

Characterization of lignin isolated from stone cells of 'Dangshansuli' pear native to China

Shutian Tao¹, Shaoling Zhang^{1*}, Jun Wu¹, Huaqing Wu¹, Shahrokh Khanizadeh², Qianwen Dong¹

1. Pear Engineering Research Centre, Nanjing Agricultural University, 1 Weigang, Nanjing, Jiangsu Province 210095, P.R.China; 2. Horticultural Research and Development Centre, Agriculture and Agri-Food Canada, 430 Gouin Blvd, St-Jean-sur-Richelieu, Quebec, J3B 3E6, Canada

Introduction

'Dangshansuli' pear (*Pyrus bretschneideri*) which accounts for 32% of the total area under cultivation in China is juicy and sweet, but it has a defect that great amount of stone cells formed by lignin deposition imparts a very gritty texture to the fruit, thus the low fruit quality.



Material and Methods

Fruit materials were harvested from the experimental orchard of Nanjing Agricultural University. Stone cells in the fruit were then isolated and estimated basing the method of Lee (2006)

Estimation of Lignin content was performed with acetyl bromide method. The amount of lignin was calculated from a linear calibration curve with commercial alkali lignin (Sigma-Aldrich, USA).

Lignin in stone cells was isolated and purified with method of Björkman (1954), and then applied to gel permeation chromatography (GPC), fourier transform infrared spectroscopy (FTIR), high performance liquid chromatography (HPLC), ¹H nuclear magnetic resonance (¹H-NMR) and ¹³C nuclear magnetic resonance (¹³C-NMR) for characterization analysis.



Result and Discussion

Proportion of stone cells in 'Dangshansuli' pear fruit was estimated up to 0.379% (Fresh Weight), in which 29.83% of the component was determined as lignin (Table1).

Table1. content of stone cells and lignin

Variety	Stone cell content (% FW)	Lignin content (%)
'Dangshansuli'	0.379	29.83

Typical composition of this lignin was determined as C, H and O, accounting for 58.38%, 5.97% and 34.18% respectively, with weight-average molecular weight (WMW) and molecular weight distribution (MWD) to be 1620g/mol and 2.6 by GPC (Table 2 and Fig.1).

Table2. Molecular weight of stone cell lignin

Variety	\overline{M}_w (g/mol)	\overline{M}_n (g/mol)	Max	Min	MWD
'Dangshansuli'	1620	700	7834	35	2.6

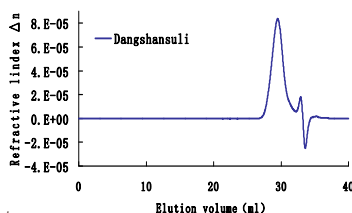


Fig. 1 Spectra of GPC. The smaller elution volume indicates the greater molecular weight

HPLC analysis of alkaline nitrobenzene oxidation products of this lignin showed vanillin was the predominant phenolic monomer, accounting for 60.77% of the products, followed by syringaldehyde (15.97%), which indicated the presence of great guaiacyl unit (Table 3 and Fig.2).

Table 3. The remain time, content and mols of the major degradation product of lignin

	Hydroxybenzoic acid	Vanillic acid	Syringaldehyde	Hydroxybenzaldehyde	vanillin	Syringic acid
Remain Time	5.087	6.229	6.753	7.824	10.328	12.481
Content%	2.97	13.48	15.97	1.51	60.77	5.26
Mols	2	8	9	1	40	3

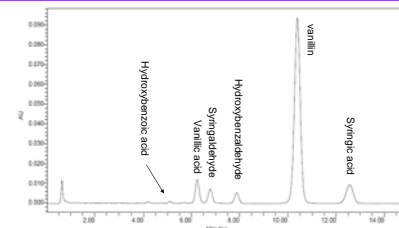


Fig.2 HPLC spectra of alkaline nitrobenzene oxidation products of stone cell lignin

Presence of guaiacyl and syringyl units in the lignin were also confirmed with the aid of FTIR (strong absorbance at $A_{1268}/A_{1228}=1.04$) and NMR (peaks δ 6.5~6.9ppm in ¹H-NMR spectra, δ 145~149ppm, δ 150~154ppm and δ 105~107ppm in ¹³C-DEPT¹³⁵ spectra).

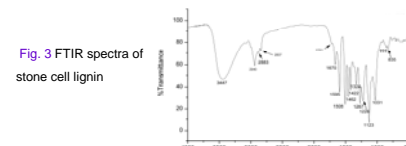


Fig. 3 FTIR spectra of stone cell lignin

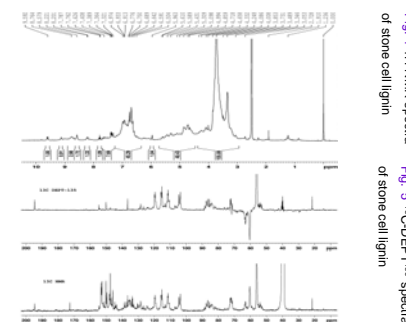


Fig. 4 1H-NMR spectra of stone cell lignin
Fig. 5 13C-DEPT135 spectra of stone cell lignin

Conclusion

lignin in pear stone cells was characterized as typical G-S lignin, however, the ratio for guaiacyl and syringyl unit was as high as 4, more closely to gymnosperm. This is speculated to result from the high temperature and humidity in the area of middle and lower reaches of Yangtse Rive, where the pear cultivated.