Iğdır Üniversitesi Fen Bilimleri Enstitüsü Dergisi, 14(4), 1636-1644, 2024
Journal of the Institute of Science and Technology, 14(4), 1636-1644, 2024
ISSN: 2146-0574, eISSN: 2536-4618
Chemistry **DOI: 10.21597/jist.1516941**
Research Article
Received: 16.07.2024 **Accepted: 01.10.2024**

To Cite: Başar, Y., Nas, M.S., Alma, M.H. & Demirtaş, İ. (2024). A Novel Approach for the Evaluation of Bio-Oils Obtained from Chestnut Shells by Different Processes. *Journal of the Institute of Science and Technology*, 14(4), 1636-1644.

A Novel Approach for the Evaluation of Bio-Oils Obtained from Chestnut Shells by Different Processes

Yunus Başar[1], Mehmet Salih Nas[1,2]*, Mehmet Hakkı Alma[1] , İbrahim DEMİRTAŞ[1]

Highlights:

- Chestnut shell is a very important source for obtaining bioactive compounds
- The most abundant ones in the chestnut shell are phenolic acids, catechin, gallic acid, and protocatechuic acid.
- Products obtained from chestnut shells can provide a very effective alternative source in the use of artificial antioxidants.

ABSTRACT:

In this study, the contents of both extraction liquid and pyrolysis liquid products of chestnut shells were characterized using Gas Chromatography-Mass Spectrometry (GC-MS), Liquid Chromatography-Mass Spectrometry (LC/MS-MS) and Fourier Infrared Spectroscopy (FT-IR). In LC-MS/MS analyses; sample samples obtained from different processes were determined using 41 phenolic standards. The results of LC-MS/MS analysis showed that both the type and amount of phenolic compounds in the liquid extract were significantly higher than those in the pyrolysis liquid. According to the analysis results, very valuable phenolic compounds such as catechin (2526.96 µg/g extract), gallic acid (175.02 µg/g extract), protocatechuic acid (76.47 µg/g extract) were obtained in high amounts. FTIR analysis of the liquid samples obtained by two different processes was performed. GC-MS analysis revealed stearic acid methyl ester (28.78%), palmitic acid methyl ester (27.81%) and o-xylene (9.29%) in the liquid extract. However, higher amounts of linoleic acid, methyl ester (32.45%), palmitic acid methyl ester (23.06%) and linolenic acid, methyl ester oleic acid, methyl ester (11.32%) compounds were detected in the liquid extract obtained by pyrolysis compared to the normal liquid extract content. All these findings show that the processing method and physicochemical conditions are very important in obtaining valuable compounds such as phenolic and organic acids from plant products such as chestnut bark.

Keywords:

- Bio-Oils
- Pyrolysis
- Phenolic
- Characterization

[1]Yunus BAŞAR **ORCID ID:** (0000-0002-7785-3242), Mehmet Hakkı ALMA **ORCID ID:** (0000-0001-7011-3965), İbrahim DEMİRTAŞ **ORCID ID:** (0000-0001-8946-647X), Igdir University, Department of Biochemistry, Igdir, Türkiye
[2*]Mehmet Salih NAS **ORCID ID:** (0000-0003-1092-5237), Igdir University, Faculty of Applied Sciences, Igdir, Türkiye

***Corresponding Author:** Mehmet Salih NAS, e-mail: msalih.nas@igdir.edu.tr

INTRODUCTION

Plant-derived waste products are a serious problem that needs to be solved all over the world due to their harmful effects on all components of the ecosystem. Especially in the food industry, a significant amount of food waste and by-products are generated at every stage from harvest to marketing. The amount of by-products worldwide is considerably high and this rate is considerable in Turkey (Capanoglu et al., 2022). These food waste and by-products contain valuable compounds in their structure. In addition, the release of these food waste and by-products into the environment creates serious pollution and also causes a significant increase in greenhouse gas emissions (Melikoglu et al., 2013). Among these food waste products, Chestnut, a hot climate fruit, plays an important role among agro-forestry-based products in terms of economic income (Piazza et al., 2023). Türkiye ranks 4th in world chestnut production in 2020, making a great contribution. Chestnut fruit is used in many different industrial areas such as boiling, roasting, puree, cream and confectionery (Suna et al., 2021). According to a study, a significant amount of food material, especially the shell, is formed during the processing of chestnuts, so releasing them into the environment poses significant difficulties in terms of impact. (Vella et al., 2018). The chestnut shell obtained during the peeling process of the chestnut fruit constitutes about 10% of the chestnut weight. The skin of the chestnut shell consists of two layers. The main components of chestnut skin are Klason lignin and carbohydrates. It contains phenolic and volatile compounds that exhibit significant antioxidant activity (Morrone et al., 2015). Especially in experimental studies, it has been determined that chestnut bark has effective antioxidant properties in inhibition studies of peroxidation. The extracts obtained from the bark have consistently shown very high antioxidant properties due to the polyphenol compounds in their content (Barreira et al., 2008). The first objective of this study is to utilize different techniques such as pyrolysis and solvent assisted extraction of chestnut shells, which is a food waste and by-product. The second objective is to determine the advantages of the extract processes applied by performing a comprehensive content analysis of the extract products obtained.

The aim of this study was to test in detail the valuable components present in chestnut bark under different processing conditions. For this purpose, chestnut shell residues were extracted for the first time using two different processes. The liquid product contents obtained from the processes were compared using some advanced analytical methods. The data obtained showed that the type of process and the operating conditions are very important in obtaining certain compounds. The presence of various compounds such as phenolic compounds, organic compounds and volatile compounds in the extracts was explained using Gas Chromatography-Mass Spectrometry (GC-MS), Liquid Chromatography-Mass Spectrometry (LC-MS/MS) and Fourier Transform Infrared Spectroscopy (FT-IR) analyzers.

MATERIALS AND METHODS

Materials

The chestnut shells used in the experimental study were supplied by the İlka Confectionery Factory in Bursa province. The chestnut shells were dried at room temperature and ground to a certain grain size. The chemical reagents methanol, acetone, ethyl acetate, ether and hexane used in this study were purchased from Sigma Aldirch and were of analytical purity. All chemicals and glassware used in this study were handled with care and under the necessary conditions.

Preliminary stages before experimental studies on chestnut shells

First, chestnut shells were left to dry under room conditions. Then the dried shell products were ground to the smallest grain size. The ground sample was washed abundantly with deionized water to

remove impurities. Then it was dried in the oven at 60 °C for two days. Dried chestnut products were stored in a moisture-free environment at approximately 24 degrees for different processes such as pyrolysis and extra.

Approaches followed

Extraction Process

50 grams of chestnut shells, previously ground to a certain size, were placed in a glass conical flask. Then, methanol-chloroform (1:1 v/w) solvent solution was prepared on the chestnut sample. The prepared solution was slowly poured onto the glass conical flask containing chestnut shells. It was kept closed in a dark environment for a week. The solvent mixture that reached the desired consistency was filtered. The obtained liquid sample was removed from the solvent using an evaporator device. Samples were taken from the solid-liquid mixture remaining at the bottom. The core sample taken was dissolved in 1 mL methanol and finally 1 mL hexane solvent was added (Başar et al., 2023; Yenigün et al., 2024). After phase separation was achieved, the hexane phase was analyzed using a GC-MS device, and the methanol phase was analyzed using an LC-MS/MS advanced technical device (Başar et al., 2024)

Pyrolysis Process

Pyrolysis processes were carried out using a specially designed fixed-bed pyrolysis (reactor) device in an inert nitrogen atmosphere designed by Defne Engineering Laboratory Devices (DefneLAB). The reactor chamber has an inner diameter of 600 x 50 mm and is made of stainless steel. The pyrolysis device operates under conditions that can accommodate 100g of solid product into the feed reactor chamber. Chestnut sample was exposed to Nitrogen gas at 450 ^{o}C for 120 min at a heating rate of 5 ^{o}C/1 min in an inert environment (Nas, et al., 2024). Then, liquid samples were collected in closed bottles in parallel cooler collection tanks. Liquid samples were dissolved homogeneously in 1/1 ml solution (methanol/hexane).

Instruments and Analyses

LC-MS/MS analysis

The samples used in the study were analyzed using highly sensitive high-performance liquid chromatography (HPLC) and Liquid Chromatography/Mass/Spectrometry (LC-MS/MS) devices. It was performed with a custom-designed reverse-phase analytical column Agilent Poroshell 120 EC-C18 (100 mm x 3.0 mm, 2.7 µm). Two solutions were prepared under two conditions: Solvent A (pure water + 5 mM ammonium formate + 0.1% formic acid) and Solvent B (methanol + 5 mM ammonium formate + 0.1% formic acid). The solvent flow rate (0.400 mL/min) and injection volume (4 µL) were adjusted at specified conditions [13,14]. Content analysis was performed based on 41 standard phenolic compounds: LC-MS/MS standard chromotogram (Shikimic acid compound (**1**), Gallic acid compound (**2**), Protocatechuic acid compound (**3**), Epigallocatechin compound (**4**), Catechin (**5**), Chlorogenic acid compound (**6**), Hydroxybenzaldeyde compound (**7**), Vanillic acid compound (**8**), Caffeic Acid compound (**9**), Syringic acid (**10**), Caffein (**11**), Vanillin compound (**12**), *o*-Coumaric acid (**13**), Salicylic acid compound (**14**), Taxifolin (**15**), Resveratrol compound (**16**), Polydatine (**17**), Trans-ferulic acid (**18**), Sinapic acid compound (**19**), Scutellarin (**20**), *p*-Coumaric acid (**21**), Coumarin (**22**), Protocatehuic ethyl ester (**23**), Hesperidin (**24**), Isoquercitrin (**25**), Rutin (**26**), Quarcetin-3-xyloside (**27**), Kaempferol-3-glucoside (**28**), Fisetin (**29**), Baicalin (**30**), Chrysin compound (**31**), Trans-cinnamic acid (**32**), Quercetin compound (**33**), Naringenin (**34**), Hesperetin compound (**35**), Morin (**36**), Kaempferol (**37**), Baicalein compound (**38**), Luteolin (**39**), Biochanin A compound (**40**), Diosgenin compound (**41**) **(**Başar et al., 2024)

GC-MS analysis

Sample analyses was gas chromatography-mass spectrometry (GC-MS) ultra model Agilent 7000 instruments. Fixed oil, free oil, and other essential oil components in chestnut shell extracts and pyrolysis oil products were examined by GC-MS analysis (Agilent 7000,). In GC-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].

Fourier Transform Infrared (FT-IR) analysis

Fourier transform infrared (FT-IR) was used to elucidate the bond structure of the compound in the bio-oil samples using the Agilan Carry60 FT-IR device. Fourier transform infrared (FTIR) spectroscopy is a method that allows the identification of bonds in the structure of molecules. This method works on the principle that infrared rays falling on intramolecular bonds are absorbed by the vibration and rotational movements of the bonds. The samples used in this study were scanned in the 4400 - 400 1/cm spectrum range with a Fourier transform infrared (FT-IR) device

RESULTS AND DISCUSSION

LC-MS/MS Analysis Results

LC-MS/MS device was used to examine the phenolic compound content of liquid samples obtained by pyrolysis of chestnut shells and aqueous extract processes. The types of phenolic compounds formed by the pyrolysis and aqueous extraction process are summarized in Table 1. Among the 41 phenolic compounds investigated, valuable compounds, especially catechin (2526.96 µg/g extract), gallic acid (175.02 µg/g extract), and protocatechuic acid (76.47 µg/g extract), were produced in high amounts in the aqueous extract process. was detected (Figure 2). However, the absence of any phenolic compounds in the pyrolysis process showed that the structure of phenolic compounds was affected or even completely deteriorated at high temperatures (Başar & Erenler, 2024).

Table 1. Phenolic content analysis of chestnut shell by Extract and pyrolysis methods

No	Compound	RT	Pyrolysis chestnut peel (µg/g liquid Extract)	extract chestnut peel (µg/g liquid Extract)
1	Gallic acid	3.15	-	175.02
2	Protocatechuic acid	5.33	-	76.47
3	Catechin	6.74	-	2526.96
4	Hydroxybenzaldeyde	7.50	-	14.48
5	Chlorogenic acid	7.38	-	14.63
6	*p*-coumaric acid	9.26	-	19.46
7	Salicylic Acid	10.31	-	27.90
8	Naringenin	14.91	-	19.39
9	Diosgenin	23.69	-	7.49

RT: Retention time

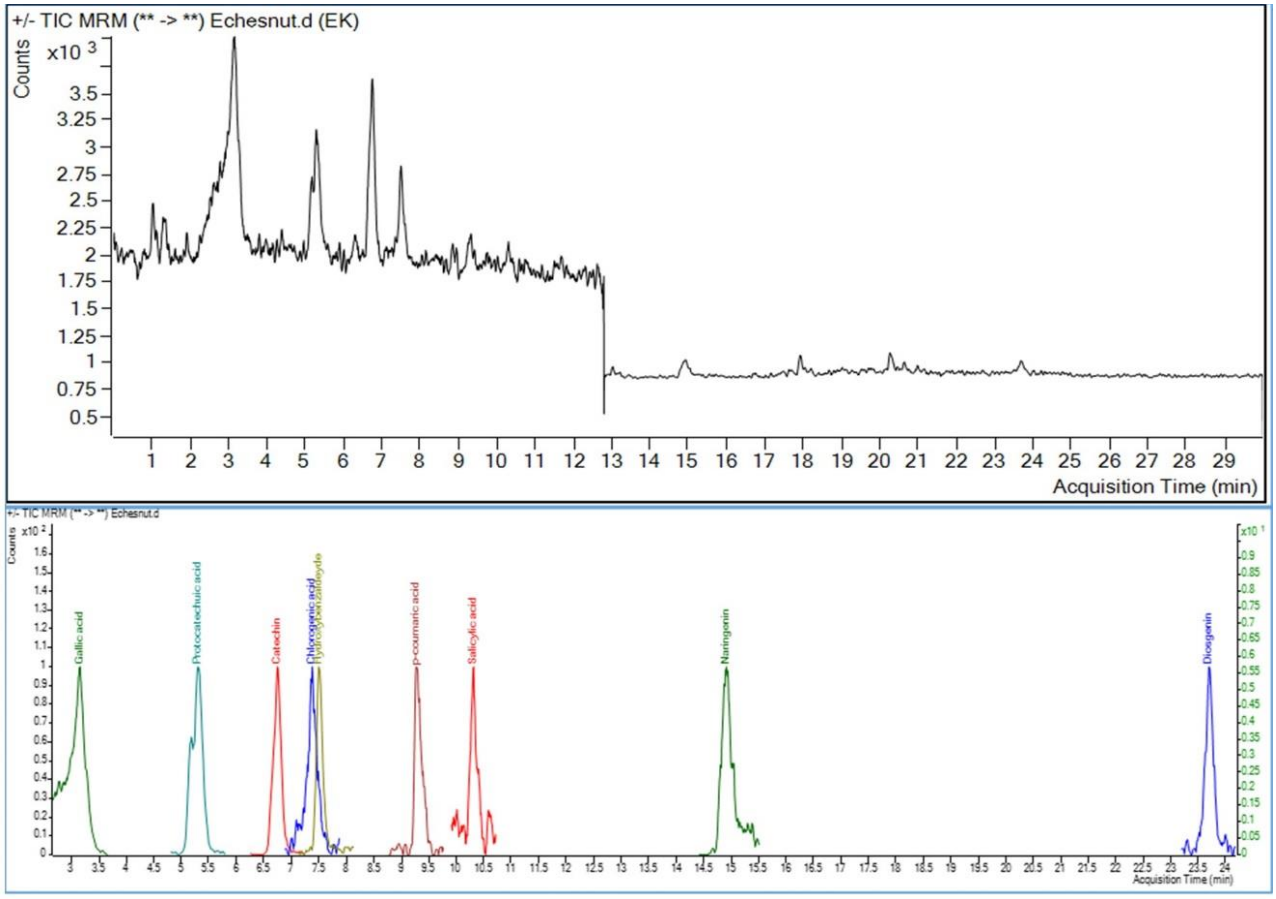

Figure 2. LC-MS/MS chromatogram of methanol-chloroform extract derived from chestnut shells

FTIR Analysis Results

FTIR analysis results of liquid samples obtained from the pyrolysis experiments performed at 550 ◦C and chestnut shells extract are illustrated in Figure 3. FT-IR analyses of the obtained extract and pyrolysis liquid samples show different peaks in the range of 500-400 1/cm. The FT-IR 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 1/cm. The vibration peaks in these ranges show different functional groups such as alcohol, phenols, and carboxylic acid. Moreover, the value of 2922 1/cm for both aqueous extracts and pyrolytic oils shows a peak band of -OH stretching (Uzun & Yaman, 2014). A sharp peak around the 1706 1/cm 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 1/cm 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 1/cm 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 (Hanafiah et al., 2012; Yang et al., 2013).

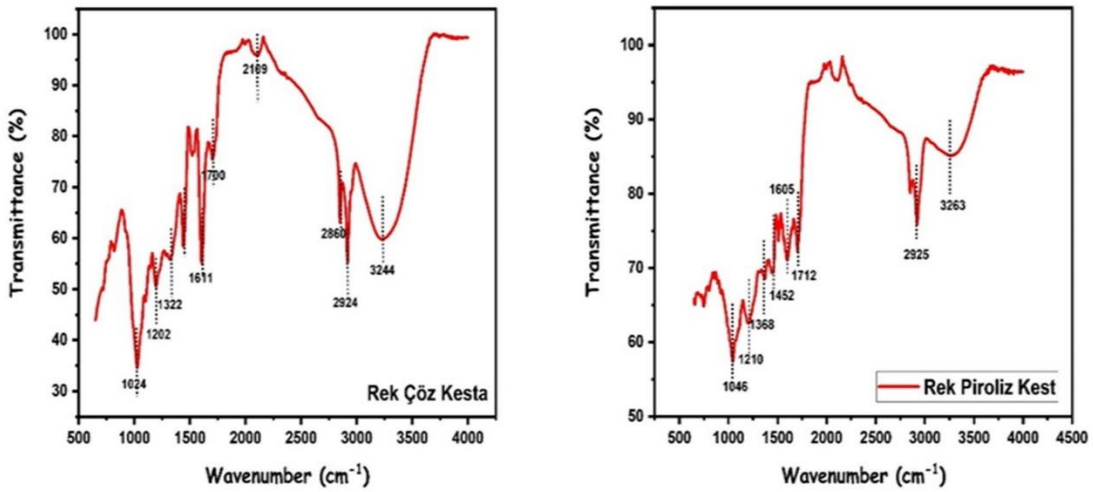

Figure 3. FT-IR analysis results of pyrolysis bio-oil obtained using chestnut shells and the products resulting from extraction

GC-MS Analysis Results

GC-MS analysis results of liquids and pyrolysis of chestnut shell extracts are given in Figure 4. According to the analysis results, palmitic acid methyl ester (27.81%), stearic acid methyl ester (28.78%), and m-Ethyltoluene (5.37%) compounds were detected in the liquid extract. High amounts of stearic acid methyl ester (8.89%), palmitic acid methyl ester (23.06%), Linoleic acid, methyl ester (32.45%), and Palmitic acid, methyl ester (23.06%) compounds in the pyrolysis liquid. has been detected. The liquid GC-MS results obtained from the extract and pyrolysis process, it was determined that the values of some compounds increased and some decreased (Table 2). However, some peaks seen in RT do not have corresponding chemical compound values. I think this situation may be related to the device to which the sample was given. Because we can see the compounds corresponding to the peaks according to how many standard compounds are defined for the examined device. We can explain this situation by the fact that hemicellulose and cellulose easily decompose due to the breakage of the structure of the material exposed to high temperatures in the pyrolysis process, and as a result, gaseous products, ketone and hydrocarbon compounds, carboxylic acids, aldehyde products and other compounds found in bio-oil are formed (Figure 4). A similar situation was observed in previous results (Ahmed et al., 2018; Naik et al., 2014)

Table 2. GC-MS analysis of chestnut shell

No	RT	Compounds	E-chestnut %	P-chestnut %
1	7.80	Ethylbenzene	-	0.25
2	7.82	m-Xylene	2.28	1.15
3	8.08	o-Xylene	9.29	0.46
4	8.92	???	3.38	-
6	11.30	???	0.87	-
7	11.64	m-Ethyltoluene	5.37	-
8	11.68	p-Ethyltoluene	-	0.58
9	11.93	Mesitylene	1.49	-
10	12.41	Pseudocumol	1.48	-
11	12.49	o-Ethyltoluene	6.24	0.59
12	13.04	o-Ethyltoluene-isomer	-	1.05
13	13.28	Decane	2.19	2.19
14	14.28	Hemimellitene	1.48	-
15	14.64	2-Ethylhexanol	0.54	2.10
16	15.60	*o*-Propyltoluene	0.54	-

Table 2. GC-MS analysis of chestnut Shell (Contiued)

18	15.89	3-Methyl-5-propylnonane	-	0.34
19	15.93	1,3-Dimethyl-5-ethyl benzene	0.55	-
21	17.16	1,2-Dimethyl-3-ethyl benzene	0.44	-
22	17.83	Undecane	1.37	1.49
23	22.43	Dodecane	1.32	0.98
24	26.01	2,6,11-Trimethyldodecane	-	0.63
26	31.12	Tetradecane	1.06	1.20
27	34.88	Pentadecane	-	1.00
28	36.45	2,6,10-Trimethyltetradecane	-	0.48
29	38.40	Hexadecane	0.95	1.57
30	41.74	Heptadecane	0.52	1.00
31	42.19	Myristic acid, methyl ester	-	0.51
32	42.97	2-Methylheptadecane	-	0.37
33	44.22	Octadecane	-	1.29
34	47.50	Palmitic acid, methyl ester	27.81	23.06
35	48.46	2-Methylnonadecane	-	0.40
36	49.24	Eicosane	-	0.84
37	51.52	Linoleic acid, methyl ester	0.44	32.45
38	51.65	Linolenic acid, methyl ester+Oleic acid, methyl ester	-	11.32
39	51.66	Oleic acid, methyl ester	1.73	-
40	51.75	Oleic acid, methyl ester-isomer	-	0.44
41	52.21	Stearic acid, methyl ester	28.78	8.89
42	53.96	Docosane	-	0.60
43	58.02	Arachidic acid methyl ester	-	0.80
44	60.86	Tetracosane	-	0.44
45	66.29	Behenic acid, methyl ester	-	1.24

RT: Retention time; E- chestnut: Extract chestnut; P- chestnut: Prolys chestnut

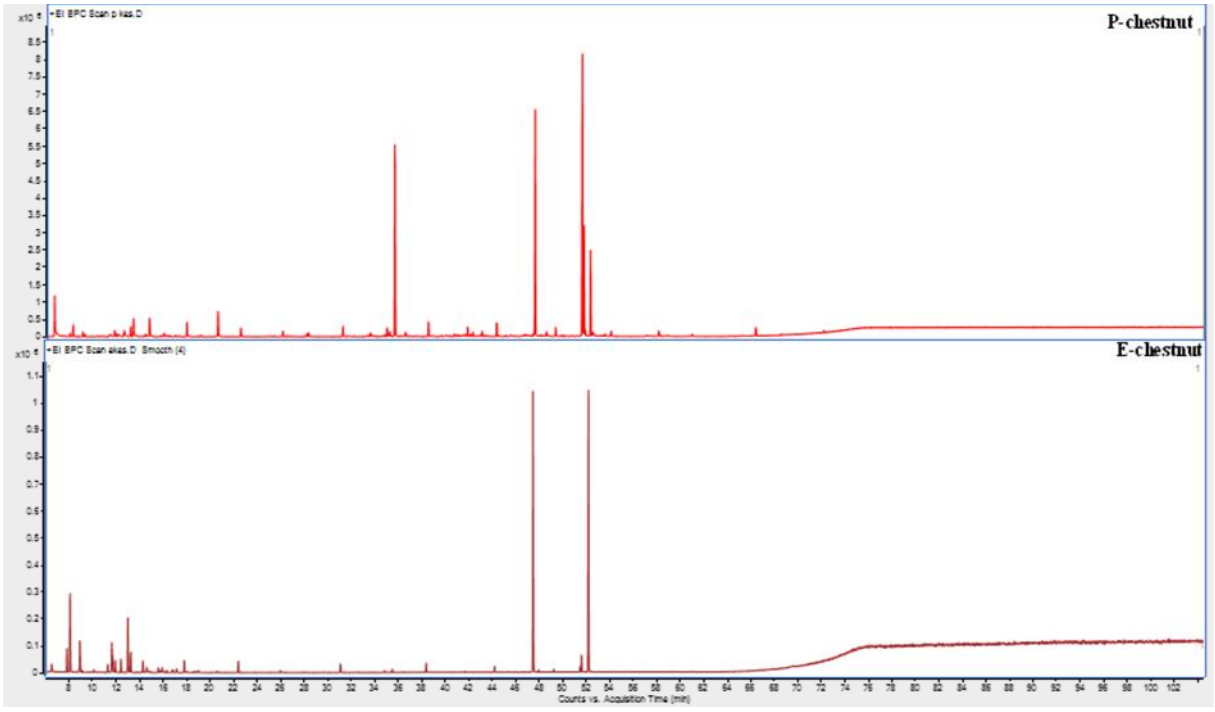

Figure 4. GC-MS chromatogram of chestnut shell methanol-chloroform extract and liquid of pyrolysis liquid

CONCLUSION

Chestnut shells are released into the environment as confectionery waste. It is extremely important to transform chestnut shells into value-added products. In this context, it is very valuable to elucidate the content of chestnut shells by aqueous extract and pyrolysis process. Liquid products obtained from two different processes were analyzed by analytical devices such as LC-MS/MS, GC-MS and FT-IR. Possible value-added compounds were identified by comparing the analysis results. Accordingly, it was concluded that the type and amount of phenolic compounds in chestnut bark extracts were higher than

the product obtained by pyrolysis process. The phenolic compounds and their amounts were catechin (2526.96 µg/g extract), gallic acid (175.02 µg/g extract), and protocatechuic acid (76.47 µg/g extract. Volatile component analysis of the liquid samples was performed by GC-MS. In the GC-MS analysis of the extract, it was determined that the pyrolysis method would contribute more to the detection and yield of volatile components. In addition, FT-IR was used to elucidate the functional structure of the samples obtained in both processes. These process products will be useful for those working in various fields such as biofuels, biochemicals, pharmaceuticals, food, textiles and cosmetics. As a result; Chestnut shell wastes will be processed and evaluated and will make an important contribution to the economy and will direct future perspectives.

ACKNOWLEDGEMENTS

This work was funded under the Specialization Project number YIP0324I06.

Conflict of Interest

The article authors declare that there is no conflict of interest between them.

Author's Contributions

In this study, experimental studies were carried out by Assoc. Prof. Dr. Mehmet Salih NAS and Researcher Yunus BAŞAR. The evaluation of the analysis results was carried out by Prof. Dr. İbrahim DEMİRTAŞ and Prof. Dr. Mehmet Hakkı ALMA.

REFERENCES

Ahmed, N., Zeeshan, M., Iqbal, N., Farooq, M. Z., & Shah, S. A. (2018). Investigation on bio-oil yield and quality with scrap tire addition in sugarcane bagasse pyrolysis. *Journal of Cleaner Production*, *196*, 927–934. https://doi.org/10.1016/J.JCLEPRO.2018.06.142

Barreira, J. C. M., Ferreira, I. C. F. R., Oliveira, M. B. P. P., & Pereira, J. A. (2008). Antioxidant activities of the extracts from chestnut flower, leaf, skins and fruit. *Food Chemistry*, *107*(3), 1106–1113. https://doi.org/10.1016/J.FOODCHEM.2007.09.030

Başar, Y., & Erenler, R. (2024). Phytochemical analysis of Silybum marianum flowers: Quantitative analysis of natural compounds and molecular docking application. *Turkish Journal of Biodiversity*, *7*(1), 20–31. https://doi.org/10.38059/BIODIVERSITY.1450643

Başar, Y., Hosaflıoğlu, İ., & Erenler, R. (2024). Phytochemical analysis of Robinia pseudoacacia flowers and leaf: quantitative analysis of natural compounds and molecular docking application. Turkish Journal of Biodiversity, 7(1), 1-10. https://doi.org/10.38059/biodiversity.1446241

Başar, Y., Nas, S., Alma, H., Demirtaş, İ., & Calimli, H. (2024). Chemical structure and characterization of bio-oils isolated from walnut shells by different processes. *Journal of Scientifi Reports-A*, *57*, 144–152. https://dergipark.org.tr/tr/pub/jsr-a

Başar, Y., Yenigün, S., Gül, F., Ozen, T., Demirtas, İ., Hakkı Alma, M., & Temel, S. (2024). Phytochemical profiling, molecular docking and ADMET prediction of crude extract of Atriplex nitens Schkuhr for the screening of antioxidant and urease inhibitory. *International Journal of Chemistry and Technology*, *8*(1), 60–68. https://doi.org/10.32571/IJCT.1389719

Başar, Y., Yenigün, S., İpek, Y., Behçet, L., Gül, F., Özen, T., Demirtaş, İ.,. (2023). DNA protection, molecular docking, enzyme inhibition and enzyme kinetic studies of 1,5,9-epideoxyloganic acid isolated from Nepeta aristata with bio-guided fractionation. *Journal of Biomolecular Structure and Dynamics*. https://doi.org/10.1080/07391102.2023.2250461

Capanoglu, E., Nemli, E., & Tomas-Barberan, F. (2022). Novel Approaches in the Valorization of Agricultural Wastes and Their Applications. *Cite This: J. Agric. Food Chem*, *2022*, 6787–6804. https://doi.org/10.1021/acs.jafc.1c07104

Hanafiah, M. A. K. M., Ngah, W. S. W., Zolkafly, S. H., Teong, L. C., & Majid, Z. A. A. (2012). Acid Blue 25 adsorption on base treated Shorea dasyphylla sawdust: Kinetic, isotherm, thermodynamic and spectroscopic analysis. *Journal of Environmental Sciences*, *24*(2), 261–268. https://doi.org/10.1016/S1001-0742(11)60764-X

Melikoglu, M., Lin, C. S. K., & Webb, C. (2013). Analysing global food waste problem: Pinpointing the facts and estimating the energy content. *Central European Journal of Engineering*, *3*(2), 157–164. https://doi.org/10.2478/S13531-012-0058-5/MACHINEREADABLECITATION/RIS

Morrone, L., Dall'Asta, C., Silvanini, A., Cirlini, M., Beghè, D., Fabbri, A., & Ganino, T. (2015). The influence of seasonality on total fat and fatty acids profile, protein and amino acid, and antioxidant properties of traditional Italian flours from different chestnut cultivars. *Scientia Horticulturae*, *192*, 132–140. https://doi.org/10.1016/J.SCIENTA.2015.04.018

Naik, D. V., Kumar, V., Prasad, B., Poddar, M. K., Behera, B., Bal, R., Khatri, O. P., Adhikari, D. K., & Garg, M. O. (2014). Catalytic cracking of jatropha-derived fast pyrolysis oils with VGO and their NMR characterization. *RSC Advances*, *5*(1), 398–409. https://doi.org/10.1039/C4RA08128E

Piazza, S., Martinelli, G., Fumagalli, M., Pozzoli, C., Maranta, N., Giavarini, F., Colombo, L., Nicotra, G., Vicentini, S. F., Genova, F., De Fabiani, E., Sangiovanni, E., & Dell'Agli, M. (2023). Ellagitannins from Castanea sativa Mill. Leaf Extracts Impair H. pylori Viability and Infection-Induced Inflammation in Human Gastric Epithelial Cells. *Nutrients*, *15*(6), 1504. https://doi.org/10.3390/NU15061504/S1

Suna, S., Avşar, B., Koçer, S., & Çopur, Ö. U. (2021). Effects of different pretreatments on the physicochemical characteristics and quality criteria of chestnut (Castanea sativa Mill.) pickle: A new value-added product. *Journal of Food Processing and Preservation*, *45*(7), e15669. https://doi.org/10.1111/JFPP.15669

Uzun, B. B., & Yaman, E. (2014). Thermogravimetric characteristics and kinetics of scrap tyre and Juglans regia shell co-pyrolysis. *Https://Doi.Org/10.1177/0734242X14539722*, *32*(10), 961–970. https://doi.org/10.1177/0734242X14539722

Vella, F. M., Laratta, B., La Cara, F., & Morana, A. (2018). Recovery of bioactive molecules from chestnut (Castanea sativa Mill.) by-products through extraction by different solvents. *Natural Product Research*, *32*(9), 1022–1032. https://doi.org/10.1080/14786419.2017.1378199

Yang, J. L., Fang, F., & Zhou, J. Bin. (2013). Effect of microstructure and surface chemistry in liquid-phase adsorptive nicotine by almond-shell-based activated carbon. *Chinese Science Bulletin 2013 58:30*, *58*(30), 3715–3720. https://doi.org/10.1007/S11434-013-6040-7

Yenigün, S., Başar, Y., İpek, Y., Behçet, L., Özen, T., & Demirtaş, İ. (2024). Determination of antioxidant, DNA protection, enzyme inhibition potential and molecular docking studies of a biomarker ursolic acid in Nepeta species. *Journal of Biomolecular Structure and Dynamics*, *42*(11), 5799–5816. https://doi.org/10.1080/07391102.2023.2229440