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Effect of ultrasound on the extraction of phenolic compounds and antioxidant activity of different parts of walnut fruit

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Abstract

Walnuts have a high nutritional value because of their high levels of essential compounds for human health. Phenolic compounds have beneficial properties, including anti-cancer and antimicrobial properties. In this study, the amount of the extracted antioxidants from different parts of walnut, including the walnut kernel, hard shell and green husk by ethanol and water was compared with those of the ultrasound-assisted extraction. This study was performed by determining the amount of polyphenols present and the free radical scavenging power of DPPH. The results showed that the effect of all factors was statistically significant at 99% statistical level. The highest rate of extraction of phenolic compounds (1.09 mg Gallic acid/g) and the highest rate of free radical scavenging of DPPH (6.86%) was related to the use of ethanol solvent for extraction. It was also shown that the hard walnut shell has the highest amount of phenolic compounds (1.1 mg Gallic acid per gram of extract) and the walnut kernel has the highest antioxidant properties (7.99%). Ultrasonic pretreatment increased the extraction efficiency of phenolic compounds and antioxidant properties so that this process increased the extraction of phenolic compounds from the green husk of walnut by 1.22 mg Gallic acid/g and increased the antioxidant properties of the kernel walnut by 13.51% compared to other parts.

Keywords: Antioxidant activity, Ultrasonic extraction, Walnut husk, Walnut waste.

Introduction

Walnut (*Juglans regia* L.) is popular all over the world for its high nutritional value and health benefits. Different parts of walnut fruit, including green walnut husk, hard shell and its leaves are also used in various industries including food, cosmetics, health and pharmaceutical industries. Green husk and hard shell are considered agricultural wastes, while various uses may be created for them.Volatile compounds and phenols of walnut green skin have anti-inflammatory and antibiotic properties. On the other hand, tannins in walnut green skin have been shown to have antioxidant and antimicrobial properties. The hard, dark inner shell of the walnut, which divides the kernel into four parts, has been used in traditional Iranian medicine to treat high blood triglycerides (Dolatabadi et al., 2014; Vahdat Shariatpanahi et al., 2013). In 2019, Iran produced 321074 tonnes of walnuts and ranked third in the world (FAO, 2020). Walnut kernels are sensitive to oxidation due to their high levels of unsaturated fatty acids (Ziaolhagh et al., 2020). There is no official report of the amount of walnut waste and loss, but it is

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estimated that about 20% of the produced walnuts are wasted because of their small size, molding and oxidation of walnut oils. On the other hand, the green husk and the hard shell contributes about 66% of the walnut fruit which are discarded after shelling the walnuts (Banaian, 2010). Researches have been done to reduce the waste and loss of nuts by proper packaging (Ziaolhagh, 2012; Ziaolhagh et al. 2020), but the waste are yet high.

Walnut kernels are rich in antioxidants such as tocopherols and phenolic compounds (namely proanthocyanidine, tannin and flavonoid) and contain more phenolic compounds than other nuts and thus can be considered a functional food (Kulacanin et al., 2020). Squalene is another compound in the walnut kernel that has antioxidant properties. Regular consumption of walnuts reduces cancer and cholesterol due to the presence of antioxidant compounds and omega- 3 and omega- 6 acids (Mohagheghi et al., 2010). Fernández-Agulló et al. (2020) recommended walnut leaves as a source of polyphenol compounds and antioxidant properties for industrial applications.

Oxidation is one of the most important and well-known causes of lipid degradation during storage or processing. The synthetic antioxidants BHA, BHT and TBHQ are used to delay or slow down the oxidation reaction process. Because their role in the development of diseases such as cancer and cardiovascular and liver diseases is well known, the replacement of these compounds with natural antioxidants of plant origin has received much attention (Ghaderi et al., 2012).

Factors such as solvent type and extraction time are very important in the extraction process of phenolic compounds. In addition, extraction by conventional methods such as soxhlet extraction and immersion requires a long time and a large amount of solvent. However, Noshirvani et al. (2015) showed that the Soxhlet extraction method leads to higher levels of polyphenols from the green husk of walnuts than the Maceration method. New extraction- assisted methods such as ultrasonic extraction and microwave extraction are fast and reliable methods for extracting effective compounds from plant tissue (Nasirifar et al., 2013).

Researchers have applied various methods to extract phenolic compounds from agricultural materials. Microwave- assisted extraction has been used for extracting phenolics from walnut leaves (Rezai Erami et al. 2015) and peanut shells (Ballard et al., 2010). Ultrasound-assisted extraction is another method that has been investigated by researchers on the extraction of phenolic, flavonoid compounds and the inhibitory power of DPPH free radicals from Daghdaghan fruit (*Celtis australis*) (Nasirifar et al., 2013), milled olive kernels (Jiménez et al., 2007), walnut leaf (Sanadgol et al., 2018), rosemary leaf (Rodriguez- Rojo et al., 2012) and pistachio green hull (Goli et al., 2005).

Polar or non-polar solvents may be used as extraction mediums. Dolat Abadi et al. (2014) investigated the effect of region, solvent and extraction time on the extraction efficiency of walnut green phenolic compounds by immersion at ambient temperature. They showed that these factors affected the amount of extracted phenolic compounds, and the activity and antioxidant properties depend on the concentration of phenolic compounds in the extract. Dolat Abadi et al. (2017b) found that a solution of 1000 ppm of walnut green husk extract showed more antioxidant activity than the synthetic antioxidant of BHA with a concentration of 200 ppm.

The results of many studies have shown that plants with high phenolic and flavonoid compounds have high antioxidant activity. The extracts of walnuts inner shells and green husk have a high antioxidant activity (Mirzaee et al., 2016). Oliveira et al. (2008) investigated the effect of solvents with different polarities on the properties of walnut green shell extract and reported that a mixture of polar and non- polar solvents increases the extraction efficiency of phenolic compounds and leads to increased antioxidant activity. As mentioned earlier, all parts of walnut fruit may be used for different purposes. The green husk and the hard shell are the parts that are disposed as waste, while they may contain phenolic compounds with antioxidant properties. In the current study, to avoid the disposal of walnut husk and shell, we aimed to discover which parts of the walnut fruit have the most antioxidant activity and if the ultrasonic treatment could help the extraction of phenolics from different parts of the walnut fruit.

Materials and methods

In this study, walnuts were prepared from Dibaj area in Damghan, Semnan. Then the different parts of the walnut, including the walnut kernel, hard shell and green husk, were completely dried in an oven at 40ºC. Walnut kernels, hard shell and green husk were completely crushed after drying with a laboratory mill (Naniwa N 95, Iran).

For extraction, the crushed samples were mixed separately with water and ethanol (1:5) and stirred at room temperature for 48 h. Based on other studies, the extraction yields of ethanol and water in extracting total phenolic compounds are higher and these solvents are safer and less toxic compared to methanol and other organic solvents. Thus we selected them as the extraction solvents (Wang et al., 2020; Han et al., 2018; Chew et al. 2011). Two samples were taken from each extract. After 48 hours, a sample of each extract was passed through a strainer, and after filtration to remove the solvent, the aqueous and the ethanolic extracts were placed in a rotary evaporator (Heidolf, 4000, Germany) for 5 and 2 hours respectively. The concentrated extract was then placed in an oven (Froilabco, France) at 40℃ for 4 days to completely remove the solvent. The second sample of the extract was treated by ultrasound (Sonica, Italy) with a frequency of 100 kHz and a temperature of 25± 3℃ for 1 h.

To determine the per cent of total polyphenols in the extract, 0.01 g of the extract was mixed with 60% methanol and the resulting solution was made up to 10 ml with distilled water. One millilitre of this diluted extract was mixed with 1 millilitre of 10% Folin-Ciocalteu reagent and after 3 min, one mL of saturated sodium carbonate was added to the solution to make a volume of 10 ml with distilled water. The final solution was then placed in a dark environment for 90 minutes and its absorption was measured by a spectrophotometer (Genoa, UK) at a wavelength of 725 nm, which indicates the per cent of antioxidants extracted from the extract. Results were expressed based on mg of gallic acid per 100 g of dried sample (Rahimipanah et al., 2011).

DPPH method was used to measure the free radical scavenging power of antioxidants. To prepare a 0.4 M DPPH solution, 0.004 g of DPPH was mixed with 100 ml of methanol. Then 2 ml of the extract was mixed with 2 mL of DPPH solution prepared by the above method and kept in the dark for 30 minutes, after which the absorbance was measured at 517 nm (Yang et al., 2014). Then, using Equation 1, the percentage of free radical scavenging was calculated.

$$\text{percentage of free radical scavenging} = \frac{(AC-AS)}{AC} \times 100 \qquad (1)$$

Where Ac and As are the adsorption of the control (without extract) and the sample, respectively.

The results were analyzed using factorial experiments in a completely randomized design with three replications. Factors included walnut parts at three levels (kernel, green husk and hard shell), solvent type at two levels (water and ethanol) and pretreatment at two levels (with or without ultrasound assistance). After analyzing the variance, the means were compared by Duncan's multiple range test. SPSS16 software was used to analyze the data and Excel software was used to draw the graphs.

Results and discussion

Total polyphenols

Table 1 shows that the effects of all treatments on the total phenols of the extracts are significant. The extracts of different parts of the walnut showed different amounts of phenolic compounds. The highest and the lowest amounts of phenolics were found in the hard shell (1.11 mg Galic acid/g extract) and the green husk (0.76 mg Galic acid/g extract) of the walnuts, respectively (Fig.1). The results showed that the ethanol solvent was more effective in extracting the phenolic compounds from different parts of the walnuts than water solvent (Fig. 2). This is related to the different polarity of solvents that affects the extraction of phenolic compounds (Rezai Erami et al. 2015). The use of water as the extraction solvent creates a completely polar environment in which some phenolic compounds with lower degrees of polarity are extracted to a lesser extent, but adding water to organic solvents is accompanied by the formation of a relatively polar environment, and more types of phenolic compounds are ensured in these conditions. In addition, the aqueous extract contains large amounts of impurities such as organic acids, proteins and soluble sugars that can interfere with the detection and quantification of phenolic compounds (Chirinos et al., 2007). Fig 2. The effect of solvent type on the total phenolic compounds. Columns with similar letters are not significant. In the case of interaction between walnut parts and solvent type, the highest amount of phenolic compounds was observed for aqueous extract of walnut kernels, and the least was found for ethanolic extract of walnut kernels. The ethanol- soluble phenolics of the green husk were more than that of other samples (Fig. 3). Rezai Erami et al. (2012) showed that the microwave-assisted extraction of walnut green husk leads to increase the extracted phenols and in this method, the alcoholic solvent was more effective than the aqueous solvent in extracting the phenolics. In this regard, Noshirvani et al (2015) showed that the extraction method affected the extracted phenolics from the green husk of walnuts. They also showed that less concentration of green husk extract is more effective than higher concentrations in reducing oxidation.

Our results showed that the ultrasonic pretreatment was effective in extracting the phenolic compounds from all parts of the walnut, and the higher phenolics were obtained from the green husk. The extraction of phenolics from the hard shell was difficult in the absence of ultrasonic pretreatment (Fig. 4). It is attributed to the increased permeability of the cells. Ultrasound speeds up the mass transmission and diffusion of the phenolic compounds from the walnut parts into the solvent. Vapour pressure increase by ultrasonic waves and this will help the penetration and transportation of the phenolic compounds into the solvent (Wang et al. 2020).

Table 1- Analysis of variance of treatments on total phenols and DPPH

Source	df	Mean Square	
		Total Phenols	DPPH
Model	12	2.057**	88.905**
Walnut Parts	2	0.248**	68.206**
Solvent Type	1	0.656**	110.510**
PreTreatment	1	0.378**	15.974**
Walnut Parts× Solvent	2	0.040**	84.494**
Walnut Parts× PreTreatment	2	0.823**	41.103**
Solvent type× PreTreatment	1	0.258**	9.985*
Walnut Parts× Solvent× PreTreatment	2	0.271**	13.411**
Error	12	0.005	1.541
Total	24		
R^2		0.997	0.983

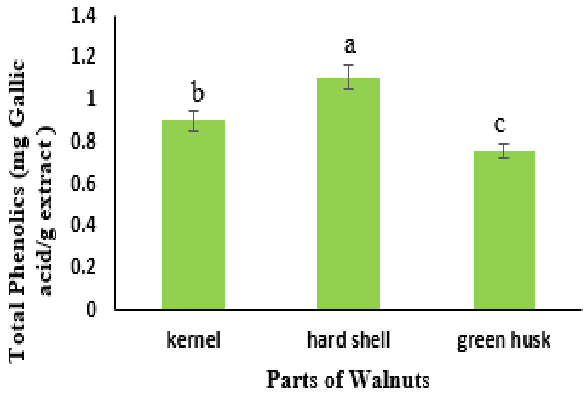

Fig. 1. Total phenolic contents of different parts of walnuts.
Columns with similar letters are not significant.

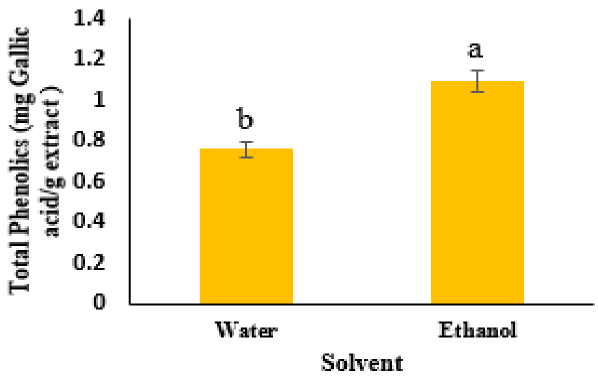

Fig. 2. The effect of solvent type on the total phenolic compounds.
Columns with similar letters are not significant.

In the ultrasonic- assisted extraction, the interaction between the ultrasound waves and the plant cells destruct the cell walls and more solvent penetrate into the cells. Ultrasound waves are sinusoidal, creating bubbles inside the environment that are full of solvent vapour. During the pressure cycle, these bubbles and the gas inside them compress and explode, increasing the pressure and temperature in the environment.

The result of the explosion of bubbles in the environment is a better mixing of solvent and plant material. In addition, ultrasound creates a mechanical force and increases the penetration of the solvent into the plant tissue. These two factors increase mass transfer and break down the cell wall (Albu et al. 2004). Ultrasound can also cause chemical changes due to the formation of free radicals during cavitation. (Paniwnyk et al., 2001). As a result, the penetration of solvents into the cells and mass transfer increases. Rapid changes in cavitation at temperature and pressure cause shear breakage and thinning of cell membranes, and these phenomena allow ultrasound to change the environment (Thompson and Doraiswamy, 1999). In accordance with the results of our research, Sanadgol et al. (2018), Rodriguez-Rojo et al. (2012) and Goli et al. (2005) respectively showed that ultrasound-assisted extraction is the best way to extract the phenolic compounds of walnut leaf, rosemary leaf and pistachio green hull. Dolat Abadi et al. (2017a) showed that the total phenolic content of the ultrasound- assisted extract of walnut green husk was more than that of extracted with the help of microwave or the maceration extraction. Ultrasound also reduces the particle size and increases the contact area, resulting in increased solvent diffusion into the tissue.

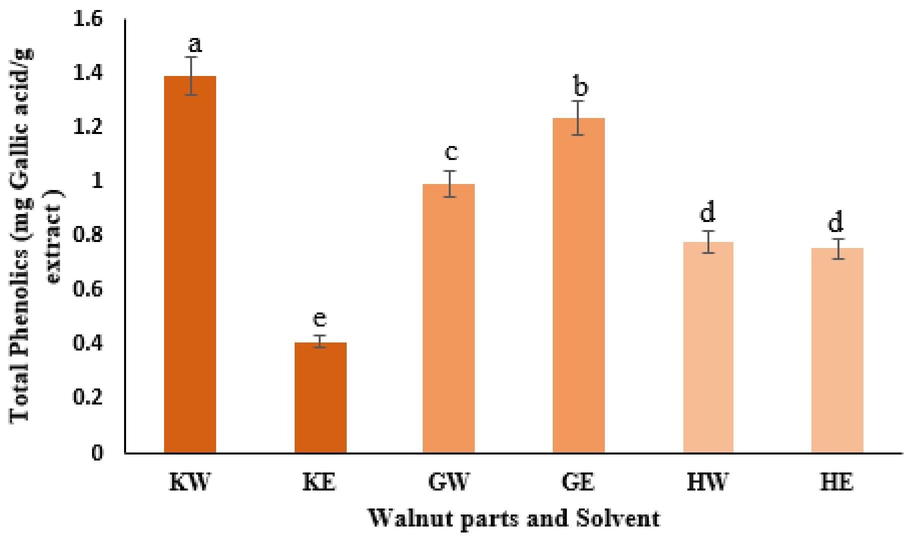

Fig. 3. The interaction effect of walnut parts and solvent on the amount of phenolic compounds. KW: Water extract from kernels, KE: Ethanol extract from kernels, GW: Water extract from green husk, GE: Ethanol extract from green husk, HW: water extract from hard shell and HE: Ethanol extract from hard shell.
Columns with similar letters are not significant.

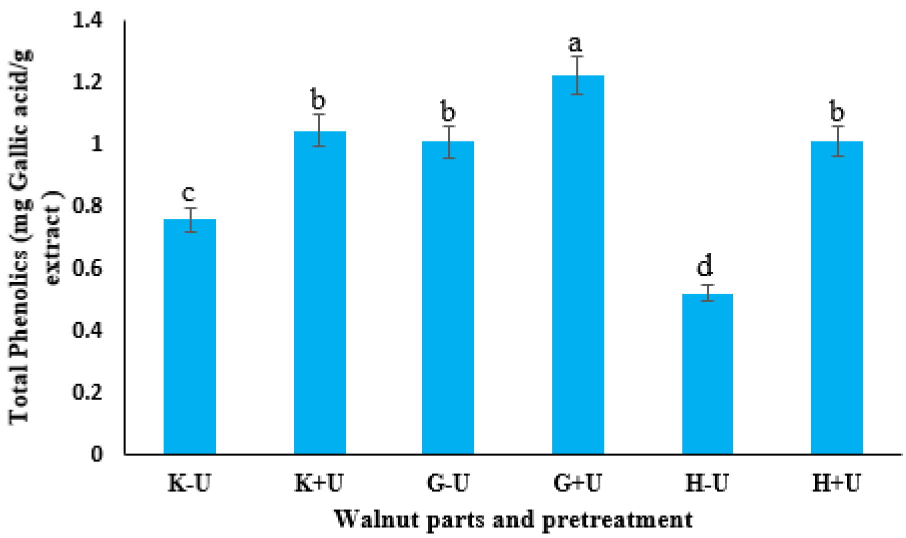

Fig. 4. The interaction effect of walnut parts and pretreatment on the amount of phenolic compounds. K-U:Kernels without ultrasonic pretreatment, K+U: Kernels with ultrasonic pretreatment, G-U: Green husk without ultrasonic pretreatment, G+U: Green husk with ultrasonic pretreatment, H-U: Hard shell without ultrasonic pretreatment and H+U: Hard shell with ultrasonic pretreatment.
Columns with similar letters are not significant.

DPPH radical-scavenging activity

As it is shown in Table 1, the effect of all factors on the DPPH radical- scavenging activity of the extracts was significant ($P<0.01$). The results showed that the kernel of walnuts had the highest DPPH radical-scavenging activity (7.9938%) followed by the hard shell (3.2287%) and the green shell (2.6875%) of the walnuts, but the difference between the DPPH radical- scavenging activity of the last two was not significant (Fig. 5).

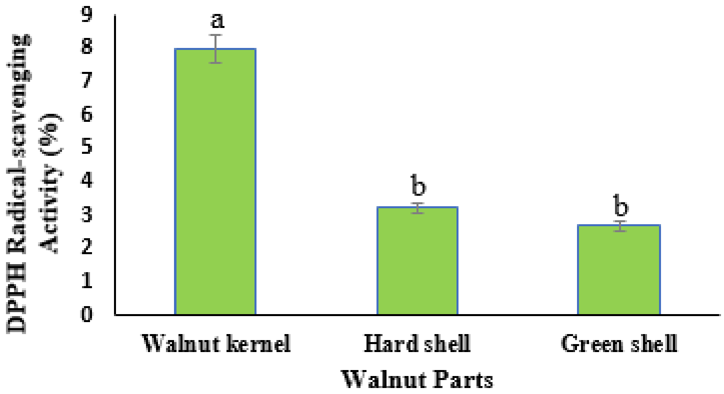

Fig. 5. The DPPH radical-scavenging activity of different parts of walnuts.
Columns with similar letters are not significant.

In addition, the ethanolic extract showed more DPPH radical- scavenging activity than the waterextract by 6.7625 and 2.4908% respectively (Fig. 6). In line with our results, Fernandez et al. (2013) showed that the highest inhibition of DPPH free radicals was related to the ethanolic extracts, which contained the most phenolic compounds. As the concentration of phenolic compounds increases, the inhibitory activity of DPPH free radicals increases, because at higher concentrations of phenolic compounds, due to the increase in the number of hydroxyl groups present in the reaction medium, the possibility of hydrogen donation to DPPH free radicals increases (Sun et al., 2007). Antioxidant activity is measured by different assays. Because the extracts contain different phenolic compounds, a number of them are identified in each method. Rezai Erami et al. (2012) showed that the antioxidant activity of the microwaved-assisted extracts were higher than that of macerated extracts, but in the conventional maceration method, the aqueous extract showed more antioxidant activity than the ethanolic extract.

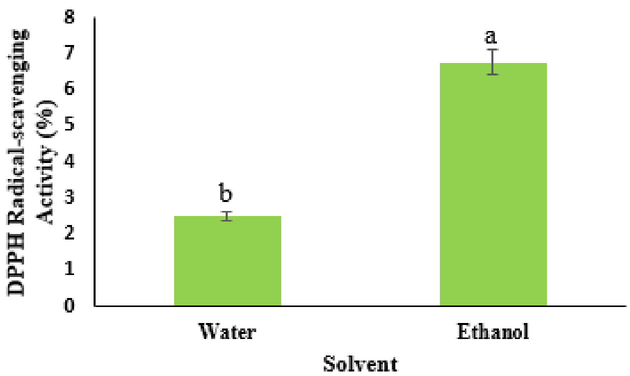

Fig. 6. The effect of solvent type on the DPPH radical-scavenging activity.
Columns with similar letters are not significant

The low level of non-polar compounds in plants is the reason for the low level of antioxidant activity of extracts prepared with non- polar solvents (Kamkar et al., 2010). In some cases, despite the high or low phenolic compounds, the antioxidant properties are not commensurate with these compounds, which indicates other influential factors that exist in these plants and affect the antioxidant properties during reactions (Mortazaie et al., 2013). Also, the phenolic content determined according to the Folin– Ciocalteu method is not a pure and definite measure of the amount of phenolic content. Different types of phenolic compounds have a variety of antioxidant activities that depend on their structure. Different plant extracts may contain different types of phenolic compounds that have different antioxidant capacities (Kaur et al. 2014).

DPPH radicals interact with antioxidants or other radicals and decrease them. The extract of different parts of walnut is rich in different phenolic compounds which may inhibit free radicals due to their hydroxyl groups positioned along the aromatic ring (Zhang et al., 2009). The potential of a solvent in dissolving a group of antioxidants vary with its polarity. The different antiradical activity of different extracts may be related to several reasons. Position of phenolic hydroxyl groups, presence of other functional groups in the molecule such as double bonds and combination of hydroxyl groups and ketone groups play an important role in antioxidant activity (Rezai Erami et al., 2015). Shabanian et al. (2021) found that with increasing concentration of ethanol solvent, the per cent of phenolic compounds undergo a whole partial downward trend. The reason is the polarity of pure ethanol compared to a mixture of ethanol and water because polar solvents have a higher ability to extract phenolic compounds from the cell structure of plants (Gharekhani et al., 2010). Since there is a direct relationship between radical receptor activity and the per cent of phenolic compounds in fruits, so by increasing the per cent of phenolic compounds in all extracts, it is expected that the percentage of active radical inhibitory DPPH of the extracts will increase (Shabanian et al., 2021).

Differences in the antioxidant activity of different methods depend to a large extent on the hydrophilic and hydrophobic nature of the phenolic compounds present and their proportions. The DPPH radical scavenging test measures the antioxidant activity of water-soluble phenolic compounds (Rezai et al., 2012). With the increasing concentration of ethanol solvent, the DPPH activity of ethanolic extract decreased (Shabanian et al., 2021).

Ultrasonic pretreatment was effective in increasing the DPPH radical-scavenging activity of the extracts ($p<0.01$). Fig. 7 shows that the DPPH radical- scavenging activity of the extracts of the kernels and hard shell was increased by ultrasonic pretreatment, and the most DPPH activity was observed for the water extracted samples pretreated with ultrasound, as it can be seen from Fig. 8. In our study, we found that the extraction method had a significant effect on both the total phenolic content and the DPPH radical- scavenging activity. The phenolic content of all samples extracted by ultrasound was higher than that of the samples extracted by dipping in solvents, with the highest for walnut green husk. However, the antioxidant activity of green husk extract was not influenced by ultrasonic treatment. The ultrasonic- treated extract of walnut kernels showed the highest DPPH radical-scavenging activity. In the ultrasound treated extracts, the aqueous extracts showed more DPPH radical- scavenging activity than the ethanolic extracts.

To extract the compounds, the cell membranes must be broken. Bubbles created by ultrasound waves create shear forces that mechanically break down cell walls and improve material transfer (Dolat Abadi et al., 2017a). Dolat Abadi et al. (2017a) showed the highest DPPH radical scavenging activity for the ultrasound-assisted extraction. Sanadgol et al. (2018) also found that the highest rate of inhibition of DPPH free radicals in walnut leaf extract was related to the ultrasound method. The free radical DPPH in an alcoholic medium has a maximum absorption of 517 nm and produces a purple color. If this radical is neutralized, the intensity of the purple color decreases and changes to pale yellow. Therefore, the reduction of light absorption will be proportional to the ability of the extracts to neutralize DPPH radicals, in other words, the antioxidant power of the sample. In line with our results, Gohari et al. (2018) showed that the antioxidant activity of extract from walnut interstitial tissue decreased during the ultrasound process. They related this fact to the increased extraction of impurities in ultrasonic treatment.

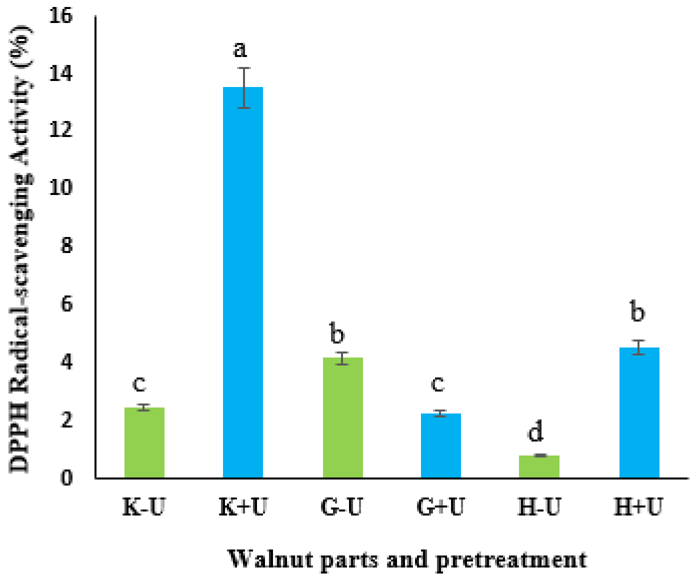

Fig. 7. The mutual effect of ultrasonic pretreatment and walnut parts on the DPPH Radical-scavenging activity. K-U:Kernels without ultrasonic pretreatment, K+U: Kernels with ultrasonic pretreatment, G-U: Green husk without ultrasonic pretreatment, G+U: Green husk with ultrasonic pretreatment, H-U: Hard shell without ultrasonic pretreatment and H+U: Hard shell with ultrasonic pretreatment.
Columns with similar letters are not significant

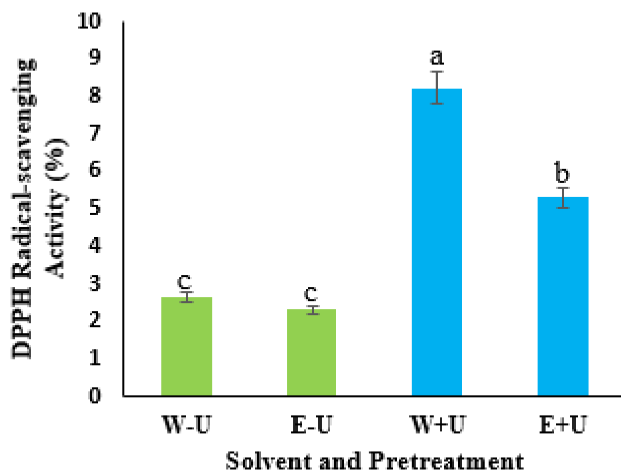

Fig. 8. The mutual effect of ultrasonic pretreatment and the solvent on the DPPH Radical-scavenging Activity. W-U: Water ectraction without ultrasonic pretreatment, E-U: Ethanol extraction without ultrasonic pretreatment, W+U: Water ectraction with ultrasonic pretreatment and E+U: Ethanol extraction with ultrasonic pretreatment.
Columns with similar letters are not significant

As it is shown in Fig. 9, the water extracts of kernels showed the most and the ethanol extract of the hard shell showed the least DPPH activity. Azadedel et al. (2018) found the lowest antioxidant activity of the pistachio green husk extract for the ultrasound-assisted extraction with ethanol solvent (which was in accordance with our results) but in the maceration method, the highest antioxidant activity was found for aqueous solvent. However, in our study, we found no significant difference between aqueous and ethanolic solvents. Contrary to our results, Azadedel et al. (2018) obtained the highest antioxidant activity of pistachio hull extract by maceration extraction in water solvent. Therefore, the soaking method is more efficient than the method of using ultrasound waves and causes less degradation of phenolic compounds with antioxidant power.

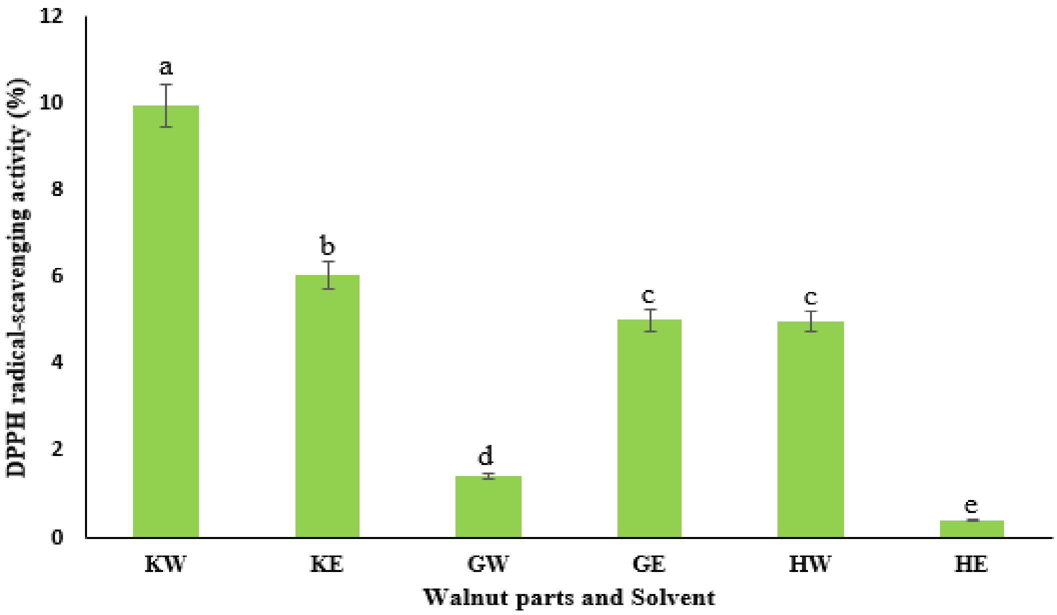

Fig. 9. The mutual effect of ultrasonic pretreatment and walnut parts on the DPPH Radical-scavenging Activity. KW: Water extract from kernels, KE: Ethanol extract from kernels, GW: Water extract from green husk, GE: Ethanol extract from green husk, HW: water extract from hard shell and HE: Ethanol extract from hard shell. Columns with similar letters are not significant.

Conclusion

Plants are a rich source of phenolic compounds (phenolic acids, flavonoids and tannins), which are the most important natural antioxidants. Antioxidants in the diet are important in protecting the body against oxidative stress and maintaining good health. In this study we found that ethanol was less effective than water in extracting phenolics from walnut kernels, but for green husk more phenolics were extracted by ethanol than water. For the hard shell of walnuts there were no significant difference between water and ethanol solvents. In the case of DPPH radical-scavenging activity the water extracts of kernels and hard shell were more effective than ethanolic extracts, but the ethanolic extract of green husk showed more antioxidant activity than aqueous extract.The results of the present study indicate that different walnut components (walnut kernel, green husk and hard shell) can be used as natural antioxidants. The green husk and hard shell of walnuts that are usually discarded as waste, are good sources of phenolics and could be used as an antioxidants source. Aqueous and ethanolic solvents are preffered for the extraction of phenolics from hard shell and green husk respectively. In addition, this study showed that ultrasound pretreatment increased the extraction yield of phenolics and antioxidant compounds.

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اثر فرایند اولتراسوند بر استخراج ترکیبات فنلی و فعالیت آنتی‌اکسیدانی بخش‌های مختلف گردو

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چکیده

گردو از نظر تغذیه، دارای ارزش غذایی بالایی است که برای سلامت انسان ضروری است. ترکیب‌های فنلی خواص سودمندی از جمله خاصیت ضدسرطانی دارند و از فعالیت میکروبی جلوگیری می‌کنند. استخراج به کمک امواج فراصوت (اولتراسوند) نسبت به سایر روش‌های استخراج مانند استخراج با مایکروویو هزینه کمتری دارد. در این پژوهش، میزان استخراج ترکیبات آنتی‌اکسیدان از قسمت‌های مختلف گردو شامل: مغز گردو، پوست چوبی و پوست سبز توسط حلال‌های اتانول و آب و با کمک دستگاه اولتراسوند مقایسه شد. این پژوهش از طریق ۲ آزمایش تعیین میزان پلی‌فنول‌های موجود و قدرت مهارکنندگی رادیکال آزاد DPPH اجرا شد. نتایج نشان داد که اثر تمام عوامل در سطح آماری ۹۹ درصد معنی‌دار بود. بیشترین میزان استخراج ترکیبات فنلی (mg Galic acid/g ۱/۰۹) و بیشترین میزان مهار رادیکال آزاد DPPH (۶/۸۶ درصد) مربوط به استفاده از حلال اتانول برای استخراج بود. همچنین نشان داده شد که پوسته سخت گردو بیشترین میزان ترکیبات فنلی (۱/۱ میلی‌گرم اسیدگالیک بر گرم عصاره) و مغز گردو بیشترین خاصیت آنتی‌اکسیدانی (۷/۹۹ درصد) را دارند. پیش‌تیمار اولتراسونیک باعث افزایش راندمان استخراج ترکیبات فنلی و خاصیت آنتی‌اکسیدانی شد، به‌طوری که این فرایند سبب افزایش استخراج ترکیبات فنلی از پوسته سبز گردو به میزان ۱/۲۲ mg Galic acid/g و افزایش خاصیت آنتی‌اکسیدانی مغز گردو به میزان ۱۳/۵۱ درصد نسبت به سایر قسمت‌ها گردید.

واژه‌های کلیدی: استخراج با اولتراسوند، پوست سبز گردو، گردو، فعالیت آنتی‌اکسیدانی.

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