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ARAŞTIRMA MAKALESİ RESEARCH PAPER

Utilization of *Trapa natans*

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Abstract: In this study, *Trapa natans* nut collected from the costs of Inkumu-Bartin was analyzed according to its lignin content. Acid-insoluble lignin content was determined according to TAPPI T222om-02 as 28.31%. TGA and FTIR analyses were performed.

FTIR analysis indicated that *Trapa natans* contained cellulose, hemicelluloses and lignin. The peaks at 1506-1510 cm^{-1} in both samples are characteristic peaks for the lignin components due to C = O and COO-non-symmetric stretching vibrations in the aromatic rings of the lignin structure. 1510 cm^{-s} peak is found in the literature as lignin's fingerprint peak. The peaks at 1230-1270 cm^{-1} band show the vibrations of guayasil ring with CO tension in lignin and hemicelluloses. Around the peaks of 900, 1025, 1030 and 1050 cm^{-1} bands cellulose's O-H, C-H and C-O-C type bonds are seen. When TGA analyzes are examined, approximately 4.5% of the sample is lost from 30 °C to 200 °C. At these intervals, samples generally lose volatile constituents and moisture. From 200 to 271 °C 4.6% of the weight is lost. The decrease in this range may be due to the decomposition of cellulose and hemicelluloses. The DTG curve shows maxima weight-loss occurring at 349 °C. The weight-loss in the range from 271-352 °C is about 50%. Pure lignin sample's TGA and DTG curve results show 10%, 50%, and 70% weight-losses at 233 °C, 489 °C, and 1167 °C respectively. According to these results, dominantly lignin and small quantities of cellulose and hemicelluloses are present in trapa samples.

Keywords: Lignin, *Trapa natans*, water-chestnut.

Trapa natans'tan Yararlanma

Öz: Bu çalışmada, Bartın-İnkumu sahillerinden toplanmış *Trapa natans* yemişi boş kabuklarının lignin miktarına bağlı analizleri yapılmıştır. Asitte çözünür lignin miktarı TAPPI T222om-02 esas alınarak yapılmış ve %28.31 bulunmuştur. TGA ve FTIR analizleri yapılmıştır.

FTIR analizleri *Trapa natans* örneklerinin selüloz, hemiselülozlar ve lignin içerdiğini göstermektedir. Her iki örnekteki 1506-1510 cm^{-1} pikleri lignin yapısının aromatik halkalarındaki C = O ve COO-simetrik olmayan gerilme titreşimlerine bağlı görülen, lignin bileşenleri için karakteristik piklerdir. 1510 cm^{-s} piki literatürde ligninin parmak izi piki olarak görülmektedir. 1230-1270 cm^{-1} bandındaki tepe noktaları, lignin ve hemiselülozlardaki CO gerilimi ile guayasil halkasının titreşimlerini göstermektedir. 900, 1025, 1030 and 1050 cm^{-1} pikleri civarında selülozun O-H, C-H ve C-O-C tipi bağları görülmektedir. TGA analizleri incelendiğinde 30 °C'den 200 °C'ye kadar numunenin yaklaşık %4,5 kadarının kaybedildiği gözlemlenmiştir. Bu aralıklarda numune genellikle uçucu maddeleri ve nemi kaybetmektedir. 200 °C'den 271 °C'ye kadar ağırlığın %4,6'sı kaybolmuştur. Bu aralıkta gözlenen kayıp, selüloz ve hemiselülozlarda meydana gelen bozunmadan kaynaklanabilmektedir. DTG eğrisi 349 °C'de maksimum ağırlık kaybını göstermektedir. 271-352 °C'deki ağırlık kaybı yaklaşık olarak %50 civarındadır. Saf lignin örneğinin TGA ve DTG eğrileri incelendiğinde, 233 °C, 489 °C ve 1167 °C'de sırasıyla %10, %50 ve %70 ağırlık kaybı görülmektedir. Bu sonuçlara göre trapa örneklerinde baskın olarak lignin ve az miktarlarda selüloz ve hemiselülozların var olduğu söylenebilir.

Anahtar sözcükler: Lignin, su kestanesi, *Trapa natans*.

INTRODUCTION

Trapa natans L. (TN); known as water chestnut or water-nut is an annual, floating–leaved plant that naturally grows in rivers, lakes, fresh-water wetlands and firths in Asia and Europe (Yasuda et al., 2014). In Turkey, it is found in trace amounds (Istanbul, Edirne and Kırklareli) (Web-1). In the last years, it was seen in the cost of North Black Sea region (TÜBİVES, 2018). It is a warm season crop and can harvest in June-September (Liu et al., 2010; Parker & Waldron, 1995).

It has 5 cm wide leaves with sharp serrate and stiffy hairs. It also has 15 cm long submersed leaves. In spring time, it has white flowers. Having an economic value, the fruit grows under water and has horned nut-like structure (Web-2).

It has an economical worth as food and raw material in different areas especially for China and India. Dried and grounded nuts can be added into flour for baking (Sturtevant & Hedrick, 1972; Web-3).Because of high water content, it is used for thirst and also used as traditional medicine. In an experimental study was showed that herbal mixture extracts which include TN decreased the pain of shingles (Hijikata et al., 2005). The outer part of nuts is utilized in pulp-paper, fish food, compost and biofuel (Hummel & Kiviat, 2004).

TN resembles a water lily at first view with its green leaves floating on the water. Floating leaves have rosette form, and leaflet is solid and triangular (5.2x6.4 cm) (Nedhuka & Kardyum, 2016). The leaves are dark green, and their bottom surface is reddish purple (Adkar et al., 2014; Zhu, 2016). The submerged stems cling to the soil by extending to 1-5 m along the roots (Adkar et al., 2014; Hummel & Kivrat, 2004).

TN, which has been used for food and therapeuthic purposes in China and India since ancient times (Zhu, 2016), is a major ingredient in many food products such as jams, beverages and biscuits in China (Sheng et al., 2006). Due to its characteristic taste and high nutritional value, it has commercial importance in the food industry (Parker & Waldron, 1995; Liu et al., 2010). The nuts has 15% protein, 7.5% fat, 52% starch, 3% sugar and 22.5% water (Zhu, 2016). TN is also an important source in terms of nutrients, protein, minerals and vitamins for fishs (Kalita et al., 2007; Kalita et al., 2008; Mukherjee, et al., 2010). It is also stated in the ancient medicine books in China that TN husks can help fight against various diseases such as diarrhea and alcohol poisoning (Sheng et al., 2006).

Its fruits and husks have a rich content consisting of starch, dietary fibre, essential amino acids and some types of phenols and minerals. Previous studies have shown that TN leaves, fruits, husks, flowers and roots have 19.5% amylose (Lertphanich et al., 2013), 1.9 mg/g phosphorus (Akao et al., 2013; Zhu, 2016), fatty acids such asnervonicacid (63.5%), α-linolenic acid (6.4%), palmitolenic acid (6.4%), linoleic acid (6.3%) (Mukherjee et al., 2010; Zhu, 2016), phenols such as caffeic acid, chlorogenic acid, ferrulic acid and 3-O-methylgallic acid (Stoicescu et al., 2012; Zhu, 2016), flavonoids such as quercetin and kaempferol (Niranjan et al., 2013; Zhu, 2016). The majority of fatty acids (69.3%) in the TN content form unsaturated fatty acids (Mukherjee et al., 2010). The fruit in the husk contains a higher amount of starch, while the husk contains higher amounts of phenolic material.

TN husks contain higher amount of C (43.4%), O (50.4%) ve H (5.7%) (Liu et al., 2010). Its husk have higher bioactivity properties (anti-cancer, anti-atherogenic, antimicrobial, antioxidant, hepatoprotective, immune enhancing, anti-imflammatory, anti-hyperglycemic, anti-skin photoaging) than fruits (Zhu, 2016). After harvesting period, pink colored TN husks change its color to dark brown in time (Huang et al., 2016). These husks contain dietary fibres and polyphenols (such as hydrolysable tannins) (Yasuda et al., 2014; Kim et al., 2014; Huang et al., 2016). The total phenolic, flavonoid and tannin amounts in TN husk extract were: 63.81% mg/g gallic acid equivalents, 21.34% mg/g of rutin equivalents and 17.11% mg/g of total tannin equivalents respectively (Malviya et al., 2010; Zhu, 2016).

Despite all of the good qualities, TN, which was exposed over large areas in North America in the 1800's, was considered as a tribulation. This is why it prevents the light permeability below the surface in the water it is spreading. With the reducing amount of light permeability, the amount of dissolved oxygen in the water reduces and that is adversely affects fish communities. It also displaces other submerged plants, making it difficult to navigate the boats, catch fish and swim (Hummel & Kiviat, 2004; Zhu, 2016).

The TN fruits must be peeled from the dark brown husks before they are cooked or canned. The peeled husks are burned or disposed of as waste. Husks thrown in this way cause water and air pollutions (Liu et al., 2010).

Lignin is the most abundant natural polymer after cellulose. It generates 10-25% of the lignocellulosic biomass. It has a complex structure composed of different types of phenol (sinapyl, p-coumaryl, and coniferyl alcohols). There are many usage areas such as emulsifiers, dyes, thermosets, dispersant agents, synthetic flooring, and paints (Watkins et al, 2015).

TN husks include 72.3 g/100 g (dry basis) of crude fiber. When this amount compared to dietary fiber contents (ranged from 10.2 to 87.9 g/100 g) of other fruits and greens (apples, citrus fruits, oat, wheat, corn), it can be said that it is a rich natural source of dietary fiber (Chau & Huang, 2004; Grigelmo-Miguel & Martin Belloso, 1999a, 1999b; Jaime et al., 2002). The previous studies have reported that the TN husks contain 6-14.6 g/100 g hemicellulose, 9-10.2 g/100 g cellulose and 35.4-48.3 g/100 g lignin (Chiang & Ciou, 2010).

In the last few years, a lot of TN shells have been seen in the Bartin coasts. The reason why these husks were not utilized is that the local people were unaware of TN. In this study, the use of TN, causing coastal pollution, as a source of valuable chemicals was investigated by determining the amount of lignin.

MATERIAL and METHODS

Materials: TN nuts were collected from the costs of Inkumu-Bartin in 2017. Identification of the plant material was performed by Prof. Dr. Z. Kaya at the Faculty of Forestry of Bartin University. Husks were dark brown, 3-5 cm wide and sclerosis and had no fruits inside. For analysis, TN husks were washed with tap water and then freezedried. Dried husks were cut with knife and grinded with Wiley Mill. Pure lignin sample was purchased from LignoWorks.

Methods:

Acid-insoluble Lignin Determination: Before the lignin analysis, extractives were removed from the TN samples. Samples were extracted in a soxhlet apparatus for 6 hours with ethanol. Acid-insoluble lignin was determined according to TAPPI T222om-02. 15 ml of 72% H_2SO_4 was added to 1 g of extractive free TN samples and stirred for 2 hours. Then the mixture was placed in 1 L of erlenmayer with adding 560 ml of distilled water and boiled on a multi-heater for 4 hours by attaching a cooler. At the end of 4 hours, the samples were filtered on glass crucibles and washed with hot distilled water.

Fourier Transform Infrared Spectroscopy (FTIR): FTIR analysis was carried out with Shimadzu IRAAffinity-1 spectrometer with single reflection Attenuated Total Reflectance (ATR) pike MIRacle sampling accessory (Fourier transform infrared spectra were obtained using a PerkinElmer Spectrum 100 with a universal ATR sampling accessory). Four accumulated spectra were collected in the wave number region of 700–4000 cm^{-1}, with a spectral resolution of 4 cm^{-1}.

Thermal gravimetric analysis (TGA) and differential thermal gravimetric (DTG) analysis: TGA/DTG analyzes were carried out with Hitachi STA7300 Thermal Analysis System. 3 mg of dried and ground sample was heated from 30 °C up to 1200 °C. Analysis was carried out at the heating rate of 10 C min^{-1} in nitrogen atmosphere.

FINDINGS and DISCUSSION

Acid-insoluble Lignin Determination: The acid-insoluble lignin content was found to be 28.31%. As seen from Table 1, TN is a good source of lignin compared with other lignocellulosic materials.

Fourier Transform Infrared Spectroscopy (FTIR): FTIR analysis indicated that TN contained cellulose, hemicelluloses and lignin as expected. 3400-3330 cm^{-1} OH stretching of alcohols, phenols, and acids (Tjeerdsma & Militz, 2005; Müller et al., 2009; Esteves et al., 2013; Mattos et al., 2015; Missio et al., 2015; Gonultas & Candan, 2018). 2970-2820 cm^{-1} C-H stretching in methyl and methylene groups (Müller et al., 2009; Esteves et al., 2013; Mattos et al., 2015; Gonultas & Candan, 2018). 1650-1652 cm^{-1} bands are absorbed water molecules of C=O stretching of the aromatic structures (Kotilainen et al., 2000; Nuopponen, 2005; Ozgenc et al., 2017). In the TN samples, it was seen that the range of 1750-1650 cm^{-1} bands a variety of pixels unlike the pure lignin was appered (Figure 1). These peaks are derived from water and extractive substances (Zhou et al., 2015). The peaks at 1506-1510 cm^{-1} in both samples are characteristic peaks for the lignin components due to C = O and COO-non-symmetric stretching vibrations in the aromatic rings of the lignin structure (Özgenç, 2014; Can & Sivrikaya, 2017; Can & Sivrikaya, 2016).

Table 1. Lignin values of some lignocellulosic materials.

Sample	Lignin (%)	Literature
Trapanatans	28.31	Detection
Pinussylvestris	27.2	Dönmez, 2010
Larix decidua Mill.	13.42-29.54	Muhcuet al., 2015
*Pinusbrutia*Ten.	25.9	Taş, 2017
Tectonagrandis	23.7-25	Dwumaa, 2016
*Quercusrobur*L.	22.6-23	Krutul et al., 2010
Betula pendula Roth.	18.8-19.8	Krutulet al., 2014
Pseudocydoniasinensis	21	Qin et al., 2018
Hybrid Pennisetum	20.8	Wang et al., 2018

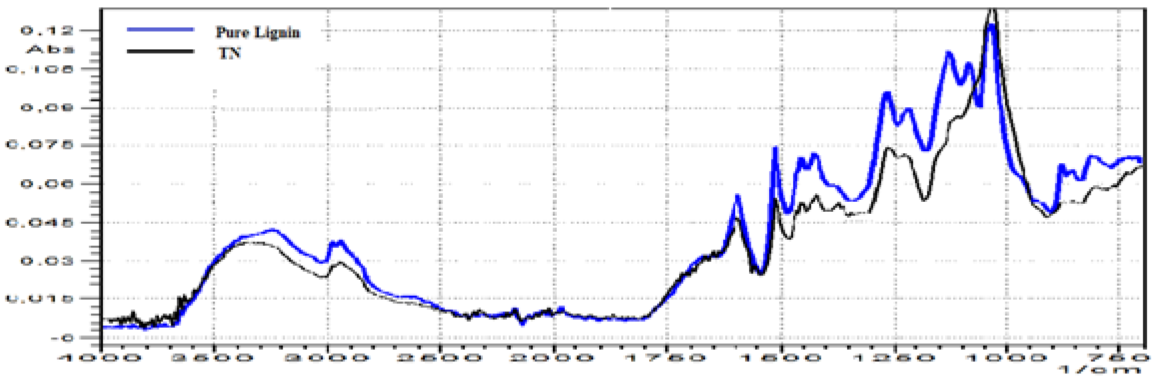

Figure 1. TN and Pure lignin ATR-FTIR spectrums.

The bands at 1452-1459 cm^{-1} belong to C=C and C=H bond, O-H in plane deformation, and asymmetric bending in lignin (Nuopponen, 2005; Ishimaru et al., 2007; Ozgenc et al., 2017). 1420-1422 cm^{-1} bands belong to aromatic skeletal vibration combined with CH inplane deformation for lignin (Muller et al., 2009; Chen et al., 2010; Herrera et al., 2014; Traore et al., 2016). 1263 cm^{-1} band belongs to guaiacyl ring breathing, C–O stretch in lignin and C–O linkage in guaiacyl aromatic methoxyl groups (Popescu et al., 2007; Muller et al., 2009; Traore et al., 2016). 1233 cm^{-1} and 1155 cm^{-1} bands show the alkyl-aryl-ether bonds, lactones and cellulose C-O-C symmetric stretching respectively (Nuopponen et al., 2003; Nuopponen, 2005; Ozgenc et al., 2017). 1110 cm^{-1} band belongs to OH association of cellulose and this peak is seen just in the TN spectrum. At the 1033 cm^{-1} band aromatic C-H deformation, C-O deformation, and C=O stretching in lignin are seen in both spectrum (Zhou et al., 2015).

As can be seen in the Figure 2; TN lignin and pure lignin samples have very similar FTIR spectrums. As far as we know, the pure lignin was obtained from pine species which shows that TN lignins chemical structure looks like pine lignin's.

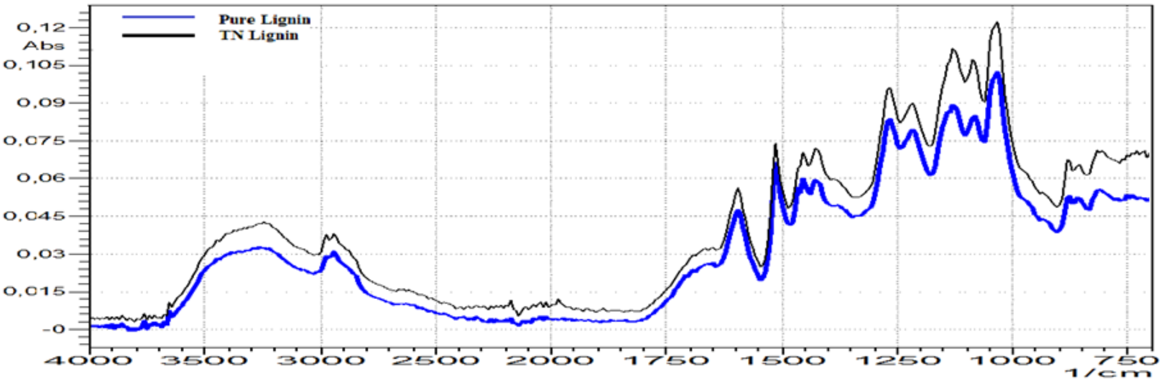

Figure 2. TN lignin and Pure lignin ATR-FTIR spectrums.

Thermal gravimetric analysis (TGA) and differential thermal gravimetric (DTG) analysis: TN and pure lignin samples's TGA/DTG curves shown in Figure 2, are sharing similarity at some temperatures. WhenTN TGA curves were examined, approximately 4,5% of the sample is lost from 30 to 200 °C. At these intervals, samples generally lose volatile constituents and moisture (El-Sayed & Mostafa, 2014). From 200 °C to 271 °C 4,6% of the weight is lost. The decrease in this range may be due to the decomposition of cellulose and hemicelluloses. The DTG curve shows maximum weight-loss occurred at 349 °C. The weight-loss in the range from 352 °C is about 50%. According to these results, dominantly lignin and small quantities of cellulose and hemicelluloses are present in TN samples.

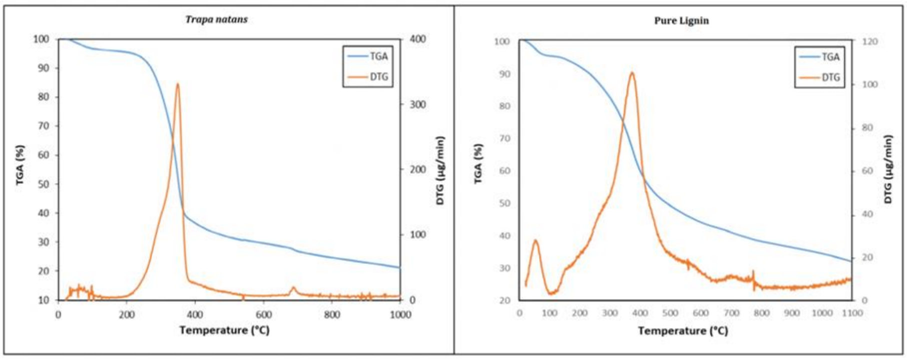

Figure 3. TGA and DTG diagrams of TN and Pure lignin samples.

Lignin's complex structure includes mostly, various branching aromatic rings. These chemical bands have a wide degradation temperature range from 100 °C to 800 °C (Yang et al., 2007). From 30 °C to 200 °C, volatile constituents and moisture removes from lignin sample. At the range of 180 °C–350 °C, carbohydrates are converted to volatile gasses (CO, CO_2, and CH_4). Following 350 °C phenolics, alcohols, and aldehyde acids are removed from lignin (Watkins et al., 2015). Pure lignin sample's TGA and DTG curve results show 10%, 50%, and 70% weight-losses at 233 °C, 489 °C, and 1167 °C respectively (Table 2). Differently from TN sample, pure lignin's 10% of degradation temperature 37,9 °C lower. It may be due to pure lignin samples contamination cause of storage conditions. 50% degradation temperature of TN has difference owing to it's cellulose and hemicelluloses content.

Table 2. Termal degredation tempratures of TN and pure lignin samples.

	T10%	T50%	T70%	Residue (%)	DTGmax
Trapa	271,5°C	352,1°C	576,7°C	82,8	349,6°C
Lignin	233,6°C	489,2°C	1167°C	100	373,1°C

CONCLUSION

In many coastal strips of Bartin such as Inkumu and Guzelcehisar, TN can not only cause an environmental pollution, but also can reach the wetlands and it may be the possibility of spreading in those areas. When considering the richness of the lignin in TN husks, the evaluation of these materials will be able to be used as a new source of valuable chemicals to produce lignin and besides can help to eliminate the environmental pollution.

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