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RESEARCH ARTICLE

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Chemical structure and characterization of bio-oils isolated from walnut shells by different processes

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Abstract

In this study, content analyses of the products obtained by pyrolysis and extraction of walnut shells were performed with some advanced analytical methods such as GS-MS, LC/MS-MS, and FTIR. In LC-MS/MS analyses. The presence of phenolic compounds in the pyrolysis liquid and extract liquid of walnut shells was determined using 41 standards. The obtained LC-MS/MS analysis results were compared, and it was found that the type and amount of phenolic compounds in the extracted liquid were more than the type and amount of phenolic compounds in the pyrolysis liquid. As a result of 41 phenolic standards investigations, the presence of a total of 10 phenolic compounds in liquids was detected and 2 of these compounds were detected in the pyrolysis liquid and 8 in the extracted liquid. As phenolic compounds, catechin (770.75 µg/g extract), hydroxy benzaldehyde (140.78 µg/g extract), and vanillic acid (114.95 µg/g compounds) were detected in the walnut extract. The existence of the compounds was supported by FTIR analyses of liquids obtained as a result of two different processes. By GC-MS analysis, linoleic acid methyl ester (44.01%), stearic acid methyl ester (14.93%), palmitic acid methyl ester (24.67%), linolenic acid and methyl ester compounds were detected in walnut shell extract liquid. GC-MS analysis of the pyrolysis liquid showed that compounds such as stearic acid methyl ester (18.97%), palmitic acid methyl ester (18.10%), o-xylene (12.17%), and o-ethyl toluene (8.14%) were formed as a result of pyrolysis. The findings revealed that walnut shell pyrolysis liquid product and extract contain very different phenolic, acid, and ester compounds. A concrete result was revealed about the use of the products obtained in this study in different areas.

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Keywords: : Walnut; Pyrolysis; Extract; LC-MS; GC-MS; Analysis

1. Introduction

Generally, tree species such as walnut, chestnut, and hazelnut are abundant in the Northern Hemisphere. Products obtained from these trees can be used in many industrial areas such as confectionery, chocolate, oil, and liquor. Since it can be consumed directly, the demand for these products is quite high. Some methods such as solvent extraction, microwave-assisted, conventional hydrothermal, and pyrolysis have been used to transform waste into value-added products [1,2]. Among these methods, the pyrolysis process is highly preferred. Pyrolysis is a process of thermally decomposing biomass residue in an airless environment and converting it into bio-oil, biochar, and gas products at different rates [3,4]. During pyrolysis, no waste or any by-products that may harm the environment are formed. Therefore, pyrolysis is a harmless process for the environment [5]. As a result of the pyrolysis process, by-products such as black carbon, coke, pyrolysis oil, and coke are formed [6]. These by-products can be used in different areas such as energy, chemical raw materials, and high-value-added products [7]. For example, black carbon; is used in areas such as fuel cells, the plastic industry, and paint raw materials. In addition, gaseous products such as hydrogen and methane resulting from pyrolysis can be used as fuel. Obtaining and using these products can provide great convenience in daily life. All these products are produced through processes such as cellulosic waste material and pyrolysis process [8]. Analytical methods that can provide highly advanced and accurate results, such as LC-MS/MS, FTIR, and GC, are widely used to elucidate the material structure. Clarifying the structure of matter by using these techniques can provide very important clues about where the materials in question will be used [9]. Additionally, qualitatively and quantitatively complex components can be detected using the solvent extraction method in biomass wastes. Tree bark and other cellulosic materials are rich in various chemicals such as biopolymers, phenolic compounds, and lignite. Detailed analysis of these complex compounds is critical to expand and evaluate the uses of cellulosic materials. Walnut, one of these tree species, belongs to the Juglandacea plant family, which is grown and consumed widely worldwide. Some researchers studies that walnuts have heart-healthy, anti-inflammatory, and cancer-reducing effects [10]. Along with the consumed walnuts, some of the walnut shells produced in large quantities are burned and cannot be adequately utilized in suitable areas [11]. Therefore, these wastes constitute a source of value-added by-products and other areas. Several studies have analyzed the components found in walnut shells. However, studies aiming to reveal the content of walnut shells are limited in the literature [12].

Herein, as far as we know, for the first time, walnut shell residues were pyrolyzed and extracted. The contents of the products obtained from pyrolysis and extraction were investigated and compared using some advanced analytical methods. The presence of various compounds such as phenolic compounds, organic, phenolic, amino acid, and volatile compounds of bio-oils was analyzed with Gas Chromatography-Mass Spectrometry (GC-MS), Liquid Chromatography Mass Spectrometry (LC/MS-MS), and Fourier Transform Infrared Spectroscopy (FT-IR).

2. Material and Method

2.1. Materials

Walnut shells collected in Iğdır province were dried at 105°C for 12 hours. Waste walnut shells were turned into powder with the help of a grinder. The chemical reagents of methanol, acetone, ethyl acetate, ether, and hexane used in this study were purchased from Sigma and were analytically pure. All chemicals used were used without any further purification processes.

2.2. Instruments and Analyses

2.2.1. LC-MS/MS Analysis

Phenolic compounds were analyzed by high-performance liquid chromatography (LC/MS-MS). LC/MS-MS combined with mass spectrometry (MS) was an Agilent Poroshell 120 EC-C18 (100 mm × 3.0 mm, 2.7 µm) reversed-phase analytical column. Eluent A (pure water + 5 mM ammonium formate + 0.1% formic acid) and eluent B (methanol + 5 mM ammonium formate + 0.1% formic acid) were set. Solvent flow rate (0.400 mL/min) and sample injection volume (4 µL) were adjusted [13,14]. The content analysis was performed with 41 standard phenolic compounds (Figure 1).

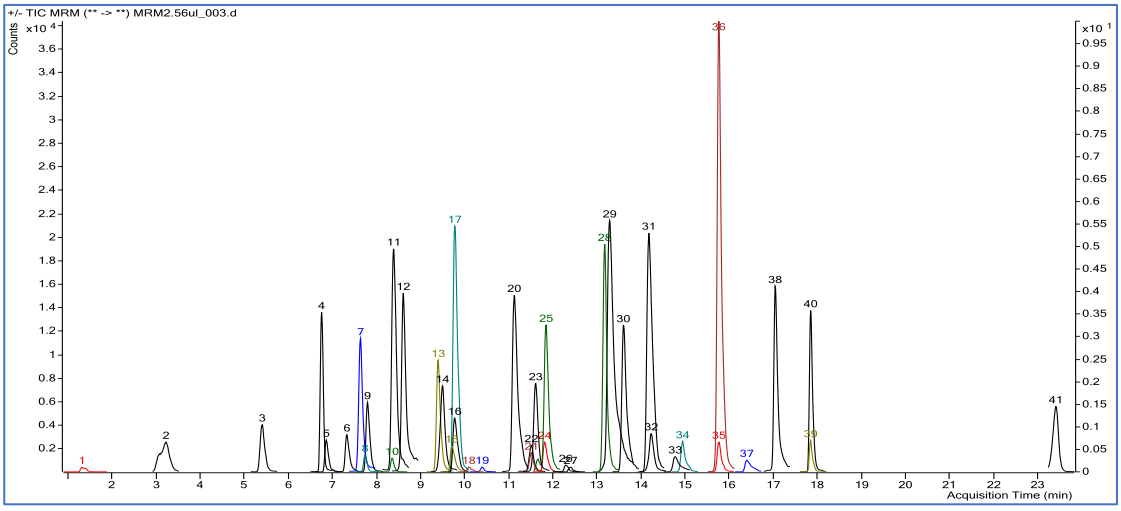

Fig. 1. LC-MS/MS chromatogram. Acids: 1. Shikimic, 2. Gallic, 3. Protocatechuic, 4. Epigallocatechin, 5. Catechin, 6. Chlorogenic, 7. Hydroxy benzaldeyde, 8. Vanillic, 9. Caffeic, 10. Syringic, 11. Caffeine, 12. Vanillin, 13. Orto Coumaric, 14. Salicylic, 15. Taxifolin, 16. Resveratrol, 17. Polydatine, 18. Transferulic acid, 18. Sinapic, 20. Scutellarin, 21. Para Coumaric, 22. Coumarin, 23. Protocathuic Ethyl Ester, 24. Hesperidin, 25. Isoquercitrin, 26. Rutin, 27. Quarcetin -3 – Xyloside, 28. Kaempferol – 3- Glucodide, 29. Fisetin, 30. Baicalein, 31. Chrysin, 32. Trans-cinnamic, 33. Quercetin, 34. Naringenin, 35. Hesperetin, 36. Morin, 37. Kaempferol, 38. Baicalein, 39. Luteolin, 40. Baichanin A, 41. Diosgenin.

2.2.2. GC-MS/MS Analysis

Fixed oil, free oil, and other essential oil components in walnut shell extracts and pyrolysis oil products were examined by GC-MS/MS analysis (Agilent 7000, 7697A Headspace Sampler, 7890 GC, 7693 Autosampler). In GC-MS/MS analysis of the samples, the initial temperature was 50 °C and the retention time was 2 minutes. The temperature was increased to 140 °C at a rate of 3 °C/min and then increased to 220 °C at a rate of 4 °C/min. It was kept at 220 °C for 10 minutes. At a constant temperature increase rate of 4 °C/min, the temperature was increased to 250 °C and finally, the temperature was increased to 270 °C and kept for 30 minutes. The ion temperature of the detector in GC-MS was 280 °C and the carrier gas was He. A 0.22 µm disposable syringe was used for sample analysis (1 µL, 1:10). The analyses were performed on a 30 m x 0.25 mm x 0.25 um, Agilant MP-5 column (5%-phenyl)-methylpolysiloxane). The process was started by taking 30 mg of the sample and dissolving it in 2 mL methanol and 2 mL n-hexane. 1 mL of 1M KOH was added to the resulting mixture and mixed using a vortex (2500 rpm, 30 sec) to ensure good mixing. 0.22 microns were taken from the upper phase of the mixture and filtered, and the filtered sample was given to the device and analysis started [15].

2.2.3. Analyses of FTIR for walnut shells

Analyses of FTIR for walnut shells were performed to elucidate the bond structure of the compound in the bio-oil samples using the Agilan Carry60 FTIR device.

2.3. Preliminary stages before experimental studies on walnut shells

The collected walnut shells were grounded using the grinding machine until they reached the smallest grain size. Then, the ground walnut shells were washed abundantly in deionized water and dried in the oven at 60 °C for 48 hours. The dried samples were prepared in a moisture-free environment at room conditions for pyrolysis and extra processing.

2.4. Approaches followed to obtain the liquid sample

2.4.1. Liquid sample preparation by extraction process

Pre-prepared ground walnut shells are crushed to a certain amount. Then, the solid sample was added to the prepared glass jar medium with methanol-chloroform (1:1 v/w) solvent. Left indoors and away from light for five days. Then, the solvent mixture was filtered and the solvents were removed with the help of an evaporator device. Finally, the obtained sample was taken into bottles. The samples taken were dissolved in 1 mL methanol and 1 mL hexane was added [13,14]. After phase separation occurred, the hexane phase was analyzed in GC-MS and the methanol phase was analyzed in GC-MS and LC/MS-MS [15].

2.4.2. Liquid sample preparation by pyrolysis process

Walnut shell grinding was carried out with a fixed bed pyrolysis (reactor) device in an inert nitrogen atmosphere. The reactor chamber is manufactured using stainless steel material with a length of 600 mm and an internal diameter of 50 mm. The reactor has a thermocouple temperature sensor design and can be operated with electrical energy. In the pyrolysis study, a 0.1 kg solid sample was taken into the feed reactor basin. Then, Nitrogen gas was exposed to 550 ◦C for approximately 2 hours under an inert environment at a heating rate of 10 ◦C/1 min [12]. Then, the liquid sample in the collection tank was taken into airtight bottles. The resulting liquid samples were dissolved in 1 ml of methanol and 1 ml of hexane was added. After phase separation occurred, the hexane phase was analyzed in GC-MS and the methanol phase was analyzed in LC/MS-MS. Additionally, an FTIR device was used to obtain detailed information about the functional structure of the sample.

3. Results and Discussion

3.1. LC-MS/MS analysis results

LC-MS/MS analyses were implemented to examine the phenolic compound content of walnut shells quantitatively and quantitatively. The type of phenolic compounds formed as a result of pyrolysis and the extraction of walnut shells, and the experimental parameters applied during the processes are summarized in Table 1. Among the 41 phenolic compounds investigated, 2 were identified in the pyrolysis sample and 8 in the extract (Fig. 2). The phenolic contents of the chloroform-methanol extract of waste walnut shells and the liquid part obtained by pyrolysis were compared. As seen in Table 1 different results were detected in phenolic compounds of the liquid of extraction and the liquid of pyrolysis of walnut shells.

Table 1. Evaluation of the content profile of the liquid of extraction and the liquid of pyrolysis of walnut shells.

No	Compound	RT	Pyrolysis walnut peel (µg/g extract)	Extract walnut peel (µg/g extract)
1	Protocatechuic acid	5.33	-	24.45
2	Catechin	6.74	-	770.75
3	Hydroxybenzaldeyde	7.50	-	140.78
4	Vanillic acid	8.81	-	114.95
5	p-Coumaric acid	9.40	-	39.84
6	Morin	12.90	-	10.88
7	Naringenin	14.88	11.93	26.92
8	Diosgenin	23.77	4.65	7.20

RT: Retention time.

When looking at the phenolic compound content of the walnut shell extract, catechin (770.75 µg/g extract), hydroxy benzaldehyde (140.78 µg/g extract) and vanillic acid (114.95 µg/g extract) compounds were detected at high levels. Naringenin (11.93 µg/g extract) and diosgenin (4.65 µg/g extract) compounds were detected in the phenolic content of the pyrolysis liquid extract. Compared to the results, it can be seen that the phenolic content decreased significantly after the pyrolysis process. It is thought that this decrease is due to the degradation of phenolic compounds with temperature. The results we obtained are compatible with previous studies. However, it should not be forgotten that differences in the methods applied in the analyses cause differences in the products obtained. For example, while only methanol is used in some studies in the literature, a methanol/hexane mixture can be used in some studies. In this study, a methanol/hexane mixture was used [16].

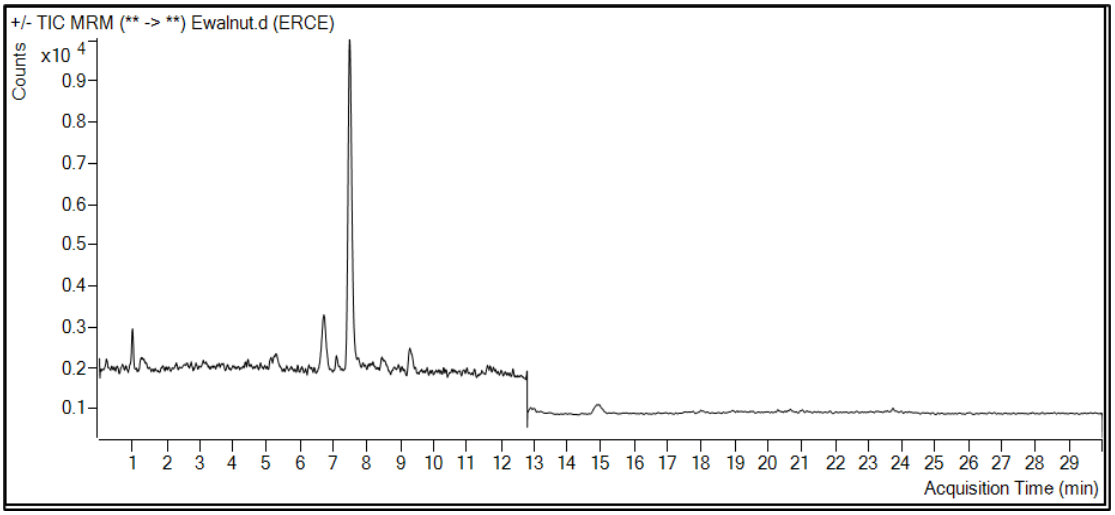

Fig. 2. LC/MS-MS chromatogram of methanol-chloroform extract derived from walnut shell.

3.2. FTIR analysis results

FTIR analysis results of liquid samples obtained from the pyrolysis experiments performed at 550 ◦C and walnut shells extract are illustrated in Fig. 3. FTIR analyses of the obtained extract and pyrolysis liquid samples show different peaks in the range of 500-400 cm-1. The FTIR results show some considerable differences between the spectra of the solution organics obtained in this study and the bio-oils of the pyrolysis. As can be seen, significant peaks were observed in some regions such as 3300 and 1028 cm-1. The vibration peaks in these ranges show different functional groups such as alcohol, phenols, and carboxylic acid. Moreover, the value of 2922 cm-1 for both aqueous extracts and pyrolytic oils shows a peak band of -CH stretching [17]. A sharp peak around the 1706 cm-1 band was observed especially in the pyrolytic oils and these vibrational peaks indicate that the bio-oils of the pyrolysis contain ketones, carboxylic acids, and aldehydes. The bands at 1214, 1208, 1030, 1028, and 1103 cm-1 indicate the presence of organic and aqueous bio-oils and -CO stretching and -OH bending. This indicates that primary-secondary alcohols, phenolic, ester, and etheric compounds are present in the structures. Finally, the intense peaks between 750 and 600 cm-1 indicate the presence of aromatic groups. It was observed from the band peak values that the pyrolytic organic bio-oils are broader and more intense compared to aqueous extract oils. The observed results are supported by similar results obtained in other studies in the literature [18,19].

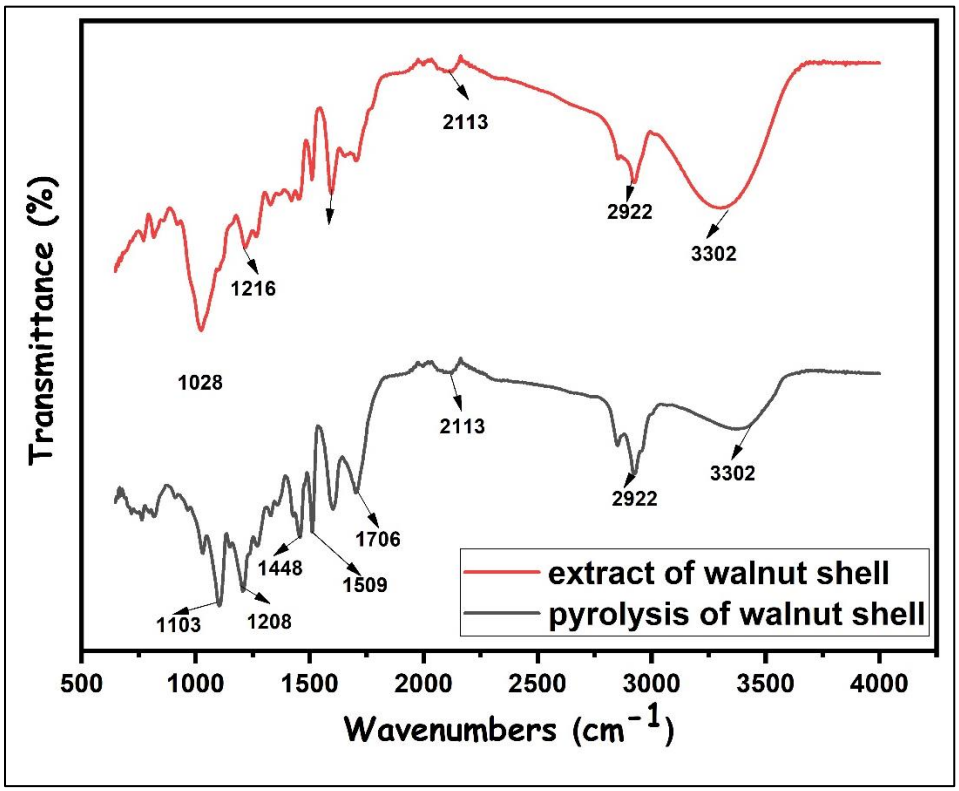

Fig. 3. FTIR analysis results of pyrolysis bio-oil obtained using walnut shells and the products resulting from extraction.

3.3. GC-MS/MS analysis results

GC-MS/MS analysis results of liquids of extracts and pyrolysis of walnut shells are given in Fig.4. According to the analysis results, linoleic acid methyl ester (44.01%), palmitic acid methyl ester (24.67%), stearic acid methyl ester (14.93%), linolenic acid and methyl ester (11.60%) compounds were detected in the liquid of extract. Stearic acid methyl ester (18.97%), palmitic acid methyl ester (18.10%), o-xylene (12.17%), and o-ethyl toluene (8.14%) compounds were determined in high amounts in the liquid of pyrolysis. The comparison between the extract and pyrolysis liquids revealed the presence of free fatty acids in the walnut shell due to the pyrolysis process. We can say that this situation occurs due to the fracture of the sample structure at high temperatures.

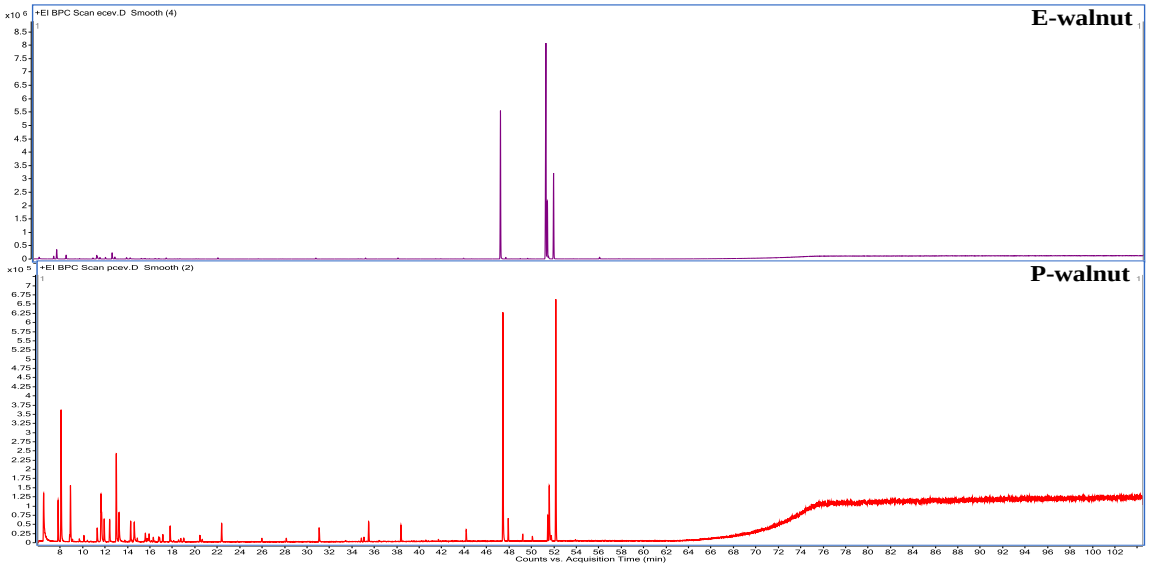

Fig. 4. GC-MS/MS chromatogram of walnut shell methanol-chloroform extract and liquid of pyrolysis liquid.

In addition, it was observed that the percentage amount of stearic acid, methyl ester, and o-ethyl toluene compounds increased as a result of pyrolysis, while the percentage amount of palmitic acid, methyl ester compound decreased (Table 2). Through the pyrolysis process, hemicellulose and cellulose are easily decomposed, resulting in the formation of gaseous products, ketone and hydrocarbon compounds, carboxylic acids, aldehyde products, and other compounds found in bio-oil. Similar results have been found in previous results [20,21].

Table 2. GC-MS analysis results of liquids of pyrolysis and aqueous extract of walnut shells.

No	RT	Compounds	Pyrolysis walnut%	Extract walnut%
1	7.82	m-Xylene	3.18	0.47
2	8.08	o-Xylene	12.17	1.84
3	8.92	???	4.69	-
4	10.12	Cumene	0.42	-
5	11.30	???	1.23	-
6	11.64	m-Ethyltoluene	6.87	0.68
7	11.93	Mesitylene	1.98	-

8	12.41	Pseudocumol	1.91	-
9	13.00	o-Ethyltoluene	8.14	1.24
10	13.25	Decane	2.91	-
11	14.29	Hemimellitene	1.84	-
12	14.60	2-Ethylhexanol	2.16	-
13	15.59	o-Propyltoluene	0.76	-
14	15.93	1,3-Dimethyl-5-ethyl benzene	0.64	-
15	17.80	Undecane	1.37	-
16	22.39	Dodecane	1.54	-
17	31.08	Tetradecane	1.35	-
18	38.37	Hexadecane	1.35	-
19	44.19	Heptadecane	0.88	-
20	47.46	Palmitic acid, methyl ester	18.10	24.67
21	51.46	Linoleic acid, methyl ester	1.90	44.01
22	51.59	Oleic acid, methyl ester	4.94	0.56
23	51.63	Linolenic acid, methyl ester	-	11.60
24	51.76	Oleic acid, methyl ester-isomer	0.70	-
25	52.17	Stearic acid, methyl ester	18.97	14.93

4. Conclusion

Walnut shell is an important environmental problem as an agricultural waste product. Analyzing liquid pyrolysis oil obtained by analytical methods such as LC-MS/MS, GC, and FTIR and determining the usage areas of walnut shells according to the analysis results will reduce waste pollution, which is an environmental problem.

The findings obtained in the study can be summarized as follows;

Pyrolysis of walnut shells and extract preparation of these shells were carried out. GC-MS, LC/MS-MS, and FT-IR analyses of the obtained liquid products were performed and their content analyses were examined. By comparing the analysis results, substance contents with high possible additive values were found. Accordingly, it was concluded that the amount and type of phenolic content in walnut extracts was higher in the pyrolysis liquid. Phenolic compounds and their amounts were found as catechin (770.75 µg/g extract), hydroxy benzaldehyde (140.78 µg/g extract), and vanillic acid (114.95 µg/g compounds). FTIR analysis was performed and it was revealed that walnut extract and pyrolysis liquids had different contents. Volatile component analyses in liquids were performed by GC-MS analyses. GS-MS analysis of the extract revealed the presence of linoleic acid methyl ester (44.01%), stearic acid methyl ester (14.93%), palmitic acid methyl ester (24.67%), linolenic acid and methyl ester compounds. Pyrolysis liquid GC-MS analyses also revealed the presence of the compounds stearic acid methyl ester (18.97%), palmitic acid methyl ester (18.10%), o-xylene (12.17%), and o-ethyl toluene (8.14%). We can probably attribute this to the deterioration of the bonds of the sample exposed to high temperatures in the pyrolysis method. The results showed us that bio-oil yield has a large impact depending on the process used. This study will be useful for those working in various fields such as bioplastics, biofuels, biochemicals, pharmaceuticals, cosmetics, fertilizers, and soil conditioners. In conclusion; this study has shown that wastes such as walnut shells can be evaluated through pyrolysis and pyrolysis products can be used in various areas. These findings could provide solutions to the problem of waste management, contribute to a new bio-economy, and contribute to sustainability.

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