Chemical Analysis of Tree Barks using ATR-FTIR Spectroscopy and Conventional Techniques

Özlem Özgenç,[a,*] Sefa Durmaz,[b] and Süleyman Kuştaş [a]

ATR-FTIR spectroscopy and conventional analysis techniques were performed to characterize the chemical structure of different coniferous (cedar, fir, Calabrian pine, and spruce) and deciduous (chestnut, oak, alder, and beech) tree barks. The cell wall components (holocellulose and lignin) and extractives of tree barks were determined using conventional analysis methods. Chemical analysis indicated that the polysaccharide contents of tree barks were very low compared to lignin and extractives content. Substantial dissolution of tree barks was brought about by 1% NaOH. FTIR analysis method is an easy and reliable way to determine the functional groups of tree bark components. The levels of carbohydrates and lignin, as determined by ATR-FTIR spectral analysis, were consistent with the results of conventional analysis. The highest content of lignin was in the alder species for the deciduous trees and in the cedar type for the coniferous trees.

*Contact information: a: Department of Forest Industry Engineering, Faculty of Forestry, Karadeniz Technical University, 61080, Trabzon, Turkey; b: Department of Forestry, Kavaklıdere Vocational School, Muğla Sıtkı Koçman University, 48570, Muğla, Turkey; *Corresponding author: oozgenc@ktu.edu.tr*

INTRODUCTION

Tree bark, which is the one of the most abundant materials in nature, covers stems, branches, and roots to preserve cambium and prevent loss of water (Harkin and Rowe 1971). It is also an important forest residue that comprises about 10 to 20% of the total weight of trunk depending on growing conditions (Fengel and Wegener 1989). Tree bark, after peeling, is usually left to rot in the forest or is burnt for energy generation. However, the increasing demand for wood and decreasing forestlands are prompting a search for new resources. At this time, tree barks are drawing attention, and several studies have been carried out.

Bark is structurally different from wood. It has a heterogeneous structure, which causes the chemical composition of bark to be different depending on growing conditions, age, region, and sampling methods (Vázquez *et al.* 1987). It is well known that it has low content of carbohydrates as compared to wood. Besides this, tree bark is also rich in phenolic and extractives compounds (Vázquez *et al.* 1987; Kofujita *et al.* 1999). The extractives and carbohydrate content decreases, while lignin and phenolic compounds increase from inner bark to outer bark (Fengel and Wegener 1989; Hafızoğlu *et al.* 1997; Gönültaş and Balaban Uçar 2012).

Tree bark has been evaluated for lots of industrial applications in recent years. It has been widely used as a fuel (Albert *et al.* 2000; Li and Liud 2000; Sippula *et al.* 2007). Meanwhile, utilization of tree bark in ethanol production has been considered because of its sugar contents (Hu *et al.* 2008). Tannin-based resin from tree bark also can provide an

alternative to formaldehyde-based resin (Tondi and Pizzi 2009). Tree bark has attracted attention due to rich extractives content, which has potential use in natural wood preservatives (Onuorah 2000; Singh and Singh 2011; Tascioglu *et al.* 2013).

Chemical characterization of tree bark is necessary for effective evaluation owing to the chemical composition of tree bark, which is affected by environmental factors. The conventional methods for determination of the chemical structure of tree bark requires a long time and large amounts of samples that are subject to various difficulties. During the past 20 or so years, infrared spectroscopy has become a very effective tool in the analysis of wood components (Moore and Owen 2001). Infrared spectroscopic analysis is a simple technique for obtaining information and conducted chemical analysis (Pandey and Pitman 2003; Petrous *et al.* 2009; Poletti *et al.* 2012).

The non-extractable components in bark include polysaccharides (cellulose, hemicellulose, and pectic substances), phenolic polymers (lignin and high-molecular-weight tannins), and cross-linked polyesters (suberin and cutin). However, there are some challenges in identification of non-extractable components in bark by conventional chemical analysis. For example, substantial amounts of hemicellulose and pectins may be removed by extraction of tannins using alkaline solutions. With this dissolution, accurate analysis of non-extractable components is also very difficult. For this reason, in recent years, identification of non-extractable components in bark by conventional methods has become greatly unpopular. Conventional methods are being replaced by spectroscopic analysis methods (Sakai 2000).

The purpose of this study was to use chemical analysis methods to reveal the differences in eight different (four coniferous and four deciduous) tree barks according to TAPPI standards. It was also aimed to support the conventional analysis method by examining the chemical bond structure of these bark species via attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy.

EXPERIMENTAL

Preparation of Bark Samples

The tree barks were peeled off from 20- to 30-year-old cedar (*Cedrus libani* A. Rich), fir (*Abies nordmanniana* (Steven) Spach), Calabrian pine (*Pinus brutia* Tenore), chestnut (*Castanea sativa* Mill.), oak (*Quercus pontica* K. Koch), spruce (*Picea orientalis* (L.) Link), alder (*Alnus glutinosa* (L.) Gaertn.), and beech (*Fagus orientalis* Lipsky) trees that were cut down in Turkey. The barks were conditioned at 20 °C and 65% relative humidity until constant weight. They were then ground using a laboratory scale Willey mill to obtain 40- to 60-mesh wood powder samples (TAPPI T 257cm-85, 1985).

Determination of Klason lignin

The Klason lignin content of bark samples was determined by sulfuric acid methods (TAPPI T222 om-11 2011). The content of clone lignin was determined using two different methods, after only alcohol-benzene dissolution and after alcohol-benzene and NaOH %1 dissolution. The Klason lignin content for each bark sample was analyzed in triplicate.

Determination of NaOH Dissolution

The solubility of bark samples in NaOH was determined according to TAPPI T 212 om-98 (2002).

ATR-FTIR Spectroscopic Measurements

The ATR-FTIR spectra were recorded using a Thermo Nicolet iS50 FTIR spectrometer (Thermo Fisher Scientific Co., Waltham, MA, USA) equipped with a single-bounce diamond crystal and a deuterated triglycine sulfate (DTGS) detector. The ATR crystal contacted the wood powder so that it could absorb evanescent waves. The ATR spectrum obtained as a result of attenuated radiation resembles a conventional absorption spectrum (Khoshhesab 2012). The FTIR spectra of all wood samples were determined in the range of 4000 to 400 cm^{-1} with a resolution of 4 cm^{-1}. Each spectrum was collected after 32 scans in the absorbance mode. The ATR-FTIR spectra of the wood samples were analyzed using the OMNICTM software (Thermo Electron Corporation, Madison, WI, USA). The peak heights constructed by connecting the lowest data points on either side of the peak were measured from the baseline. The intensities of the lignin-associated band were rationed against the carbohydrate band in untreated and heat-treated samples for the decay test (Pandey and Nagvani 2007).

RESULTS AND DISCUSSION

Conventional Analysis

The chemical compositions of eight different tree barks are given in Table 1. The contents of the main cell wall components of the tree barks varied depending on species, as well as growing conditions, region, and age. However, the differences in the ratios of cell wall components were noteworthy in comparison to wood. As known, tree bark generally has high extractives and lignin content and relatively low holocellulose (Vázquez *et al.* 1987; Hafızoğlu and Usta 2005; Valentín *et al.* 2010).

Conventional analysis methods may cause incorrect results because of tree barks' chemical structure (Fengel 1989). In particular, phenolic components dissolve in sulfuric acid, which causes an increase in lignin content in tree barks (Solar and Kačik 1993). As may be seen in Table 1, the lignin content of the tree barks was significantly high.

Table 1. Chemical Composition of Different Tree Barks (%)

Species	Holocellulose	Lignin*	Lignin**	Extractives	NaOH 1% Dissolution
Calabrian pine	34.91	37.64	19.23	19.02	50.63
Fir	50.13	28.05	20.53	17.01	25.54
Spruce	46.95	20.44	11.70	22.50	48.50
Cedar	40.95	40.36	23.75	13.02	43.21
Alder	51.96	45.78	33.55	9.23	37.63
Chestnut	51.48	25.23	14.55	15.20	46.13
Beech	63.52	32.87	24.25	5.50	26.93
Oak	54.87	23.88	15.45	12.50	39.79
*Klason lignin content after the alcohol-benzene dissolution.					
**Klason lignin content after the alcohol-benzene and NaOH 1% dissolution.					

bioresources.com

Different solvents such as water, toluene, ethanol, benzene, hexane, and 1% NaOH are utilized to remove extractives from wood (Fengel 1989). Tree barks are rich in extractives, which protect the stem against biological threats. According to the results, solubility of the tree barks in the solvents was relatively high, which enabled evaluation of the extracts of tree barks for different parameters such as nutrition, health, and protection (Jerez *et al.* 2007).

Moreover, it is well known that phenols can be dissolved in a NaOH solution. After the alcohol-benzene and NaOH dissolution, there was a remarkable decrease in the lignin content of the tree barks. Accurate lignin contents of the tree barks, which are similar to wood, were obtained with removal of phenolic acids from the wood structure after the NaOH dissolution.

After only alcohol-benzene and alcohol-benzene and NaOH 1% dissolution, the highest lignin content in a coniferous tree was found in the cedar bark (40.36%) while the highest content in a deciduous tree was determined in the alder bark (45.78%). On the other hand, the highest holocellulose content in a coniferous tree was found in the fir bark (50.13%), while the highest content in a deciduous tree was determined in the beech bark (63.52%). Among all species, the extractives were determined to be maximal in spruce (22.50%) and minimal in beech bark (5.50%).

ATR-FTIR Spectroscopy Analysis

FT-IR spectroscopy has been often utilized to examine the chemical structure of tree barks because it is a non-destructive, fast, and reliable technique (Pandey and Pitman 2003; Petrou *et al.* 2009).

Common lignin bands were also seen in the 1400 to 1700 cm^{-1} region, as shown in Table 2.

Table 2. Characteristic FT-IR Bands of Tree Bark Components

Wavenumber cm^{-1}	Assignment	Attributed to	Reference
1736	C=O stretch of acetyl and carbonyl groups	Hemicellulose	a, b, c
1603-1608	Aromatic skeletal and C=O stretch vibration	Lignin	d, c, b
1508-1510	C=C stretching of aromatic skeletal vibration	Lignin	d, c, e, b, h
1450-1453	C=C and C-H bond. O-H in plane deformation	Lignin and Hemicellulose	f, c, g, c, b, h
1369-1372	C-H deformation vibration	Cellulose	h, c, e, k
1315-1317	CH$_2$ rocking vibration	Cellulose	h, c, k
1264	G-ring plus C=O stretch (1268)	G-Lignin	h, c, e, k
1224	Syringyl ring and C-O stretch	Lignin and Xylan	a, g, k
1157-1159	C-O-C symmetric stretching	Cellulose and Hemicellulose	f, e, j, h
1101-1102	Ring asymmetric valence vibration	Polysaccharides	h, k
1024-1026	C-O stretch	Cellulose and Hemicellulose	g, e, l, k
893-895	Aromatic C-H out of plane deformation	Cellulose, hemicellulose, and pectin	h, f, e, b, k
a: Popescu *et al.* 2010; b: Naumann *et al.* 2005; c: Pandey 1999; d: Rowell 2012; e: Pandey 2007; f: Pandey 2003; g: Mohebby 2005; h: Özgenc *et al.* 2017; i: Kacurakova *et al.* 2000; j: Martín *et al.* 2005; k: Durmaz *et al.* 2016			

The bands at 1508 to 1510 and 1603 to 1608 cm^{-1} were recognized as the characteristic lignin peaks, which contain aromatic skeletal vibrations of benzene rings (Pandey 1999). However, the higher amounts of extractives in the tree barks might have contributed to the absorption to bands at around 1508 to 1510 cm^{-1} due to benzoic acids in the wood tannins comprising aromatic rings (Poletto *et al.* 2012). For this reason, peak heights may be observed as much higher than the actual. This may be misleading for the results. This is why this issue should be taken into consideration during interpretation of the findings.

There were various bands related to the main tree bark components in the "fingerprint" region. The specific polysaccharide bands were determined in the 890 to 1400 cm^{-1} region where a stretch of side groups (C-OH) and (C-O-C) glycosidic bond vibrations were dominant. As shown in Figs. 1 and 2, FT-IR analysis was performed to determine the characteristic plant cell wall bands from the tree barks in the fingerprint region (1800 to 800 cm-1) and support the conventional analysis results.

As seen in the figures, the spectra of tree barks were nearly similar, except for intensity of peaks, which shows the differences in the cell wall chemistry of the tree barks. The lignin and carbohydrate related peak heights are also shown in Tables 3 and 4. The peaks at 1157-1159 and 1369-1373 cm^{-1} were characteristic carbohydrate bands, and they were assigned to C-O-C symmetric stretching and C-H deformation vibration, respectively. The peak intensity may be related to the chemical bond density in the chemical structure of tree barks (Pandey 1999).
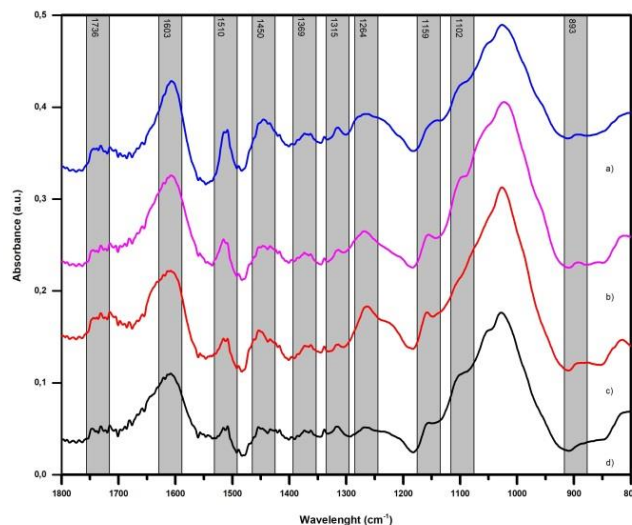

Fig. 1. FT-IR spectra of the coniferous species' tree barks; a) cedar, b) fir, c) Calabrian pine, d) spruce
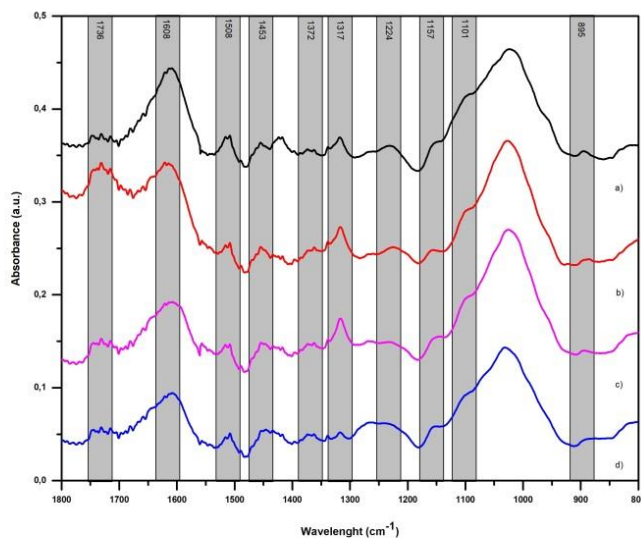
Fig. 2. FT-IR spectra of the deciduous species' tree barks; a) alnus, b) beech, c) oak, d) chestnut.

Table 3. Lignin and Carbohydrate Related Peak Heights of Coniferous Species

Species	1603 cm⁻¹	1510 cm⁻¹	1159 cm⁻¹	1102 cm⁻¹
Cedar	0.0847	0.0470	0.0121	0.0078
Fir	0.0735	0.0315	0.0184	0.0142
Calabrian Pine	0.0665	0.0281	0.0102	0.0021
Spruce	0.0514	0.0227	0.0151	0.0119

Table 4. Lignin and Carbohydrate Related Peak Heights of Deciduous Species

Species	1608 cm⁻¹	1508 cm⁻¹	1157 cm⁻¹	1101 cm⁻¹
Alder	0.6940	0.0246	0.0071	0.0090
Beech	0.0531	0.0208	0.0090	0.0101
Chestnut	0.0392	0.0186	0.0077	0.0059
Oak	0.0400	0.0200	0.0085	0.0099

Therefore, the intensities of peaks related to lignin decreased for the coniferous tree species of cedar, fir, Calabrian pine, spruce, respectively and the deciduous tree species of alder, beech, oak, and chestnut, respectively. On the other hand, the highest peaks for holocellulose were obtained from fir, spruce, cedar, and Calabrian pine, respectively, among the coniferous tree species, and beech, oak, chestnut, and alder for deciduous tree species.

CONCLUSIONS

1. Bark is the most common waste in nature. Its cell wall components are similar to wood, while the amounts of these components vary. The two methods used to characterize tree barks of four different coniferous and four different deciduous species were found to be appropriate for evaluation of the differences in the structures of the bark components.

2. High amounts of lignin and extractives content in tree barks have attracted attention. It is well-known that tree bark has low holocellulose content. Low sugar is also important in evaluation of tree barks. In this study alder tree bark was found to have the highest lignin content before and after the NaOH 1% dissolution procedure, while the lowest content was found in the spruce tree bark. Therefore, the highest holocellulose content was in beech, while the lowest content was in Calabrian pine tree bark. Additionally, the highest extractives content was found to be in the spruce tree bark while the lowest was found to be in beech.

3. The high NaOH 1% solubility greatly affects the amount of cell wall components determined by conventional methods. Especially the lignin content of the cell wall observed to be much higher than it actually was.

4. This study used ATR-FTIR spectroscopy to identify chemical differences among eight different tree barks. The highest peaks in the lignin bands of 1508-1510 and 1608-1610 were identified in the alder bark among the deciduous trees and in the cedar bark among the coniferous trees. In the 1157-1159 cm^{-1} cellulose and hemicellulose and 1101-1102 cm^{-1} polysaccharide bands, the highest peak was found in the fir bark among the coniferous trees, and in the beech bark among the deciduous trees.

5. The ATR-FTIR spectroscopy analysis was demonstrated to be a fast, reliable, and easy method for determination of functional groups in tree bark components. However, organic extractives may be absorbed, and this may cause misinterpretation of results. Therefore, it is necessary to make an evaluation based on consideration of this issue.

ACKNOWLEDGMENTS

REFERENCES CITED

Albert, R. M., Weiner, S., Bar-Yosef, O., and Meignen, L. (2000). "Phytoliths in the middle palaeolithic deposits of Kebara Cave, Mt Carmel, Israel: Study of the plant materials used for fuel and other purposes," *Journal of Archaeological Science* 27(10), 931-947. DOI: 10.1006/jasc.2000.0507

Durmaz, S., Özgenç, Ö., Boyacı, İ. H., Yıldız, Ü. C., and Erişir, E. (2016). "Examination of the chemical changes in spruce wood degraded by brown-rot fungi using FT-IR and FT-Raman spectroscopy," *Vibrational Spectroscopy* 85, 202-207. DOI: 10.1016/j.vibspec.2016.04.020

Fengel, D., and Wegener, G. (eds.). (1989). "Wood: Chemistry, ultrastructure, reactions," Walter de Gruyter.

Gönültaş, O., and Balaban Uçar, M. (2012). "Tannin composition of stone pine (*Pinus pinea*)," *KSU, Journal of Natural Science*, Special Issue, 80-84.

bioresources*.com*

Hafızoğlu, H., Usta, M., and Bilgin, Ö. (1997). "Wood and bark composition of *Picea orientalis* (L.) Link," *Holzforschung* 51, 114-118. DOI: 10.1515/hfsg.1997.51.2.114

Hafızoğlu, H., and Usta, M. (2005). "Chemical composition of coniferous wood species occurring in Turkey," *Holz als Roh- und Werkstoff* 63, 83-85. DOI: 10.1007/s00107-004-0539-1

Harkin, J. M., and Rowe, J. W. (1971). "Bark and its possible uses," Forest Products Laboratory, Forest Service U.S. Department of Agriculture.

Hu, G., Heitmann, J. A., and Rojas, O. J. (2008). "Feedstock pretreatment strategies for producing ethanol from wood, bark, and forest residues," *BioResources* 3(1), 270-294. DOI:10.15376/biores.3.1.270-294

Jerez, M., Selga, A., Sineiro, J., Torres, J. L., and Nùnez, M. J. (2007). "A comparison between bark extracts from *Pinus pinaster* and *Pinus radiata*: Antioxidant activity and procyanidin composition," *Food Chemistry* 100, 439-444. DOI: 10.1016/j.foodchem.2005.09.064

Kacurakova, M., Capek, P., Sasinkova, V., Wellner, N., and Ebringerova, A. (2000). "FT-IR study of plant cell wall model compounds: Pectic polysaccharides and hemicelluloses," *Carbohydrate Polymers* 43(2), 195-203. DOI:10.1016/S0144-8617(00)00151-X.

Kofujita, H., Ettyu, K., and Ota, M. (1999). "Characterization of the major components in bark from five Japanese tree species for chemical utilization," *Wood Science and Technology* 33(3), 223-228. DOI: 10.1007/s002260050111

Khoshhesab, Z.M. (2012). *Reflectance IR Spectroscopy*, Intech Open Access Publisher.

Martín, J. A., Solla, A., Coimbra, M. A., and Gil, L. (2005). "Metabolic distinction of *Ulmus minor* xylem tissues after inoculation with *Ophiostoma novo-ulmi*", *Phytochemistry,* 66(20), 2458-2467. DOI:10.1016/j.phytochem.2005.08.004.

Mohebby, B. (2005). "Attenuated total reflection infrared spectroscopy of white-rot decayed beech wood," *International Biodeterioration & Biodegradation* 55, 247-251, DOI:10.1016/j.ibiod.2005.01.003.

Moore, A. K., and Owen, N. L. (2001). "Infrared spectroscopic studies of solid wood," *Applied Spectroscopy Reviews* 36(1), 65-86.

Naumann, A., Navarro-González , M., Peddireddi, S., Kües, U., and Polle, A. (2005). "Fourier transform infrared microscopy and imaging: Detection of fungi in wood," *Fungal Genetics and Biology* 42, 829-835. DOI:10.1016/j.fgb.2005.06.003.

Onuorah, E. O. (2000). "The wood preservative potentials of heartwood extracts of *Milicia excelsa* and *Erythrophleum suaveolens*," *Bioresource Technology* 75(2), 3-171.

Özgenç, Ö., Durmaz, S., Boyaci, I. H., and Eksi-Kocak, H. (2017). "Determination of chemical changes in heat-treated wood using ATR-FTIR and FT Raman spectrometry," *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* 171, 395-400. DOI: 10.1016/j.saa.2016.08.026

Pandey, K. K. (1999). "A study of chemical structure of soft and hardwood and wood polymers by FTIR spectroscopy," *Journal Applied Polymer Sci.* 71(12), 1969-1975. DOI: 10.1002/(SICI)1097-4628(19990321)71:12<1969::AID-APP6>3.0.CO;2-D

Pandey, K. K., and Pitman, A. J. (2003). "FTIR studies of the changes in wood chemistry following decay by brown-rot and white-rot fungi," *International Biodeterioration & Biodegradation* 52(3), 151-160. DOI: 10.1016/S0964-8305(03)00052-0

Pandey, K. K., and Nagveni, H. C. (2007). "Rapid characterisation of brown and white rot degraded chir pine and rubberwood by FTIR spectroscopy," *Holz Roh Werkst* 65, 477-481. DOI: 10.1007/s00107-007-0181-9

Petrou, M., Edwards, H. G., Janaway, R. C., Thompson, G. B., and Wilson, A. S. (2009). "Fourier-transform Raman spectroscopic study of a Neolithic waterlogged wood assemblage," *Analytical and Bioanalytical Chemistry* 395(7), 2131-2138. DOI: 10.1007/s00216-009-3178-x

Poletto, M., Zattera, A. J., and Santana, R. M. C. (2012). "Structural differences between wood species: Evidence from chemical composition, FTIR spectroscopy, and thermogravimetric analysis," *Journal of Applied Polymer Science* 126 (1), 337-344. DOI: 10.1002/app.36991

Popescu, C. M., Popescu, M. C., and Vasile, C. 2010. "Characterization of fungal degraded lime wood by FT-IR and 2D IR correlation spectroscopy," *Microchemical Journal* 95(2), 377-387, DOI:10.1016/j.microc.2010.02.021.

Rowell, R. M. (ed.). (2012). *Handbook of Wood Chemistry and Wood Composites*, CRC Press.

Singh, T., and Singh, A. P. (2011). "A review on natural products as wood protectant," *Wood Science and Technology* 46(5), 70-851. DOI: 10.1007/s00226-011-0448-5

Sippula, O., Hytönen, K., Tissari, J., Raunemaa, T., and Jokiniemi, J. (2007). "Effect of wood fuel on the emissions from a top-feed pellet stove," *Energy & Fuels* 21(2), 1151-1160. DOI: 10.1021/ef060286e

Solar, R., and Kačik, F. (1993). "Comparative study of Caribbean pine (*Pinus caribaea* L.) wood and bark dioxane lignin," *European Journal of Wood and Wood Products* 51(5), 347-352. DOI: 10.1007/BF02663807

Tascioglu, C., Yalcin, M., Sen, S., and Akcay, C. (2013). "Antifungal properties of some plant extracts used as wood preservatives," *International Biodeterioration & Biodegradation* 85, 23-28. DOI: 10.1016/j.ibiod.2013.06.004

TAPPI T 212 om-98 (2002). "One percent sodium hydroxide solubility of wood and pulp," TAPPI Press, Atlanta, GA.

TAPPI T 222 om-11 (2011). "Acid-insoluble lignin in wood and pulp," TAPPI Press, Atlanta, GA.

TAPPI T 257 cm-85 (1985). "Sampling and preparing wood for analysis," TAPPI Press, Atlanta, GA.

Tondi, G., and Pizzi, A. (2009). "Tannin-based rigid foams: Characterization and modification," *Industrial Crops and Products* 29(2), 356-363.

Valentín, L., Kluczek-Turpeinen, B., Willför, S., Hemming, J., Hatakka, A., Steffen, K., and Tuomela, M. (2010). "Scots pine (*Pinus sylvestris*) bark composition and degradation by fungi: Potential substrate for bioremediation," *Bioresource Technology* 101(7), 2203-2209. DOI: 10.1016/j.indcrop.2008.07.003

Vázquez, G., Antorrena, G., and Parajó, J. C. (1987). "Studies on the utilization of *Pinus pinaster* bark," *Wood Science and Technology* 21(2), 155-166. DOI: 10.1007/BF00376195