CHEMICAL CHARACTERISTICS OF THE CONE AND WOOD OF *PINUS PINEA*

Oktay Gonultas[a] and Mualla Balaban Ucar[a,*]

The cell wall components and solubility characteristics of *Pinus pinea* cone and wood fibers harvested from three regions in Turkey were determined. The ash content of the cones was higher than that of the wood fibers, almost similar to the ash content of softwood bark. The total extractives that were generated by successive extractions were approximately 23 to 30% of the weight of the cones. Both the cones and wood fibers contained almost equal amounts of holocellulose. However, the alpha-cellulose content was higher in the wood samples. The cones contained more acid-soluble lignin than did the wood, but after 1% NaOH-extraction, the Klason lignin content in cones was lower than that in wood. With the exception of those from the Coruh region, the chemical analysis of the cones from other regions gave almost identical results, and the values of the measured components in the wood exhibited only small differences.

Keywords: Pinus pinea; Cone; Wood; Chemical components

*Contact information: a: Department of Wood Chemistry, Faculty of Forestry, Istanbul University, 34473, Bahcekoy, Istanbul, Turkey; *Corresponding author: mbalaban@istanbul.edu.tr*

INTRODUCTION

Pinus pinea (stone pine) is one of the five native pine species growing in Turkey. This species is spread throughout the Mediterranean region from Portugal up to Syria and has a wide distribution around the Bergama-Kozak, Aydin, and Mugla provinces of Turkey. Some small stands can be encountered in Manavgat, Gemlik, Onsen Kahramanmaras, Kalenema Trabzon, and in the valleys of the Coruh river in Artvin (Ansin and Ozkan 1997).

After the removal of the seeds, called pine nuts, the remaining cones are used as fuel or in traditional arts and crafts making. The pine nuts are one of the most important non-wood forest products, as they have export value as well. The nuts contribute significantly to the income of forest village residents. *Pinus pinea* has also been widely planted in many regions of Turkey as an important wood-producing species for private forestry (Bozali *et al.* 2006).

Cone formation in the trees begins around the ages of 13 to 15 years and continues until the ages of 80 to 100 years. However, the cone yield decreases considerably during the latter years (GDF, 1991).

The available literature provides a wealth of information on the chemical composition of different pine cones. The carbohydrate composition of some pine cones was determined by Michales *et al.* (1994). Eberhardt and Young (1996) investigated the

lignin and polysaccharides from some pine cones. Ucar and Ucar (2008) studied the main components and the lipophilic extractives of old and fresh black pine cones.

Recently, the polysaccharide composition of some pine cones grown in Turkey was studied by Kilic *et al.* (2010). The same authors investigated the phenolic compounds of some Turkish coniferous species, including the extractive composition of some pine cones. However, there is still a lack of knowledge about the main components (lignin, acid soluble lignin, alpha cellulose, and extractive content) of several pine cones grown in Turkey. The annual residue of stone pine cones was estimated to have reached some six thousand metric tonnes per year. As stone pine cones possess an appreciable potential in Turkey, the objective of this work was to investigate the chemical composition of the fibers from the cone and the wood of *Pinus pinea*.

EXPERIMENTAL

The cone and wood samples of *Pinus pinea* were obtained from Bursa-Armutlu Muftuciftligi, Artvin-Coruh Cerattepe and Izmir-Bergama Kozak Gobeller, Asagicuma. Three year-old cones were collected from five trees in each region. The trees (55, 126, and 30 years-old) were felled and three discs were taken from the bottom, center and top of each stem. The cones were spread and stored at room temperature until the brackets had opened up, and the seeds were released and removed.

The wood and cone samples were ground in a Wiley mill and sieved, and 40 to 100 mesh-fractions were used for the analysis. The ash content was determined according to the Tappi method T-211 om-85. The extraction was performed in a Soxhlet system according to the Tappi method T-204 om-88. In order to determine the total extractive content, this method was repeated using several different solvents in succession. The solubilities in hot water and in 1% NaOH were determined according to Tappi methods T-207 om-88 and T-212 om-88. The delignification process as defined by Jayme (1942) and Wise *et al.* (1946) was applied in order to determine the chlorite holocellulose portion, and this was done by adjusting the active chlorine content of the $NaClO_2$-solution (ca. 20%) iodometrically.

The acid-soluble lignin content was determined spectroscopically according to the Tappi method UM-250, and the acid-insoluble lignin content was estimated, according to the method of Runkel and Wilke (1951), using a 72% sulfuric and 40% hydrobromic acid mixture. An assay of alpha-cellulose was performed according to Tappi test methods T-203.

RESULTS AND DISCUSSION

The cell wall components and solubility characteristics of the stone pine cones are given in Table 1. While the ash content of the cones was close to that of the bark of softwoods, these values for the stone pine wood were much lower (Table 5). A comparison of the hot water and 1 % NaOH solubilities of stone pine and black pine cones showed that stone pine cones had a higher solubility (black pine cones showed

solubilities of 5.6 % in hot water and 18.7% in 1% NaOH) (Ucar and Ucar 2008). The hot water solubility of the stone pine cones was almost twice the average hot water solubility value of the black pine cones. This is perhaps a distinctive feature of the species *Pinus pinea*. The results further showed that the extractive content of the cones was somewhat lower in those taken from the Coruh region, whereas the extractive content of the other samples differed only slightly from each other.

Table 1. Ash content and Solubilities of *Pinus pinea* Cones

District	Ash+ (%)	Solubility+					
		Hot Water (%)	1% NaOH (%)	Et-OH/ Cyclo-hexane (%) -I-	Et-OH (%) -II-	1% NaOH (%)* -III-	Total I+II+III
Coruh I	0.81	8.60	20.01	5.82	0.69	16.30	22.81
Coruh II	1.32	10.65	21.93	5.19	0.92	18.32	24.43
Armutlu I	1.49	13.38	27.46	7.33	1.50	20.67	29.50
Armutlu II	1.34	11.75	26.05	7.53	1.54	19.08	28.15
Kozak I	1.31	11.08	27.31	6.57	0.98	21.40	28.95
Kozak II	1.40	13.42	27.20	7.28	1.19	20.74	29.21

+ based on oven-dry original material, * 1 % NaOH extraction conducted after successive Et-OH/cyclohexane and Et-OH extractions

In Table 1, two kinds of 1 % NaOH solubility values are listed: those collected using original material and those collected using the material pre-extracted with ethanol-cyclohexane followed by ethanol. Since some material had already been removed with organic solvents during pre-extractions, the 1 % NaOH solubility dropped after the subsequent extraction. By adding this particular NaOH solubility value to the Et-OH/cyclohexane and Et-OH values, the total extractible material in *Pinus pinea* cones was calculated (column 8, Table 1). Approximately 23 to 30% of the cones were composed of extractive substances. A remarkable difference was observed in the cones from the Coruh region regarding 1% NaOH solubility and total extractives.

The cones may have contained some flavonoids, *i.e.*, condensed tannins that could not be entirely removed by standard ethanol-benzene extractions. This kind of acid-insoluble substance would interfere with lignin determination. Therefore, a determination of lignin was also conducted using the extractive-free cone material that was extracted with 1% NaOH. The results of the determinations of lignin in the cones are summarized in Table 2. As can be seen, all cone samples contained larger amounts of residual lignin when the material was prepared by organic solvent extraction only. An additional extraction step involving 1% NaOH caused a drop in the acid-insoluble residue, indicating the dissolution of some material, in particular phenolic compounds, by alkali treatment. Since the phenolic compounds, like tannins, can condense and remain with lignin in acidic conditions, it was likely that the Klason lignin content would be somewhat overestimated if the cone material were prepared without an additional 1% sodium hydroxide extraction. The lignin contents determined after three extraction steps would be considered to be possibly true lignin values. As can be seen from Table 2, the lignin

contents were close to those of hardwoods but notably lower than those of softwoods. Eberhardt and Young (1996) examined the chemical compositions of different pine cones and stated that the high lignin contents would contribute to the durability of the cones.

Furthermore, a comparison of the acid-soluble lignin contents of the cones and wood showed that the values were higher for cones than they were for softwoods (0.2 to 0.5% higher), but they were much lower than those of hardwoods (3 to 5% lower). Eberhardt and Young (1996) studied the precursors of pine cone lignin and found that the lignin of the cones had the same precursors, guaiacyl and hydroxyphenyl, as did the softwoods.

The total lignin yield was estimated by adding the corrected residual lignin to the acid-soluble lignin values. As can be seen from the Table 2, there isn't so much variation in the total lignin yields from region to region.

Table 2. Klason and Acid-Soluble Lignin Content in Cones (Based on Oven-Dried, Extracted Cones)

District	Klason Lignin I	Acid-Soluble Lignin II	Klason Lignin after 1% NaOH extraction III	Total Lignin A I+II	Total Lignin B II+III
Coruh I	33.47	1.19	25.44	34.66	26.63
Coruh II	33.52	1.04	24.68	34.56	25.72
Armutlu I	36.90	1.56	24.32	38.46	25.88
Armutlu II	35.47	1.32	24.57	36.79	25.89
Kozak I	37.23	1.34	24.72	38.57	26.06
Kozak II	36.25	1.46	24.79	37.71	26.25

A comparison of the lignin content of some pine cones is given in Table 3. As can be seen from Table 3, the Klason lignin content of stone pine cones was similar to that of other pine cones. According referenced works in table 3, lignin determined according to the acetyl bromide method resulted lower values comparing Klason lignin but resembled Klason lignin after 1% NaOH extraction shown in the fourth column of Table 3.

Table 3. Klason and Acetyl Bromide Lignin Content in Some Pine Cones (Based on Oven-Dried, Extracted Cones)

Species	Klason Lignin (%)	Lignin (Acetlyl Bromide Method) (%)	Klason Lignin after 1%NaOH extraction (%)	**References**
Pinus banksiana	26.9	23.0	-	Eberhardt and Young, (1996)
Pinus nigra	26.9	20.4	-	Eberhardt and Young, (1996)
Pinus ponderosa	35.0	24.7	-	Eberhardt and Young, (1996)
Pinus resinosa	30.8	21.2	-	Eberhardt and Young, (1996)
Pinus taeda	37.6	27.9	-	Eberhardt and Young, (1996)
Pinus nigra	29.7	-	22.9	Ucar and Ucar, (2008)
Pinus pinea (Coruh)	33.5	-	25.1	Present work
Pinus pinea (Armutlu)	36.2	-	24.4	Present work
Pinus pinea (Kozak)	36.7	-	24.8	Present work

Table 4. The Main Components of *Pinus pinea* Cones (Based on Oven-Dried, Extracted Cones)

District	Original Holocellulose Yield (%) I	Lignin in Holocellulose (%)			Corrected Holocell. Yield (%) (I-IV)	α-cellulose (%)	Hemicellulose (%)*
		Klason Lignin (II)	Acid-Soluble Lignin (III)	Total Lignin (IV)			
Coruh I	70.25	4.42	8.75	13.17	57.08	39.03	18.05
Coruh II	69.24	4.46	8.68	13.14	56.10	37.94	18.16
Armutlu I	67.68	3.86	8.74	12.60	55.08	36.85	18.23
Armutlu	67.56	3.91	8.70	12.61	54.95	37.54	17.41
Kozak I	65.97	5.21	8.42	13.63	52.34	35.99	16.35
Kozak II	66.56	5.11	8.14	13.25	53.31	35.82	17.49

*Hemicellulose values were calculated as the difference between corrected holocellulose and alpha cellulose values.

The holocellulose content and the percentages of residual and acid-soluble lignin in the holocellulose are summarized in Table 4. The holocellulose content of the cones was almost the same as that of *Pinus pinea* wood (Table 6). In contrast, the alpha-cellulose yield was distinctly lower in cones than it was in wood (Table 4). Thus, it could be concluded that the cones contained higher amounts than wood of polysaccharide fractions solubilized by alkali, particularly polyoses. Perhaps a part of the cellulose in the cones was also removed with polyoses from cones holocellulose by concentrated NaOH solution during the alpha-cellulose isolation.

The results of the measurements of polyoses in the cones concurred with the polysaccharide composition of *Pinus nigra* cones (Ucar and Ucar 2008). Kilic *et al.*

(2010) demonstrated that some pine cones had even more polyoses than they had cellulose. Of the cone samples, the Coruh samples had the most holocellulose, whereas the Kozak samples had the least.

The yield of main components and extractives for *Pinus pinea*, both heartwood and sapwood, are also given in Table 5. As expected, the heartwood contained higher amounts of extractives than did the sapwood. Both the sapwood and the heartwood contained the same amounts of acid-soluble lignin, while small differences between the two were observed in the residual lignin values.

The yields of holocellulose and alpha-cellulose showed some differences that should be considered acceptable among trees of the same stand. The results of this study were in agreement with a previous study by Gumuskaya *et al.* (2011), which reported that the holocellulose and alpha-cellulose contents in *Pinus pinea* wood were 70.8% and 46.08%, respectively.

Although the hot water solubility values of both heartwood and sapwood were lower than those of the cones, the heartwood had higher values of direct alkali solubility, probably due to the presence of high amounts of resinous compounds.

Table 5. Ash, Solubilities and Lignin Content of *Pinus pinea* Wood

District	Ash+ (%)	Solubility+				Klason Lignin (%)‡	Acid Soluble Lignin (%)‡
		Ethanol-Cyclohexane (%)	Ethanol (%)	Hot Water (%)	1% NaOH (%)		
Coruh SW	0.26	2.95	0.10	2.51	12.45	27.60	0.35
Coruh HW	0.17	17.69	0.18	5.66	26.45	28.26	0.40
Armutlu SW	0.33	4.93	0.12	4.98	14.92	27.39	0.32
Armutlu HW	0.16	22.49	0.15	9.24	30.18	29.46	0.36
Kozak SW	0.23	4.80	0.15	3.62	15.57	29.56	0.39

SW: sapwood, HW: heartwood, + based on oven-dry original material, ‡ based on Et-OH/cyclohexane and ethanol extracted material

The holocellulose content and the percentages of residual and acid-soluble lignin in the holocelluloses of *Pinus pinea* heartwood and sapwood are listed in Table 6. The values of corrected holocellulose were calculated by subtracting the residual lignin (acid-insoluble and acid-soluble) from the holocellulose value. The results indicated that the corrected holocellulose yield for these woods was notably low.

Table 6. . The Main Components of *Pinus pinea* Wood (Based on Oven-Dried, Extracted Wood)

District	Original Holocellulose Yield (%) I	Lignin in Holocellulose (%)			Corrected Holocell. Yield (%) (I-IV)	α-Cellulose (%)	Hemicellulose Yield (%)*
		Klason Lignin (II)	Acid-Soluble Lignin (III)	Total Lignin (IV)			
Coruh SW	71.99	2.65	6,71	9.36	62.63	45.45	18.18
Coruh HW	71.42	1.55	7.36	8.91	62.51	43.99	18.52
Armutlu SW	74.05	2.22	7.54	9.76	64.29	47.45	16.84
Armutlu HW	70.75	1.51	8.15	9.66	61.09	43.07	18.02
Kozak SW	72.88	2.11	7.48	9.59	63.29	45.13	18.16

SW, sapwood; HW, heartwood., *Hemicellulose values were calculated as the difference between corrected holocellulose and alpha-cellulose values.

CONCLUSIONS

1. Notable differences in the ethanol-cyclohexane, ethanol, and alkali solubility of stone pine cones from the three different regions were observed. However, the variation in the contents of the main components, *i.e.*, holocellulose, alpha cellulose, and lignin, was limited and depended on the region.
2. The solubility values of the heartwood and sapwood differed considerablyly, with the heartwood showing higher values. Five to six times more extracts were removed from heartwood with EtOH/cyclohexane than from sapwood. With regard to lignin content, the differences between both woods were negligible. However, sapwood showed higher alpha-cellulose contents than did the heartwood.

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