Optimization of ultrasound-assisted extraction of ascorbic acid from fennel (*Foeniculum vulgare*) seeds and evaluation its extracts in free radical scavenging

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Abstract: The main objective of this study was to compare ultrasound assisted extraction with soxhlet extraction method as control in the extraction ascorbic acid of fennel seeds. Treatment conditions were performed using soxhlet method (240 min at 85°C) and ultrasound treatment (20 kHz, 39, 64 and 96 W cm^{-2}, 40°C and 60°C), for 15, 30 and 45 min. No significant differences were observed for total soluble solids among the samples studied. A similar trend was observed for both the amount of ascorbic acid extract and its ability to DPPH free radical scavenging. The greatest amount of ascorbic acid obtained in 15 minutes sonication (96 W cm^{-2} at 60°C), with equivalent of 1.73 mg mL^{-1} fennel seeds extraction. There was a significant difference between soxhlet and ultrasound method, which caused an increase of 69.94 mg mL^{-1} ascorbic acid in the extraction compared to the soxhlet. The highest percentage of hydrogen peroxide scavenging was observed in 15 min sonication (96 W cm^{-2} at 60°C) equivalent to 74.13% without any significant difference with soxhlet method (6.93% increase in free radical). The highest percentage of DPPH free radical scavenging was observed in 15 min sonication (96 W cm^{-2} at 60°C) equivalent to 98.88% without any significant difference with soxhlet method (0.18% increase in free radical). The results of the present study demonstrate that ultrasound assisted extraction is an alternative affordable for yield extraction compared to soxhlet method.

Keywords: ascorbic acid, DPPH, fennel, hydrogen peroxide, total soluble solids, soxhlet, ultrasound-assisted extraction

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1 Introduction

Fennel (*Foeniculum vulgare*) belonging to Apiaceae (Umbelliferae) family, is native to the Mediterranean areas (Zahid et al., 2009). The fruit is a dry and grooved seed from 4-10 mm long (Blamey and Grey-Wilson, 1989). Fennel is also used in folk medicine as a stimulant, diuretic, carminative and sedative (Charles et al., 1993). Nowadays it is cultivated for industrial uses such as cosmetic and pharmaceutical product. It is widely used for its medicinal properties (Lazouni et al., 2007). Fennel has shown antimicrobial, antioxidant, and anticholinesterase activity in vivo and in vitro experiments (Agarwal et al., 2013; Gulfraz et al., 2008). Phytochemical analysis of fennel showed the presence of terpenes, alkaloids, flavonoids, tannins, and saponins (Kaur and Arora, 2009).

Classical methods of extraction are based on the suitable placing of the plant in solvent which is used to speed up the process of mixing or heating. The soxhlet method is a standard one to asses other methods. This method is common which is mainly used for extraction compounds with low or moderate volatility that are stable

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against heat (Luque-Garcia and Luque de Castro, 2003).

The application of power ultrasound has been recognized as a promising processing technology to replace or complement conventional thermal treatment in the food industry. When high power ultrasound propagates in a liquid, cavitation bubbles are generated due to pressure changes. These micro bubbles collapse violently in the succeeding compression cycles of a propagated sonic wave resulting in localized high temperatures up to 5000 K, pressures up to 50,000 kPa, and high shearing effects (Suslick, 1988). The mechanism of ultrasound processing is mainly by physical (cavitational, mechanical effects) and/or chemical (formation of free radicals such as OH^-, H^+ due to sonochemical reactions). Advantages of sonication include reduced processing time, higher throughput and lower energy consumption while reducing thermal effects (Zenker et al., 2003). Ultrasound has been recognized for potential industrial application in the phyto-pharmaceutical extraction industry for a wide range of herbal extracts. Vinatoru (2001) published an overview of the ultrasonic-assisted extraction (UAE) of bioactive principles from herbs. The improvement in extractive value by UAE compared with classic methods in water and ethanol for fennel, hops, marigold and mint was 34%, 18%, 2%, and 3% respectively in water, whereas 34%, 12%, 3%, and 7%, respectively in ethanol.

Ascorbic acid (Vitamin C) is a water-soluble natural antioxidant that has been proposed to have beneficial effects on many age-related diseases such as atherosclerosis, cancer, neurodegenerative and ocular diseases (Rose et al., 1998). It is believed that ascorbic acid can scavenge reactive oxygen- and nitrogen species and thereby prevent oxidative damage to important biological macromolecules such as deoxyribonucleic acid (DNA), lipids and proteins (Whiteman et al., 1996). Ascorbic acid is thermo labile and highly sensitive to various processing conditions. The mechanism of vitamin C degradation follows aerobic and/or anaerobic pathways and depends upon several processing conditions (Vieira et al., 2000). Ascorbic acid and its derivatives are used in many foods for various purposes. Ascorbic acid has a potent antioxidant capacity by acting as a singlet oxygen quencher (Elliott, 1999). Ascorbic acid is also used as an index of the nutrient quality of fruit and vegetable products.

Shortcomings of existing extraction technologies, like high energy consumption and extraction time, possible degradation of bioactive compounds including ascorbic acid, and high consumption of harmful chemicals, have forced the food and chemical industries to find new separation ‘‘green’’ techniques, such as ultrasound extraction, which typically use less solvent and energy. Recently, UAE has been widely employed in the extraction of target compounds from different materials owing to its facilitated mass transfer between immiscible phases through super agitation at low frequency (Tsochatzidis et al., 2001). It offers high reproducibility at shorter times, simplified manipulation, and lowered energy input, as well as solvent consumption (Vilkhu et al., 2008). By using conventional extraction under ultrasound irradiation (20-100 kHz), structural changes and degradation of ascorbic acid can be avoided (Khan et al., 2010; Zhou and Ma, 2006). Thus, UAE may be an effective and advisable technique for the extraction of ascorbic acid.

So far, there is no report on UAE from fennel (*Foeniculum vulgare*) seed. The objective of this research is to compare ultrasound and conventional (soxhlet) methods by evaluation the efficiency of ascorbic acid and antioxidant properties of the extractions, which are extracted by these methods.

2 Materials and methods

2.1 Sample preparation

The effects of extraction methods using ultrasound on ascorbic acid extracted from the fennel seeds (FS) evaluated with FS collected from research farm of college of Aburaihan, University of Tehran in 2014.

All required chemicals including metaphosphoric acid, sodium bicarbonate, 2,6-dichloroindophenol, hydrogen peroxide, 1,1-diphenyl-2-picrylhydrazine (DPPH) and methanol were purchased from Merck company, Germany.

Two methods of soxhlet and ultrasound were applied to prepare FS extraction. In both methods the ethanol

70% solvent was prepared by mixing alcohol 97% and distilled water in a ratio of 73:27 mL (V/V). The ratio of 1:10 (W/V) for fennel powder/ethanol 70% were considered for all treatments.

The experiments were performed as a factorial in a completely randomized design with three replications. The outputs were evaluated for different treatment levels of ultrasound method including two levels of temperature (40°C and 60°C), three levels of extraction times (15, 30 and 45 min) and three levels of power (100, 200 and 300 W) in comparison with soxhlet at 85°C for 240 min as control. The ultrasound intensity was predicted according to Equation (1) (Li et al., 2004):

$$I = P/(\pi r^2) \quad (1)$$

where, I (W cm^{-2}) is the ultrasound intensity; P (W) is the input power, and r (cm) is the radius of the ultrasound probe. The ultrasonic powers were set at 100, 200 and 300 W, corresponding to ultrasonic intensities of around 39, 64 and 96 W cm^{-2}, respectively.

2.2 Ultrasound-assisted extraction

An amount of 10 grams FS weighed by using a balance with an accuracy of 0.001 gram and milled by a grinder (Moulinex MC 300132, France) for one minute. Then the powder was sieved with mesh 16 and finally was mixed with 100 mL of ethanol 70%. A 1000 W ultrasound equipment (MPI, Switzerland) with a titanium ultrasonic probe (2 cm diameter) was used in order to make ultrasonic waves while the nominal frequency was 20±0.5 kHz. The temperature was verified with a thermometer and non-significant increase in temperature (below 2°C) was detected in water bath (Memmert WNB 14, Germany) during extraction. The input energy was controlled by setting the power of generator. The ultrasound probe was submerged to a depth of one cm in the liquid sample (Figure 1).

Solid/liquid extractions were carried out using a benchtop centrifuge (SiGmA 2-16P, Germany) for 30 min at 7800 rpm, then, ethanol was removed from the extracts by evaporation under vacuum at 40°C (preventing damage phenolic compounds) using a rotary evaporator (Beals Hei-VAP Value, Germany). Subsequently, the residual solvent was removed and dried in an oven (BINDER, Germany) at 40°C for 1 hour. The sample was then spread in a very thin layer (film) on a round closed plate, with Para film which is covered with aluminum foil to prevent completely against the light. The samples kept in a freezer (–18°C) for experiments (Bimakr et al., 2012).

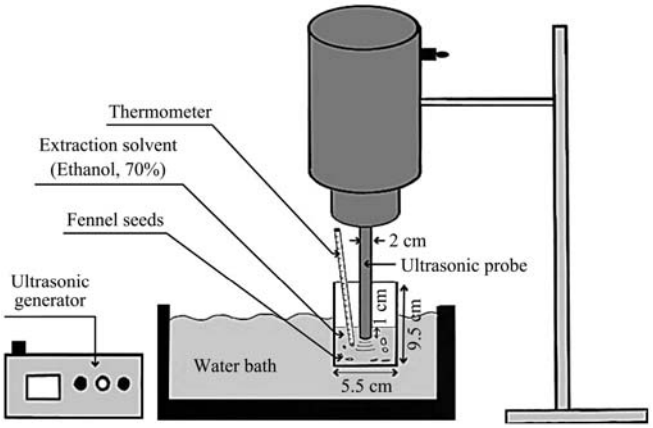


Figure 1 Schematic diagram of experimental set-up for ultrasound-assisted extraction

2.3 Conventional soxhlet extraction

An amount of 30 grams FS powder, sifted with mesh 16, was plugged with filter papers and placed in the soxhlet chamber which was fitted with 300 mL of 70% ethanol. Extraction continued until the solvent became colorless and extraction time of 4 hours after seeing the first drop of condensation was calculated. The obtained extract was released in a rotary evaporator under vacuum and other stages done similar to ultrasound method.

2.4 Total soluble solids (Brix)

Soluble solids were measured using a refractometer (ATAGO R500, Japan). Refractive index was recorded and converted to °Brix by using a conversion table. Measurements were performed at 25±0.5°C. The refractometer prism was cleaned with distilled water after each analysis.

2.5 Ascorbic acid

Ascorbic acid was determined according to the method of Klein and Perry (1982) with some modifications. Extracts samples (1.5 mL) was extracted with metaphosphoric acid (1%, 10 mL) for 45 min at room temperature. The resulting solution (1 mL) was mixed with 2,6-dichloroindophenol (9 mL) and the absorbance was measured within 30 min at 515 nm against a blank. Content of ascorbic acid was calculated on the basis of the calibration curve of authentic

L-ascorbic acid (0.025-0.1 mg mL^{-1}; $y = 4.4283x - 0.0066$; $R^2 = 0.9995$), and the results were expressed as l mg of ascorbic acid per 1 mL of the extracts.

2.6 Hydrogen peroxide scavenging activity

The ability of FS extracts to scavenge hydrogen peroxide was determined according to the method of Ruch et al. (1989) with some modifications. A solution of hydrogen peroxide (2 mmol L^{-1}) was prepared in phosphate buffer (pH 7.4). Extracts samples (0.25 mL) were added to a hydrogen peroxide solution (0.6 mL). Absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing in phosphate buffer without hydrogen peroxide. The percentage of scavenging of hydrogen peroxide of FS extracts was expressed according to Equation (2).

$$\text{Inhibition (\%)} = 100 \times [(A_0 - A_1)/(Abs_0)] \quad (2)$$

where, A_0 was the absorbance of the control, and A_1 was the absorbance in the presence of the sample of FS extracts.

2.7 DPPH· radical scavenging activity

In this test the ability of the hydrogen atom or electron was measured by different compounds and extracts with the amount of achromatize DPPH solution in methanol (Burits and Bucar, 2000). The antioxidant properties of the extract was measured with some changes using the method Brand-Williams. According to this method, 0.1 mL extract sample was mixed with 9.9 mL of distilled water, then 0.1 mL of the final solution was mixed with 3.9 mL of a methanolic solution of DPPH (25 mg L^{-1}). The mixture was strongly shaken and left to stand at room temperature for 30 min in the darkness.

In order to prepare the control sample, an amount of 0.1 mL methanol was mixed with 3.9 mL of methanolic solution of DPPH. The absorbance was measured using the UV-Vis spectrophotometer at 515 nm. The radical-scavenging activity was expressed as percentage of inhibition according to Equation (3) (Brand-Williams et al., 1995).

$$\text{Inhibition (\%)} = 100 \times [(Abs_{control} - Abs_{sample})/(Abs_{control})] \quad (3)$$

2.8 Statistical analysis

All treatments were carried out in three replications. The results were compared by analysis of variance (ANOVA) using SAS.9.1 software. The significant differences among the treatments were determined at $p<0.05$ levels using Duncan's multiple range tests.

3 Results and discussion

3.1 Total soluble solids (Brix)

The results of sonication treatments were analysis and no significant differences were observed among the samples studied for total soluble solids. In similar researches, no significant differences were observed in Brix for tomato juice (Adekunte et al., 2010), and in total soluble solids of guava juice (Cheng et al., 2007). The total soluble solids for different measurements do not differ significantly from one another because the samples were freshly prepared and no microbial fermentation had taken place in the samples prior to analysis.

3.2 Ascorbic acid

Figure 2 shows significant difference of ascorbic acid between ultrasound and soxhlet methods at both temperatures of 40°C and 60°C. By increasing time at 40°C, the ascorbic acid decreased at sonication power 39 W cm^{-2} sonication power, while at 64 and 96 W cm^{-2} sonication power the ascorbic acid was destroyed completely (Figure 2a). Degradation of ascorbic acid is mainly related to sonochemical reactions and the extreme physical conditions which occur during sonication. It is well known that during the sonolysis of water molecules hydrogen ions (H^+), free radicals (O^-, OH^-, HO_2^-) and hydrogen peroxide (H_2O_2) are formed (Feril and Kondo, 2004) and are present in juice samples. The ascorbic acid degradation during ultrasonic processing could be related to oxidation reactions, promoted by the interaction with free radicals formed during sonication (Hart and Henglein, 1985). Hydroxyl radicals produced by cavitation may be involved in the degradation of ascorbic acid.

The degradation of ascorbic acid is corresponding to aerobic and anaerobic degradation (Kennedy et al., 1992; Ariahu et al., 1997; Blasco et al., 2004). Sonication results in a reduction of dissolved oxygen, a critical parameter influencing the stability of ascorbic acid (Solomon et al., 1995). Hydroxyl radical formation is found to increase with degassing. Sonication cavities can

be filled with water vapour and gases dissolved in the juice such as O_2 and N_2 (Korn et al., 2002). The interactions between free radicals and ascorbic acid may occur at the gas-liquid interfaces. In summary ascorbic acid degradation may follow one or both of the following pathways:

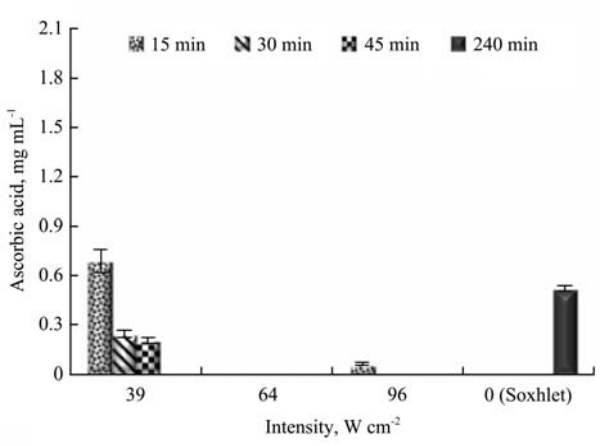
Ascorbic acid→thermolysis (inside bubbles) and triggering of Maillard reaction.

Ascorbic acid → reaction with OH^- → HC–OH and production of oxidative products on the surface of bubbles.

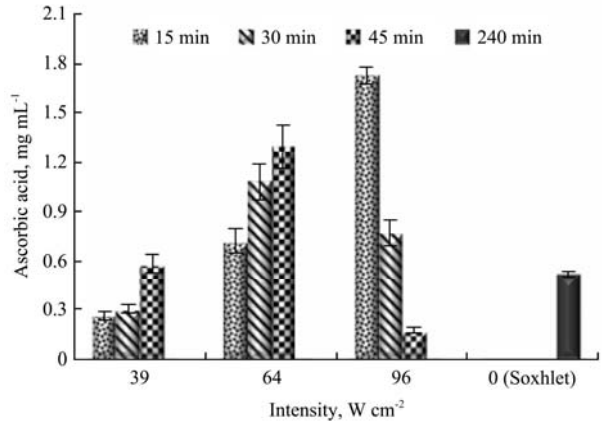
However sonication can be related to advanced oxidative processes since both pathways are associated with the production and use of hydroxyl radicals (Pétrier et al., 2007).

At 60°C, by increasing sonication time the amount of ascorbic acid increased at sonication powers of 39 and 64 W cm^{-2} while decreased at 96 W cm^{-2} (Figure 2b). It goes to the phenomenon that at high intensity the cell walls ruptured, impurities such as insoluble substances, suspend in the extract, lowering the solvent's permeability into cell structures (Tian et al., 2013). Furthermore, target components also re-adsorb into the ruptured tissue particles due to their relatively large specific surface areas, lowering extraction of ascorbic acid (Dong et al., 2010). Also at intensity of 96 W cm^{-2} contact severity increased cavitation bubbles, also the vapor pressure of the solvent increased with the increase of temperature and the vapor pressure had a great influence on the occurrence and the intensity of acoustic cavitation. At lower temperature, the vapor pressure is lower. Ultrasound produces a few cavitational bubbles as a result of high acoustic cavitation threshold. However, the bubbles explode with relatively greater force, which enhanced cell tissues disruption during extraction. At higher temperature, the vapor pressure was higher and more bubbles were created, but they collapsed with less intensity due to a smaller pressure difference between inside and outside of bubbles (Zhang et al., 2008). So both the intensity and the high-temperature treatment reduce the activity of bubbles and the extraction of antioxidant compounds.

In a study about the effect of ultrasound on the amount of ascorbic acid in tomato juice, the results showed that at a constant intensity by increasing sonication duration the amount of ascorbic acid increased (Adekunte et al., 2010).



a. Effect at temperatures of 40°C



b. Effect at temperatures of 60°C

Note: SE is the abbreviation for standard error.

Figure 2 Effect of intensity and time on the changes of ascorbic acid by ultrasound-assisted extraction and soxhlet method (mean±SE, *n*=3): at temperatures of 40°C (a) and 60°C (b)

The main reasons of temperature rise during ultrasound treatment from 40°C to 60°C are due to improvement in mass transfer, increase in solubility and more exit of antioxidant compounds from plant. Increasing the amount of ascorbic acid in the extraction at 60°C temperature compared to ascorbic acid degradation at this temperature prevailed during ultrasound treatment and causing increase the amount of vitamin during extraction has been compared to temperature 40ºC.

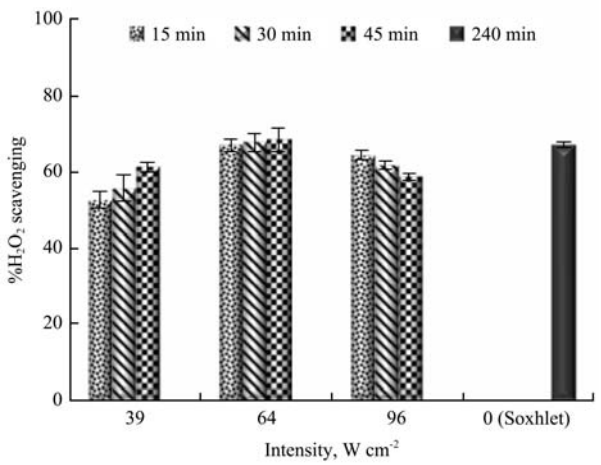
Results of this study showed at ultrasound method the greatest amount of ascorbic acid was observed for 15 min sonication (96 W cm^{-2} and 60°C), equal to 1.73 mg mL^{-1} FS extract, that was significantly different than soxhlet method (240 min and 85°C) which increased 69.94% more ascorbic acid.

Extraction efficiency by ultrasound goes to the propagation of ultrasound pressure waves, and resulting cavitation phenomena. High shear forces cause increase in mass transfer of extractants (Ji et al., 2006). The implosion of cavitation bubbles causes macro-turbulence, high-velocity inter-particle collisions of the biomass which accelerates internal diffusion. Furthemore, the cavitation near the liquid-solid interface sends a fast moving stream of liquid through the cavity at the surface. This effect provides exposure of new surfaces further increasing mass transfer. Scanning electron micrography confirmed this phenomenon on peppermint plant leaves and trichomes. After these were ultrasonically treated for menthol extraction, microscopy results indicated that there were two mechanisms involved in extraction: (a) the diffusion of product through the cuticle of peppermint glandular trichomes and (b) the exudation of the product from broken and damaged trichomes (Shotipruk et al., 2001).

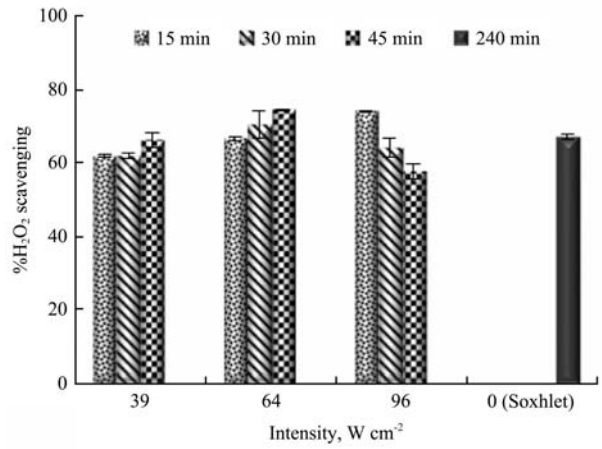
Several studies have shown that non-thermal process technologies including high pressure, pulsed electric fields and sonication retain a higher level of ascorbic acid relative to thermally processed juices (Yeom et al., 2000; Torregrosa et al., 2006; Cheng et al., 2007). Ultrasound treatment of orange juices is reported to have a minimal effect on the ascorbic acid content during processing and results in improved stability during storage when compared to thermal treatment (Tiwari et al., 2009). This positive effect of ultrasound is assumed to be due to the effective removal of occluded oxygen from the juice (Knorr et al., 2004), a critical parameter influencing the stability of ascorbic acid (Solomon et al., 1995).

3.3 Hydrogen peroxide scavenging activity

Similar trends were observed for both temperatures of 40°C and 60°C for the effects of intensity, temperature and sonication time on percentage of scavenging H_2O_2 (Figure 3a,b). In a fixed time, the percent scavenging of hydrogen peroxide initially increased and then decreased with increasing intensity. At 39 and 64 W cm^{-2} sonication with increasing the time, the percentage of scavenging increasing trend while the power of 96 W cm^{-2} had decreasing trend.



a. Effect at temperatures of 40°C



b. Effect at temperatures of 60°C

Figure 3 Effect of intensity and time on the scavenging H_2O_2 by ultrasound-assisted extraction and soxhlet method (mean±SE, n=3) at temperature of 40°C (a) and 60°C (b)

A similar trend was observed between the extraction of ascorbic acid and hydrogen peroxide scavenging at 60°C (Figure 2b, 3b). The reason could be the efficient destruction of O_2^- and H_2O_2 requires the action of several antioxidant enzymes acting in synchrony. Superoxide produced in the different compartments of plant cells is rapidly converted to H_2O_2 by the action of superoxide dismutase (SOD) (Bowler et al., 1992). In organelles such as chloroplasts, which contain high concentrations of ascorbate, direct reduction of O_2^- by ascorbate is also rapid (Buettner and Jurkiewicz, 1996). Also dismutation of O_2^- simply serves to convert one destructive AOS to another. Since H_2O_2 is a strong oxidant that rapidly oxidizes thiol groups, it cannot be allowed to accumulate to excess in organelles such as chloroplasts, where photosynthesis depends on thiol-regulated enzymes (Kaiser, 1979). Catalases (CAT) convert H_2O_2 to water and molecular oxygen (Willekens

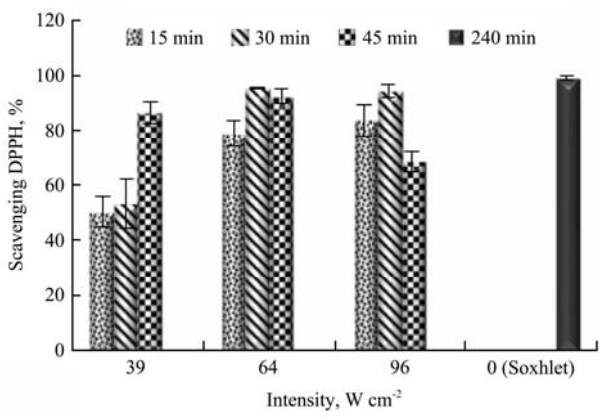
et al., 1995). In plant cells, the most important reducing substrate for H_2O_2 detoxification is ascorbate (Mehlhorn et al., 1996). Therefore it is expected that hydrogen peroxide scavenging percent increase by increase the amount of ascorbic acid in extracts. In a study report on pea plant H_2O_2 collectors increased in the presence of ascorbate, glutathione-ascorbate cycle activity (Dixit et al., 2001).

In a study on effects of hydrogen peroxide on the stability of ascorbic acid during storage in pomegranate juice, the degradation was slow at 0.5 ppm H_2O_2 concentration, but increasing of the H_2O_2 concentration from 0.5 to 5 ppm accelerated ascorbic acid degradation tremendously (Özkan et al., 2004).

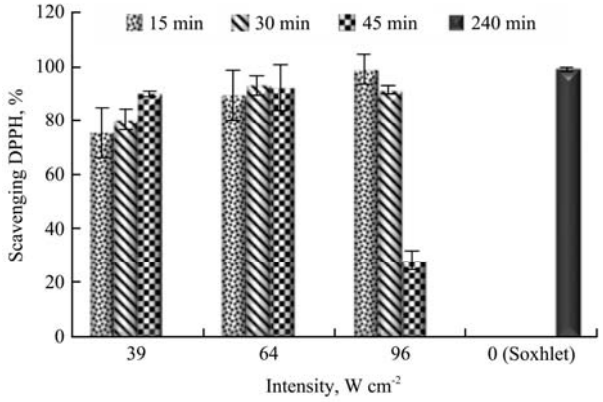
Results of this study showed the greatest hydrogen peroxide scavenging activity equivalent to 74.58% was observed for ultrasound method with 45 min sonication (64 W cm^{-2} and 60°C). But results showed the ultrasound treatment with 300 W cm^{-2}, 15 min and 60°C equivalent to 74.13% of hydrogen peroxide scavenging was only 0.45 percent less than the maximum sonication treatment. Due to less sonication time and no significant differences, this treatment was introduced as the best ultrasound treatment compared to soxhlet method. Significant difference was found between ultrasound and soxhlet method (240 min and 85°C) which hydrogen peroxide scavenging was 6.93% higher.

3.4 DPPH$^{\cdot}$ radical scavenging activity

The effect of intensity, temperature and sonication duration on percentage of scavenging DPPH was studied. Similar trends were observed for both temperatures of 40°C and 60°C (Figure 4a, b). A lot of antioxidant compounds were extracted during 15 minutes. It is worthy of note that the UAE mechanism consists of two main stages of "washing" which is dissolution of soluble compounds on surfaces of the plant matrix and "slow extraction" which defined as mass transfer of the solute from the plant matrix into the solvent by diffusion and osmotic processes (Veličković et al., 2008). These two phenomena were clearly observed in Figure 4. Washing was happening at the beginning of the extraction with a rapid increase and then slow extraction was observed by a low raise in extraction of antioxidant compounds.



a. Effect at temperatures of 40°C



b. Effect at temperatures of 60°C

Figure 4 Effect of intensity and time on the scavenging DPPH by ultrasound-assisted extraction and soxhlet method (mean±SE, n=3) at temperature of 40°C (a) and 60°C (b)

Cavitational activity decreases with increasing temperature by reducing cavitational threshold. Furthermore, the great number of cavitation bubbles formed could serve as a damper the ultrasonic energy. Several mechanisms can act concurrently when ultrasound is applied in liquid systems, i.e., thermal effects produced by bubble implosion, which produce implosion shock waves, and free radical production. Nevertheless, radical productions have been considered the most probable mechanism (Vercet et al., 2001).

Also a similar trend was observed between the extraction of ascorbic acid and DPPH free radical scavenging at 60°C (Figure 2b, 4b). Vitamin C, L-ascorbic acid, is a well-known antioxidant that efficiently scavenges free radicals, for example reactive oxygen species, formed in cellular metabolism or derived from the atmosphere (Horemans et al., 2000; Smirnoff, 2005). Therefore expected to increase the amount of ascorbic acid in extracts, DPPH free radical scavenging percent increase.

Results of this study showed that by ultrasound method the greatest percentage of DPPH free radical equal to 98.88% was obtained at the scavenging time of 15 min, temperature of 60°C and intensity of 96 W cm^{-2} which was not significantly different compared to soxhlet method. There was only 0.18% increase in free radical scavenging in soxhlet method at 85°C for 240 min compared to ultrasound method with time 15 min and temperature 60°C which is negligible.

In a study phenolic compounds and flavonoids extracted from Elaeagnus umbellate examined using ultrasonic bath (30, 60 and 90 min) and three levels of ultrasonic probe (5, 10 and 20 min). The results showed that with increase the time, the percentage of free radical scavenging increased for both methods and the highest percentage of inhibition was 77.66% in ultrasonic bath for 90 min, and 95.43% for the method of ultrasound probe for 20 min (Kamali et al., 2015). The effect of temperatures (30°C, 40°C, 50°C, 60°C and 70°C) on cherry seeds revealed that at 60°C temperature DPPH radical scavenging increased and then decreased due to decomposition antioxidant compounds (Yao et al., 2011). In another study the effect of ultrasound duration on DPPH radical scavenging ability was significant, which inhibition increased by increasing extraction time (Shaddel et al., 2011).

4 Conclusions

In the present study, intensification was carried out for extraction of antioxidant compounds including ascorbic acid from FS using ultrasound. Process parameters which affect the extraction yield like extraction time, extraction temperature and ultrasound intensity were investigated. Comparing the ultrasound and soxhelt methods showed that optimum conditions of ultrasound extraction for ascorbic acid, hydrogen peroxide scavenging and DPPH free radical scavenging was observed at 15 min sonication traeatment and 96 W cm^{-2} intensity at 60°C temparature. In these conditions amount of ascorbic acid and hydrogen peroxide scavenging percent were 69.94% higher ultrasound method and 6.93% higher compared to soxhlet method while the extraction time was 16 times less. The percentage of DPPH free radical scavenging in soxhlet method showed only 0.18% increase compared to ultrasound method despite of low temperature and decrease in extraction time. Results showed that ultrasound method provide the opportunity of enhanced extraction and improved retention of ascorbic acid in FS at lower processing duration and temperature compared to thermal processing.

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