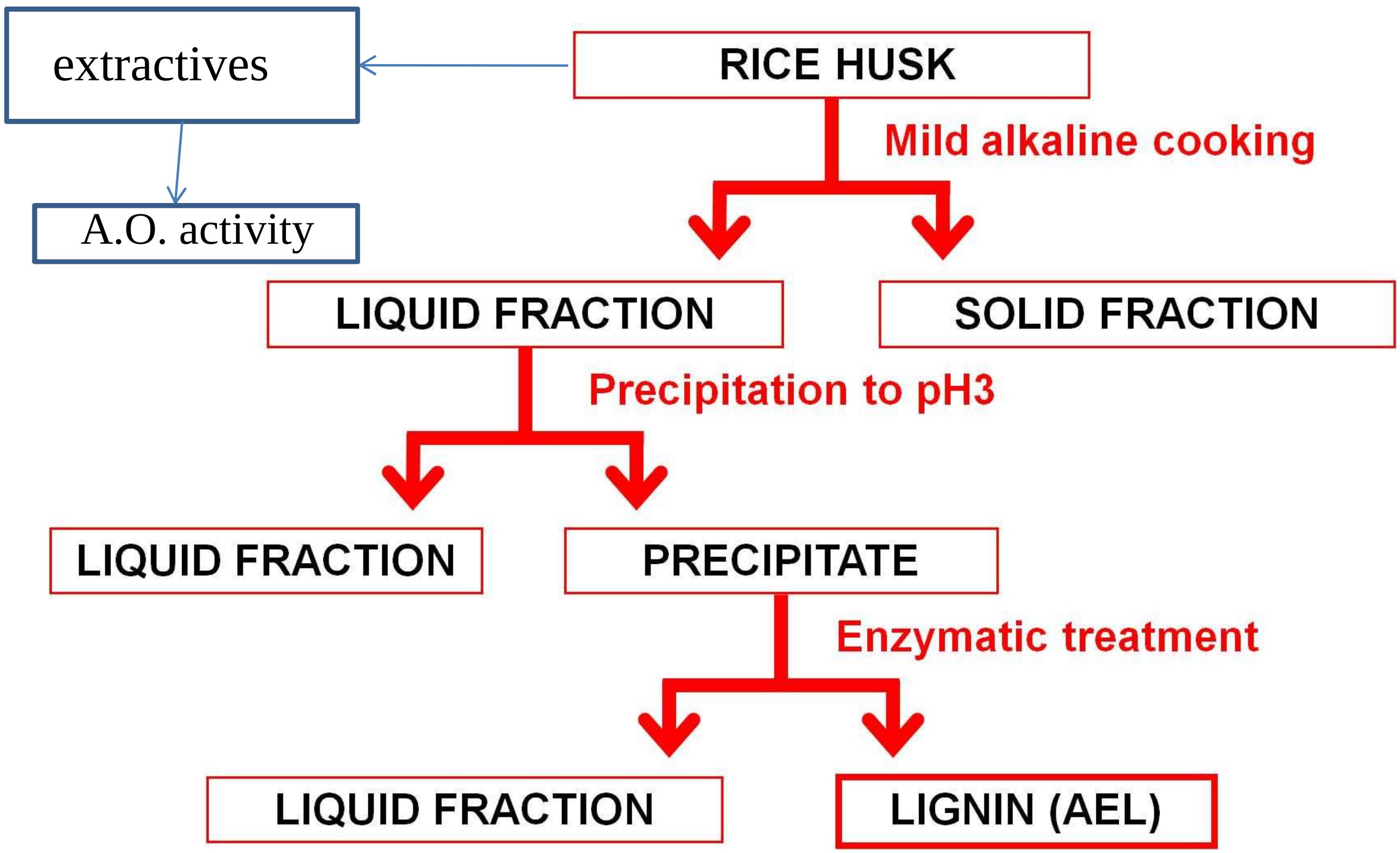
PRELIMINARY RICE HUSK ANALYSIS



Color	Component	%
(white)	Water extractives	3,5
(dark dotted)	Ethanol extractives	1,2
(light grey)	Acid insoluble lignin	23,3
(light dotted)	Acid soluble lignin	2,7
(black)	Ashes	16,8
(grey)	Carbohydrates	52,6

- **Lignin: more than 20%**
- **Ashes: about 16%, constituted of around 85-90% amorphous silica**
- **Carbohydrates: about 52%**

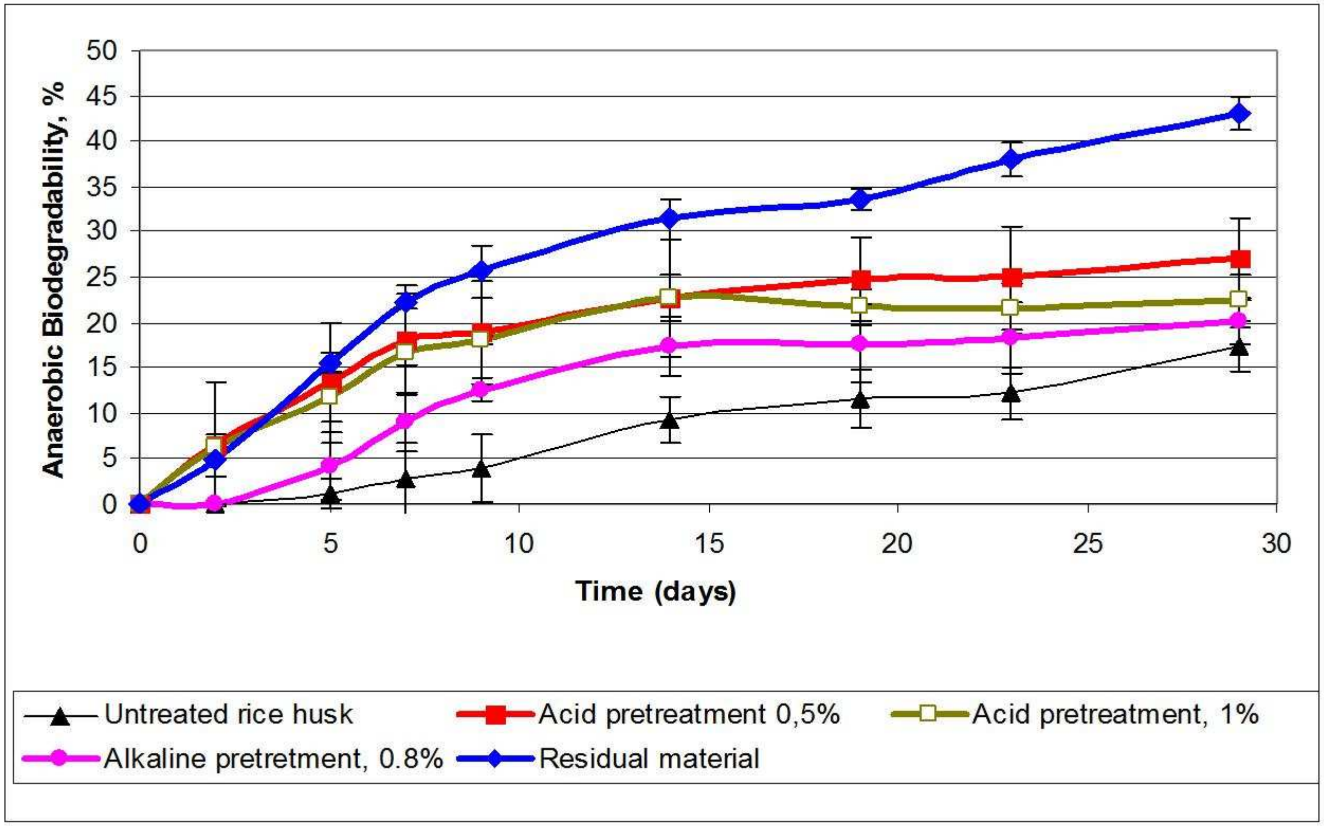
OUTLINE OF RICE HUSK PROJECT



ANTIOXIDANT ACTIVITY OF EXTRACTIVES FROM RICE HUSK

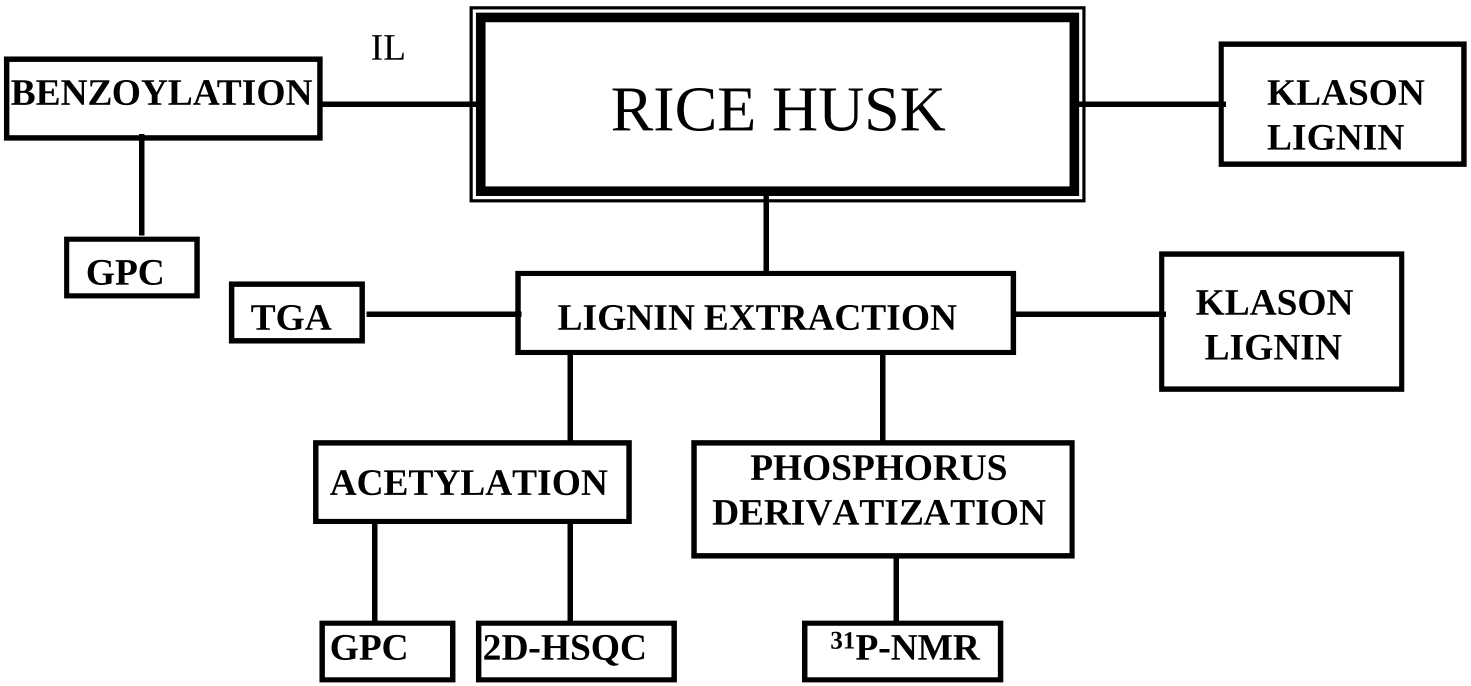
	IC_{50} (µg/mL) by DPPH radical scavenging activity	AAC By B-carotene bleaching test
Water extract	82.9	632
Ethanol extract	112.4	565
Acetone extract	195.2	503
BHA reference	7.6	633

ENHANCED BIOGAS PRODUCTION AFTER LIGNIN REMOVAL



The residual material, after lignin removal, was digested faster in anaerobic conditions in comparison to the untreated raw material

ANALYSIS PROTOCOL



IL = **1-allyl-3-methylimidazolium chloride - [amim]Cl**

NATIVE RICE HUSK CHARACTERIZATION

Benzoylation – GPC characterization:

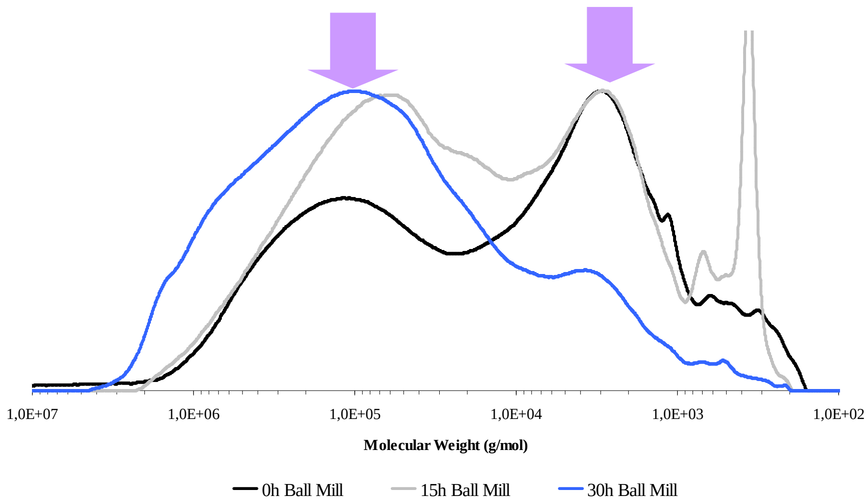
Milling time (h)	WPG (%)	Benzoylated soluble fraction (%)
0	28	19,1
5	31	21,3
10	49	26,4
15	80	37,2
20	122	56,3
30	130	63,3

↓ Particle size ↑ More effective esterification (WPG)

↑ WPG ↑ Solubilized rice husk fraction

CELLULOSE / LIGNIN-CARBOHYDRATE COMPLEXES

"FREE" LIGNIN



Reduced milling time: mainly benzoylated lignin
Higher milling time: enhanced benzoylated carbohydrate content

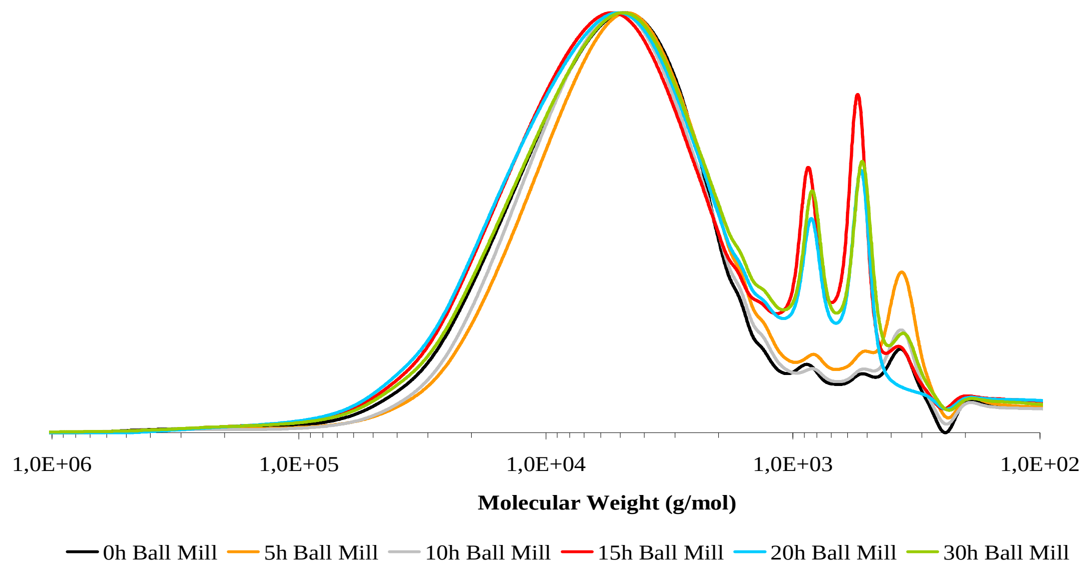
WPG = Weight Percentage Gain

LIGNIN EXTRACTION

- **Acidolysis Lignin (AL).** Dry, extratives-free (blended rice husk milled in a planetary ball mill for different periods of time at 300 rpm. Differently milled rice husk samples were refluxed under nitrogen for 2 hours in a **0.1 M HCl dioxane – water solution (9:1)** and then cool to room temperature. The insoluble material remained after lignin solubilization was collected by centrifugation The supernatant was added dropwise into a 0,01 M HCl aqueous solution which was then kept at + 4 °C overnight to allow for a complete lignin precipitation
Modified parameters: **Milling Time (h)**

- **Alkali Enzyme Lignin (AEL) .** *Mild alkaline cooking* (5-10% solid consistency, 0.1-0.3 M NaOH, 90°C, 4 hours) followed by *Enzymatic hydrolysis* (two-3 hours cycles with 50U/g of crude cellulase from *Trichoderma reesei* ATCC 26921 in 50 mM Na-acetate buffer pH5 at 40°C).
Modified parameters: **Temperature (°C), NaOH concentration**

GEL PERMEATION CHROMATOGRAPHY: ACETYLATED SAMPLES

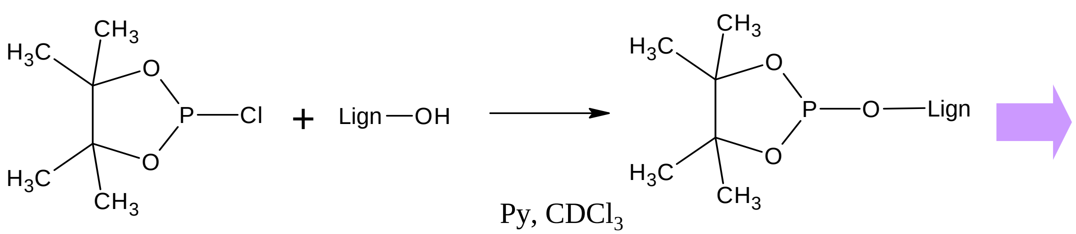


Milling time (h)	M_w (g/mol)	M_n (g/mol)	M_p (g/mol)	I
0	31500	9000	4780	3,5
5	30300	7900	4680	3,8
10	29500	8300	5070	3,5
15	37200	9900	5410	3,8
20	41000	10200	5090	4,0
30	36300	9300	4880	3,9

SAME REPRESENTATIVITY

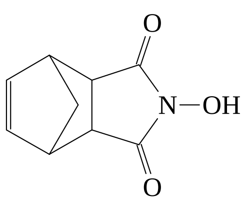
M_n (number-average molecular weight, M_w (weight-average molecular weight,

^{31}P-NMR QUANTIFICATION: Sample derivatization



2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane

DIFFERENTIATE: ALIPHATIC HYDROXYLS, DIFFERENTLY METHOXYLATED PHENOLS, ACIDIC GROUP



endo-N-hydroxy-5-norbornene-2,3-dicarboximide

\+ phospholane

INTERNAL STANDARD, QUANTITATIVE ANALYSIS

Yields, purity, ashes content, average molecular weight indexes and labile hydroxyls composition of AL lignin extracted from differently milled rice husk samples.

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Milling Time (h)	**0**	**5**	**10**	**15**	**20**	**30**
Yield (%)	**16.0**	**26.8**	**34.0**	**31.2**	**46.3**	**41.9**
Purity (Klason, %)	**> 85**	**> 85**	**> 85**	**> 85**	**> 85**	**> 85**
Ashes (%)	**< 2**	**< 2**	**< 2**	**< 2**	**< 2**	**< 2**
GPC (g/mol)						
M_n	9000	7900	8300	9900	10200	9300
M_w	31500	30300	29500	37200	41000	36300
M_p	4800	4700	5100	5400	5100	4900
I	3.5	3.8	3.5	3.8	4.0	3.9
^{31}P NMR (mmol/g)						
Aliphatic -OH	3.08	2.89	3.40	2.98	3.03	2.88
Cond. PhOH + S-OH	0.23	0.21	0.34	0.27	0.23	0.31
G-OH	0.47	0.61	0.70	0.61	0.65	0.60
P-OH	0.66	0.66	0.74	0.65	0.65	0.63
COOH	0.23	0.22	0.23	0.22	0.27	0.23

Optimization of AEL extraction: effect of different reaction temperatures and NaOH concentrations on yields, purity, and morphological and chemical features.

	Reaction Temperature (°C)			NaOH Concentration (M)		
	70	**80**	**90**	**0.1**	**0.2**	**0.3**
Yield (%)	**11.2**	**15.3**	**22.3**	**11.2**	**22.3**	**29.1**
Purity (Klason, %)	**65.2**	**65.2**	**74.3**	**49.7**	**74.3**	**77.9**
Ashes (%)	**< 2**	**< 2**	**< 2**	**< 2**	**< 2**	**< 2**
GPC (g/mol)						
M_n	11300	12300	12000	7400	12000	13600
M_w	106000	113000	96300	39000	96300	115000
M_p	4200	4600	4600	3800	4600	4500
I	9.4	9.2	8.0	5.4	8.0	8.4
^{31}P NMR (mmol/g)						
Aliphatic -OH	1.23	0.86	2.58	0.77	2.58	3.71
Cond. PhOH + S-OH	0.06	0.06	0.18	0.05	0.18	0.13
G-OH	0.14	0.10	0.34	0.09	0.34	0.38
P-OH	0.15	0.07	0.23	0.08	0.23	0.14
COOH	**0.27**	**0.20**	**0.62**	0.16	0.62	0.59

If not otherwise indicated: reaction period, concentration of soda and reaction temperature set at 4 h, 0.2 M, and 90°C.

CONCLUSIONS ABOUT THE AEL EXTRACTION

The parameters chooses for AEL extraction from rice husk were

4 h, 90 °C, 0.3M NaOH as a compromise between

•Yield

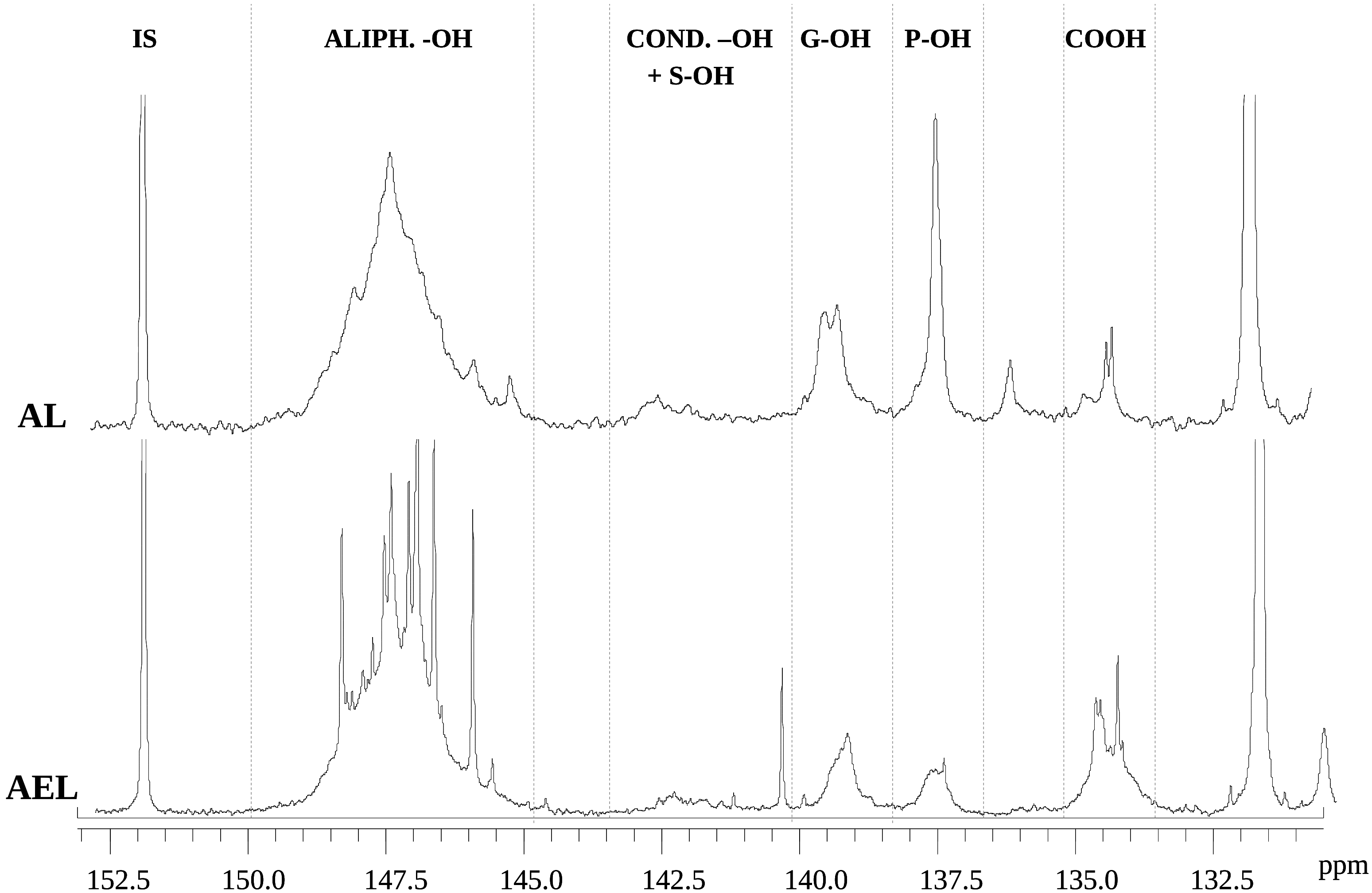
•Purity

•Oxidative conditions

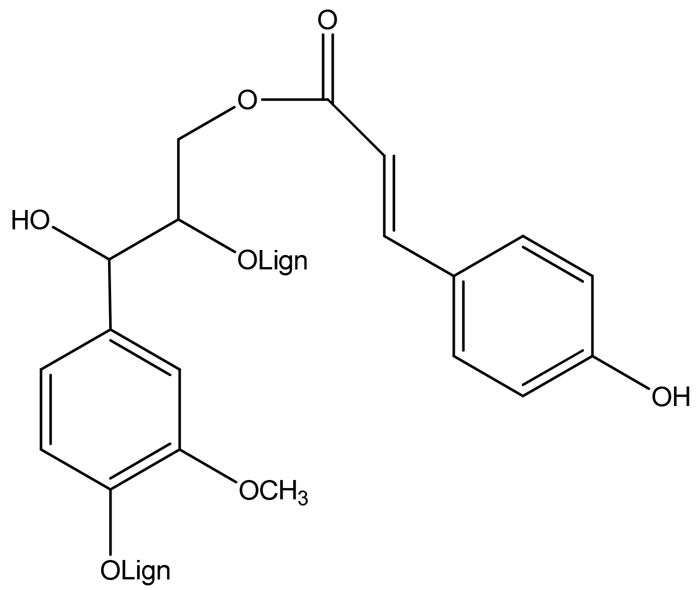
Comparison among yields, compositional evaluation, and morphological and chemical features of rice husk lignin specimens by gravimetric, GPC and ^{31}P NMR analyses.

	AL	AEL
Milling time (h)	**20**	**blended**
Yield (%)	**46.3**	**29.1**
Purity (Klason, %)	**86.0**	**77.9**
Ashes (%)	**< 2**	**< 2**
Carbohydrate (%)	**12.0**	**20.0**
GPC (g/mol)		
M_n	10200	13600
M_w	41000	115000
M_p	5100	4500
I	4.0	8.4
^{31}P NMR (mmol/g)		
Aliphatic -OH	3.03	3.71
Cond. PhOH + S-OH	0.23	0.13
G-OH	0.65	0.38
P-OH	0.65	0.14
COOH	0.27	0.59

COMPARISON AMONG ^{31}P NMR SPECTRA OF AL AND AEL SAMPLES

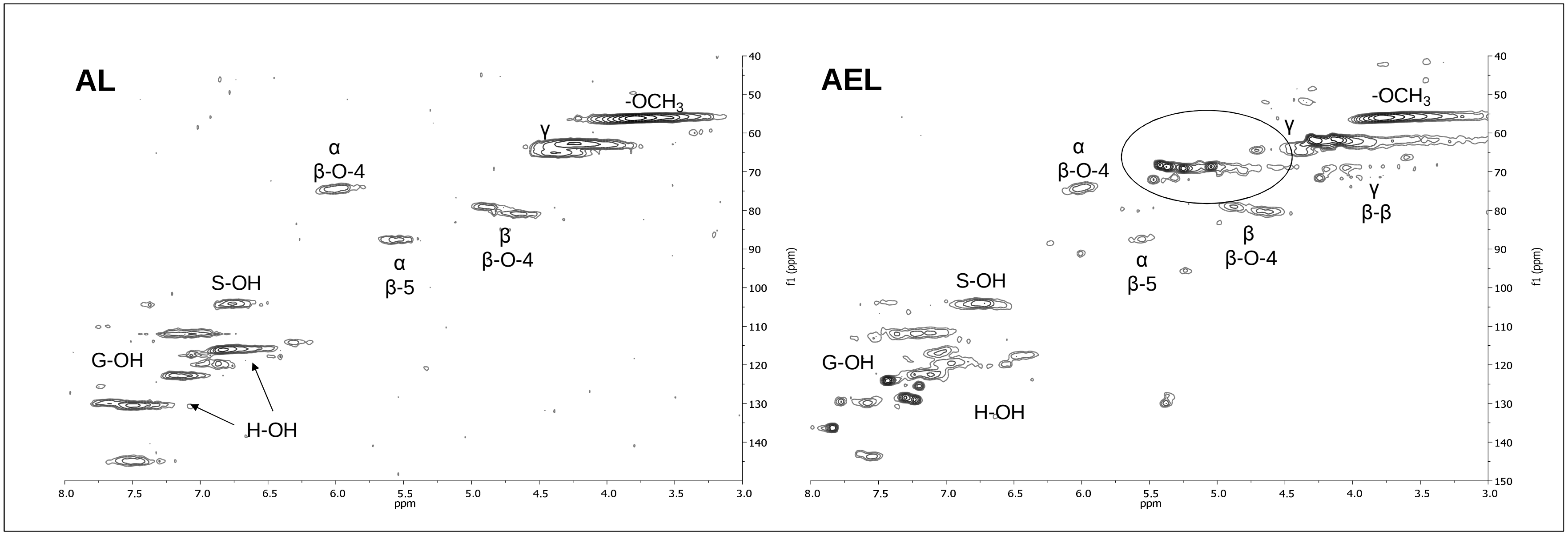


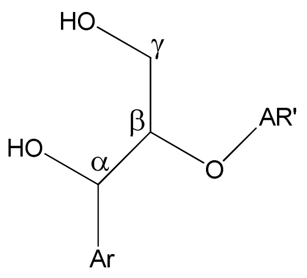
Ester bonds on wheat straw lignin terminal units



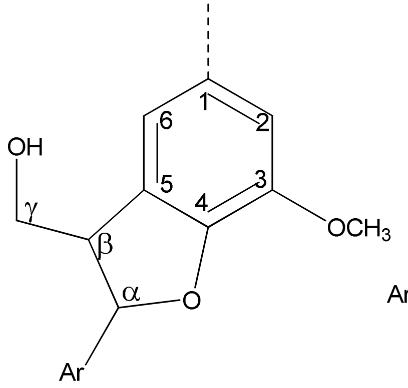
Crestini C.; Argyropoulos D.S. Structural Analysis of Wheat Straw Lignin by Quantitative ^{31}P and 2D NMR Spectroscopy. The Occurrence of Ester Bonds and β-O-4 Substructures. *J. Agric. Food Chem*. **1997**, 45, 1212-1219

2D-HSQC-NMR SPECTRA OF ACETYLATED AL AND AEL SAMPLES FROM RICE HUSK

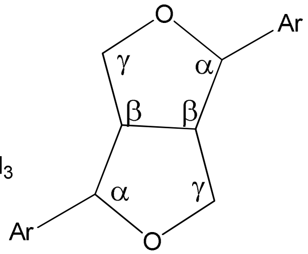




(β-O-4)

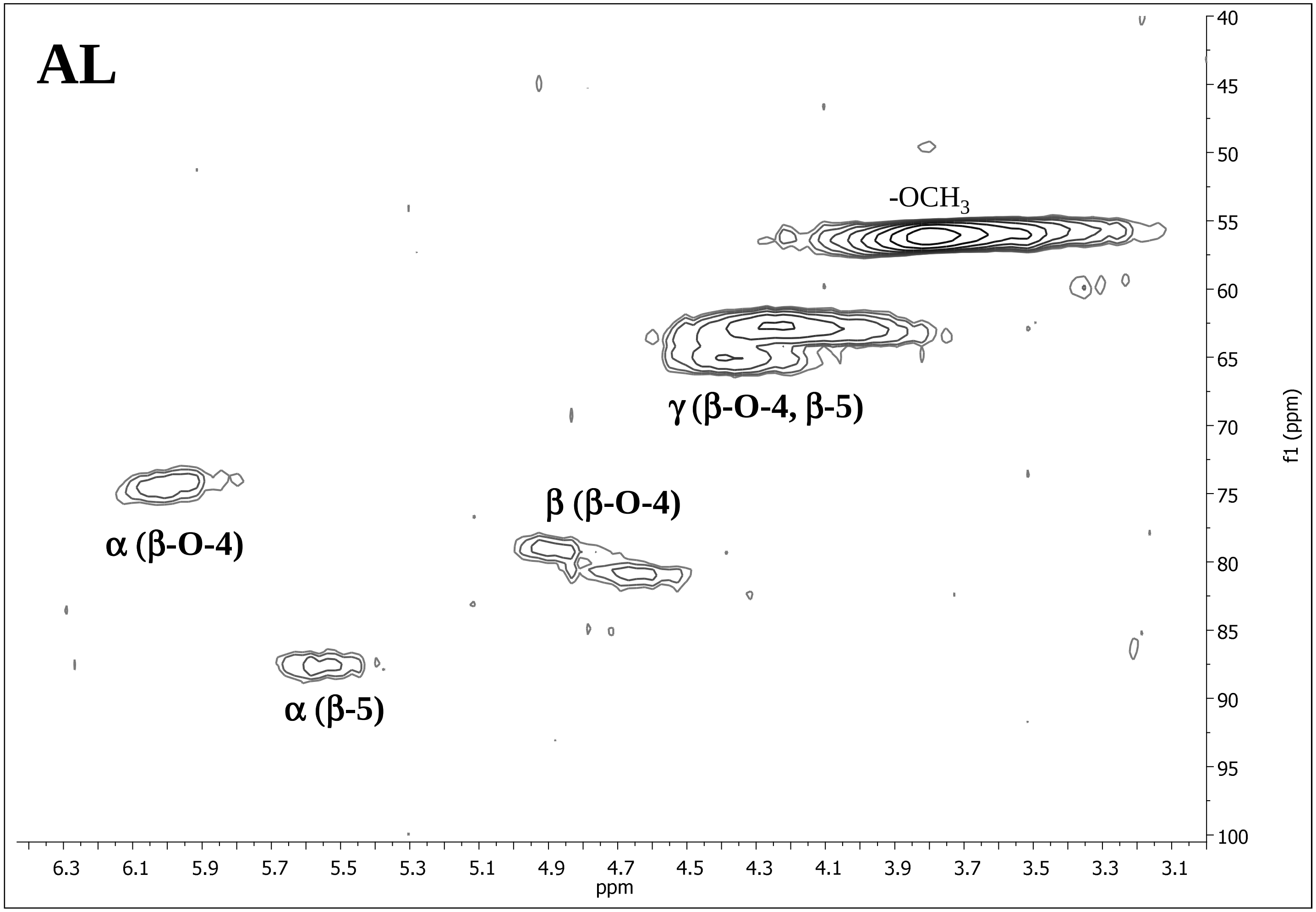


(β-5)

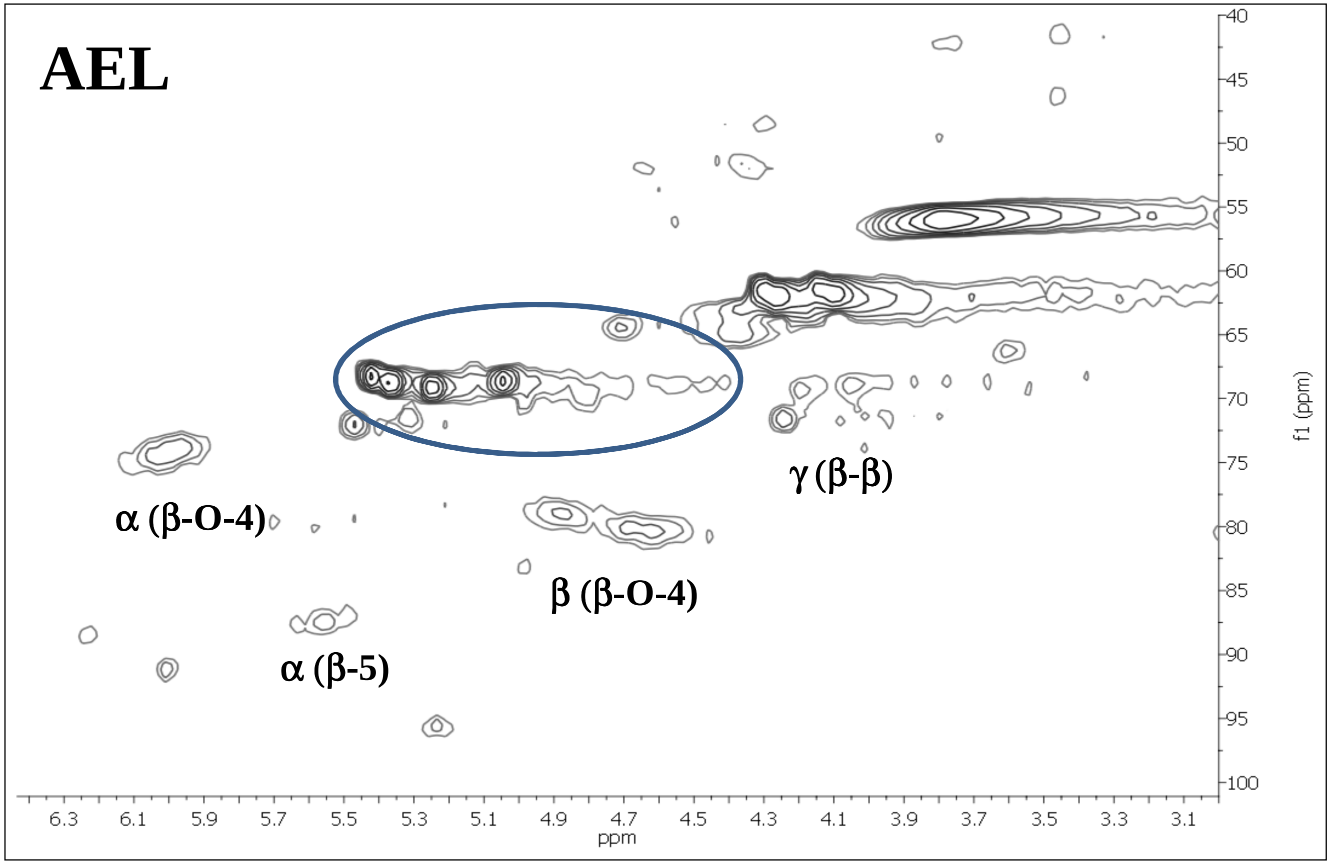


(β-β)

2D HSQC SPECTRUM OF AL ACETYLATED LIGNIN SAMPLE FROM RICE HUSK: INTERMONOMERIC BONDS AREA



2D HSQC SPECTRUM OF AEL ACETYLATED LIGNIN SAMPLE FROM RICE HUSK: INTERMONOMERIC BONDS AREA



CONCLUSIONS

•husk lignin is mainly formed by guaiacyl and p-hydroxyphenyl units, not depending by the applied extraction procedure, and by β-O-4 and β-5 intermonomeric bonds

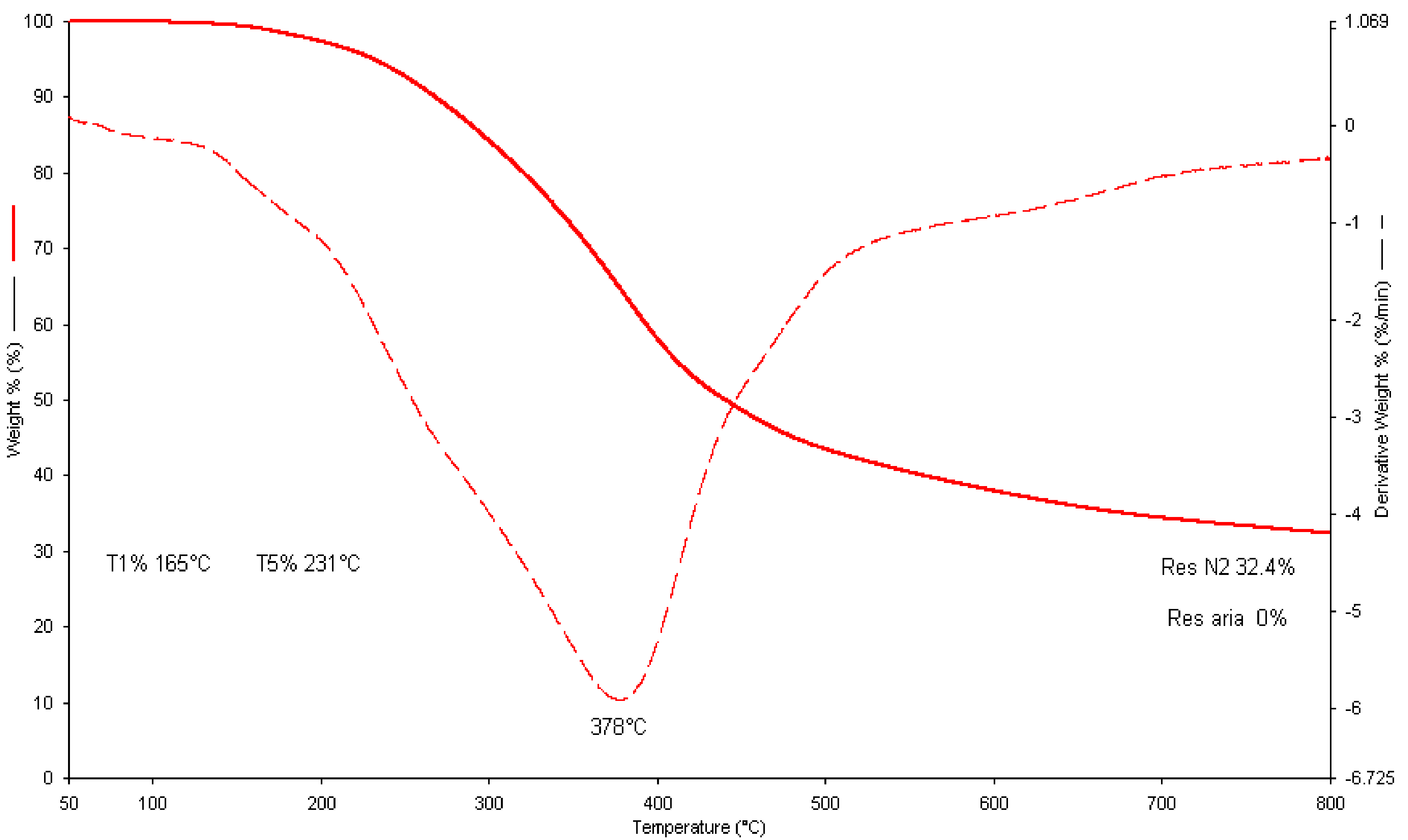
•AEL sample is characterized by a molecular weight distribution shifted toward higher molecular weight

•AEL sample is contaminated by the presence of residual carbohydrate

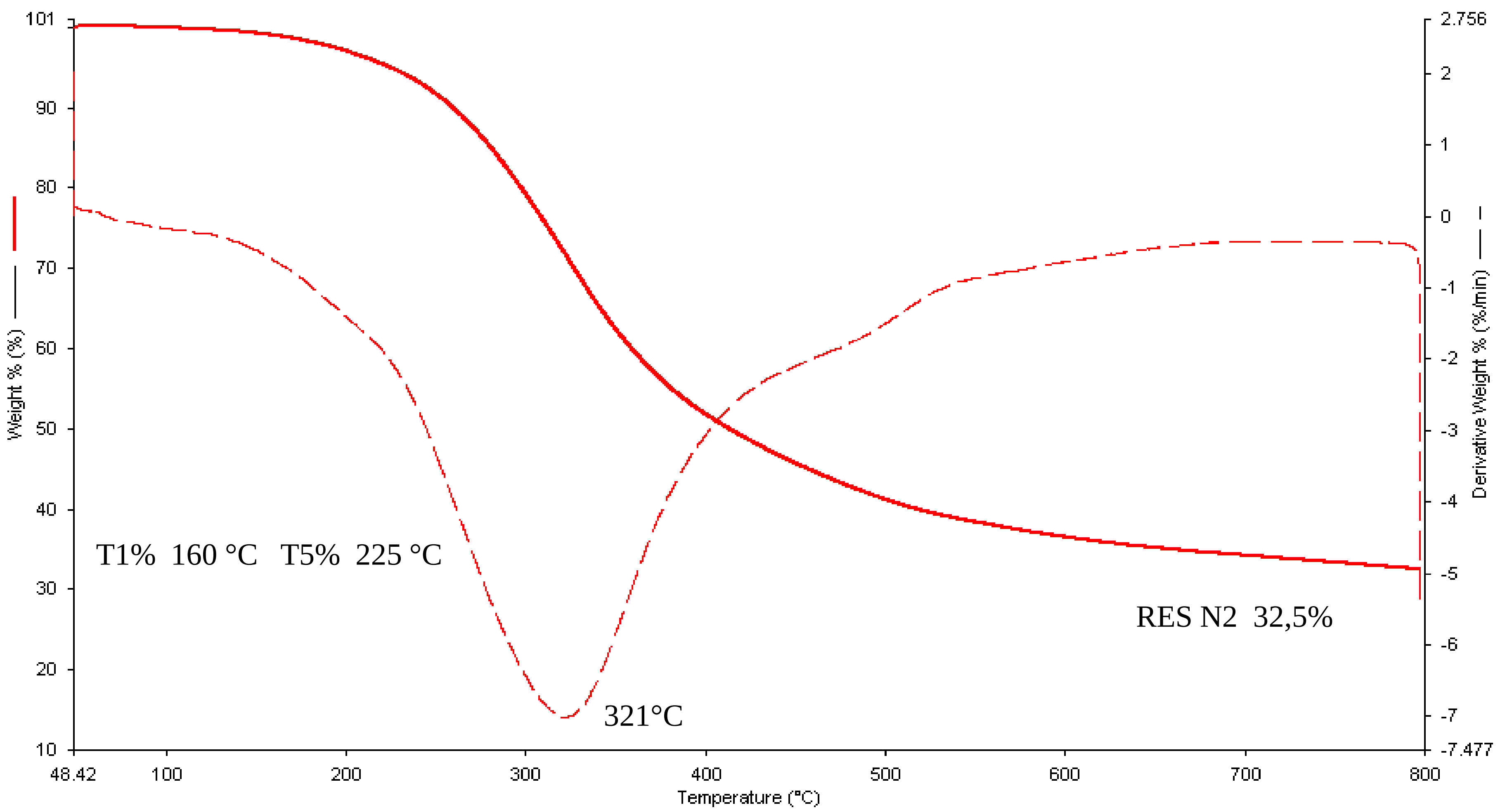
•AEL sample has lower amount of free phenolic groups

•In AEL lignin there are lignin-carbohydrate bond that the alkaline treatment is not able to cleave.

TGA/DTG OF RICE HUSK ACIDOLYSIS LIGNIN (AL)



TGA/DTG OF RICE HUSK ENZYMATIC ALKALINE LIGNIN (EAL)



ANTIOXIDANT ACTIVITY OF RICE HUSK

	IC_{50} (µg/mL) by DPPH radical scavenging activity	AAC By B-carotene bleaching test
Water extract	82.9	632
Ethanol extract	112.4	565
Acetone extract	195.2	503
AEL total	92.4	N.D
AEL > 10kDa	183.0	N.D
AEL < 10kDa	51.0	608
BHA reference	7.6	633

What is *Arundo donax* (Giant Cane)

- *Arundo donax* is a tall perennial cane growing in damp soils, either fresh or moderately saline

- *Arundo donax* is strong candidate for use as a renewable biofuel source because of its fast growth rate, ability to grow in different soil types and climatic conditions.

Comparison among yields, compositional evaluation, and morphological and chemical features of rice husk, Arundo donax and Wheat straw

	RH	AD	WS
Klason Lignin , %	26.0	22.8	16.6
Ashes, %	16.8	4.7	9.4
Extractives, %	4.7	1.7	2.9
Carbohydrates, %	52.5	70.8	71.1

Lignin			
Milling Time (h)	20	blended	blended
Yield, %	46.3	44.5	59.0
Purity, % (Klason)	86.0	> 85	> 85

Comparison among yields, compositional evaluation, and morphological and chemical features of rice husk, Arundo donax and wheat straw lignins, specimens by, GPC and ^{31}P NMR analyses

	RH	**AD**	**WS**
GPC (g/mol)			
M_n	10200	15000	10200
M_w	41000	81800	57200
M_p	5100	5000	4000
I	4.0	5.5	5.6
^{31}P NMR (mmol/g)			
Aliphatic -OH	3.03	3.42	4.35
Cond. PhOH + S-OH	0.23	0.29	0.32
G-OH	0.65	0.67	0.61
P-OH	0.65	0.43	0.53
COOH	0.27	0.29	0.15

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