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Thermosonication Process Design for Recovering Bioactive Compounds from Fennel: A Comparative Study with Conventional Extraction Techniques

Adela Cristina Martinez Urango, Monique Martins Strieder, Eric Keven Silva * and Maria Angela A. Meireles

School of Food Engineering, University of Campinas, Rua Monteiro Lobato, 80, Campinas 13083-862, SP, Brazil; adelmartinez08@gmail.com (A.C.M.U.); monique_strieder@hotmail.com (M.M.S.); maameireles@lasefi.com (M.A.A.M.)

* Correspondence: engerickeven@gmail.com

Abstract: This study aimed to examine the impact of the combination of acoustic energy at the nominal powers of 100, 200, 300, and 400 W with moderate heat processing at 40, 50, and 60 °C on the extraction of phytochemical compounds from *Foeniculum vulgare*. Thermosonication processing, based on high-intensity ultrasound combined with an external heat source, can potentialize the extraction of soluble solids from plant material. However, the excessive temperature increase generated by the two energy sources during thermosonication treatment may degrade the thermolabile bioactive compounds. Regardless of the temperature condition, fennel extracts obtained at 400 W presented lower total phenolic content (TPC) than those obtained at 300 W. The cavitation heat and mechanical stress provided at 400 W may have degraded the phenolic compounds. Thereby, the best extraction condition was 300 W and 60 °C. The fennel extract presented the highest content of TPC (3670 ± 67 µg GAE/g) and antioxidant activity determined by DPPH and ABTS methods (1195 ± 16 µg TE/g and 2543.12 ± 0.00 µg TE/g, respectively) using this treatment. Thermosonication can be an innovative technique for extracting phytochemicals because it provides good results in shorter processing times, with 73% and 88% less energy consumption than Percolation and Soxhlet techniques, respectively.

Keywords: *Foeniculum vulgare*; high-intensity ultrasound; thermal history; percolation; Soxhlet

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1. Introduction

Plants and plant extracts have been used to prevent and treat various diseases for thousands of years. Fennel (*Foeniculum vulgare*) is an aromatic and medicinal plant known for its flavor, odor, and wide use in several products [1]. The seeds, leaves, fruits, and the entire plant have been used to prepare infusions. The consumption of these infusions, popularly denominated as tea, contributes to preventing diseases related to the digestive, endocrine, reproductive, and respiratory systems, such as cancer, conjunctivitis, gastritis, colic in children, kidney ailments, and laxatives [2]. The different parts of the plant have also been used as a spice in food preparation. The secondary metabolites of plants responsible for their protection also provide their pharmacological effects. These are bioactive compounds classified into three groups: phenolic compounds, terpenes, and alkaloids [3]. Anethole, fenchone, estragole (methyl-chavicol), myrcene, and limonene are the volatile bioactive compounds reported in fennel [1,4]. These compounds have demonstrated beneficial effects, such as anti-inflammatory, analgesic, antioxidant, diuretic, antispasmodic, antithrombotic, and antitumor [2,5].

The extraction of phytochemical compounds from fennel allows obtaining extracts applicable in pharmaceutical and food products. The extraction processes used include conventional techniques such as percolation, maceration, and solid–liquid extraction, or Soxhlet extraction that requires high solvent volumes, long extraction times, and the use of temperatures generally above room temperature [6]. Thereby, these conditions can favor the thermal degradation of thermolabile compounds. Thus, emerging technologies have been evaluated to extract these compounds to overcome the drawbacks of traditional techniques. Innovative techniques include extractions that use supercritical CO_2 or pressurized liquids as the solvent, microwave, ultrasound, etc. These techniques enable the use of a smaller solvent volume, promoting higher yield, increasing process reproducibility, and generating fewer toxic residues, besides shorter extraction times [3].

Among the emerging technologies, ultrasound stands out for providing highly efficient processes. The application of low-frequency (16 to 100 kHz) and high-power ultrasound (>1 W/cm^2) waves in liquid media generates the acoustic cavitation phenomenon. Sonication promotes the sonoporation effect in plant materials. It increases the temperature in the extraction system, favoring the release of phytochemical compounds linked to plant cells and their diffusion into the solvent [7]. The rupture of cellular organelles and the temperature rise can increase the extraction yields [8]. Likewise, acoustic energy with moderate heat processing can be an innovative technique for recovering phytochemicals. This combined treatment is known as thermosonication [9,10].

Thermosonication is a relatively new technique that has been used for kinetic, microbiological, and enzymatic stabilization of foods and beverages [11,12]. Thermosonication treatments are based on the combined impact of the acoustic and thermal energies to inactivate pathogenic and spoilage microorganisms and endogenous enzymes. Acoustic energy is provided by an ultrasound system and the thermal energy from an external heat source. Thus, the application of combined acoustic and thermal energies increases the temperature of the sample, favoring the diffusion rate of bioactive compounds into the solvent. The temperature rise may also be unfavorable since some phytochemicals are thermolabile. Thereby, an accurate thermosonication process design is required to know the potential impacts of the combined action of acoustic and thermal energies on the target phytochemical compounds.

Thermosonication treatments applied for the extraction of phytochemicals remain scarce. Some studies technically performed thermosonication but did not call it as such; maybe the setup was not the exact setup of the technique [13]. Other authors reported having extracted bioactive compounds by thermosonication. They performed an extraction assisted by ultrasound [14]. The authors did not use an external heat source beyond the heat generated by the acoustic cavitation. In this way, the design of thermosonication processing for the extraction of phytochemical compounds was uninvestigated. Therefore, this study aimed to evaluate the impact of combining acoustic energy with an external heat source to extract bioactive compounds from fennel. The effects of nominal power (100, 200, 300, and 400 W) and temperature (40, 50, and 60 °C) were examined. Furthermore, the thermosonication technique was compared with the conventional solid–liquid extraction techniques of percolation and Soxhlet regarding the quality of their phytochemical extracts and energy performance.

2. Materials and Methods

2.1. Raw Materials

The fennel seeds were purchased from Empório Figueira Produtos Naturais (São Paulo, Brazil). The seeds were ground in a knife mill model MA-340 (Marconi, Piracicaba, Brazil) and sieved in an 1868 vibratory system (Bertel, Caieiras, Brazil) using sieves from 9 to 80 mesh (Tyler series, Wheeling, WV, USA). The selected fennel particles were those retained in the n° 48 sieve with an opening of 0.287 mm. The ground particles were stored at −18 °C until the extraction assayed.

2.2. Thermosonication-Assisted Extraction of Phytochemicals

The thermosonication-assisted extraction of phytochemical compounds from fennel was carried out with four nominal power levels (100, 200, 300, and 400 W) and three temperature levels (40, 50, and 60 °C) for 15 min. The acoustic powers provided for samples at the nominal powers of 100, 200, 300, and 400 W were 4.6 ± 0.4, 8.5 ± 0.1, 14.5 ± 0.3, and 20 ± 1 W, respectively [15]. All experiments were performed using a solvent mass to feed mass (S/F) ratio of 10. The extraction assisted by thermosonication was performed by mixing 2 g of ground fennel and 20 g of ethanol in a 50 mL Falcon tube. The sample was heated in a water bath until reaching the extraction temperature. After the sample reached the working temperature, an ultrasound probe, with a 13 mm diameter, at 19 kHz (Unique, Indaiatuba, Brazil), was dipped in the Falcon tube center. The external heat source was kept constant during the thermosonication treatments by a jacketed Becker connected to a heating water bath. After the extraction, the sample was filtered using paper filters to remove the fennel particles. The extracts were stored at −18 °C until analysis. The thermosonication-assisted extraction experiments were carried out according to a full factorial design (4 × 3). All experiments were performed in duplicate. Therefore, 24 experiments were performed.

The thermal history of the thermosonication treatments was determined for each thermosonication process condition to evaluate the effect of the working temperature and the nominal power on the temperature rise of the samples throughout the extraction time. The sample temperature was measured during the extraction process using a stainless-steel thermocouple probe immersed in the Falcon tube and connected to a portable data logger.

2.3. Conventional Solid–Liquid Extraction Techniques: Soxhlet and Percolation

Fennel extracts were obtained using the Soxhlet and percolation solid–liquid extraction techniques. The extractions were performed using the same S/F ratio of 10. Soxhlet extraction was performed using 10 g of ground fennel and 90 g of ethanol under condensing temperature. The samples were placed in a paper cartridge to be refluxed for 6 h in a Soxhlet apparatus. Extraction by percolation was performed at 60 °C and 200 rpm using an incubator Shaker model MA-420 (Marconi, Piracicaba, Brazil). Twenty grams of ground fennel were added to 180 g of ethanol in a hermetically sealed vessel. The percolation was also carried out for 6 h. After the assays, the ethanolic fennel extracts were stored at −18 °C until analysis.

2.4. Extraction Yields

The extraction yields were determined according to Equations (1) and (2). The ethanol from the fennel extracts was removed by evaporation at 60 °C. The samples were then cooled at room temperature and weighed.

$$\text{Global yield } (\text{g}/100\text{ g}) = \frac{\text{Dried extract mass}}{\text{Ground fennel mass}} \times 100 \tag{1}$$

$$\text{Extracted solids } (\text{g}/100\text{ g}) = \frac{\text{Dried extract mass}}{\text{Extract mass}} \times 100 \tag{2}$$

2.5. Characterization of Fennel Extracts

2.5.1. Total Phenolic Content

The total phenolic content (TPC) was determined using the Folin–Ciocalteu colorimetric method according to Arruda et al. [16]. An aliquot of 300 µL of diluted ethanolic extract (1:3 *v/v*), 300 µL of Folin-Ciocalteu reagent, and 2400 µL of sodium carbonate (5%, *w/v*) was mixed. Then, the reaction solution was kept in the dark for 20 min. The absorbance was measured at 760 nm against a blank using a UV-VIS spectrophotometer 800XI (FEMTO, São Paulo, Brazil). A calibration curve using gallic acid (10–90 µg/mL, R^2 = 0.999) as the standard was used to quantify the TPC. The results were expressed as µg gallic acid equivalents per gram of ground fennel (µg GAE/g).

2.5.2. In Vitro Antioxidant Capacity by DPPH Radical Scavenging Assay

Free radical scavenging activity was measured using the methodology reported by Brand-Williams et al. [17]. The experiments were performed on freshly prepared ethanolic solutions of DPPH (2,2-Diphenyl-1-picrylhydrazyl) (0.004% *w/v*). In brief, 600 μL of the diluted ethanolic extract (1:10 *v/v*) was mixed with 3000 μL of DPPH solution using a vortex. After 30 min of the reaction, the absorbance of the remaining DPPH was measured at 517 nm on a UV-VIS spectrophotometer 800XI (FEMTO, São Paulo, Brazil). Antioxidant capacity was expressed as a percentage of the absorbance of the control DPPH solution, obtained from the following equation:

$$\%\ \text{Activity} = [(A_{DPPH} - A_{sample})/A_{DPPH})] \times 100$$

where A_{DPPH} is the absorbance value of the control and A_{sample} is the absorbance value of the test solution. Trolox was applied as a standard using a calibration curve (5–50 μg/mL, $R^2 = 0.998$). The results were expressed as microgram of Trolox equivalents per gram of fennel (μg TE/g).

2.5.3. In Vitro Antioxidant Capacity by ABTS Radical Scavenging Assay

The $ABTS^{\bullet+}$ scavenging capacity assay was determined as described by Le et al. [18]. The method is based on the decolorization of the ABTS (2,2-Azino-bis-(3-ethylbenzothiazoline)-6-sulphonic acid) radical cation to determine the antioxidant potential of the samples. The ABTS radical cation solution was prepared in advance by reacting aqueous ABTS solution (7 mM) with potassium persulfate (2.45 mM). In the analysis, diluted ABTS + solution with an absorbance of 0.70 ± 0.02 at 734 nm was used. The reaction system was composed of 800 μL of diluted ethanolic extract in ethanol (1:5 *v/v*) and 4000 μL of ABTS + solution, followed by incubation for 6 min at room temperature. The absorbance values were measured by a UV-VIS spectrophotometer 800XI (FEMTO, São Paulo, Brazil) at 734 nm. The free radical scavenging activity was expressed as a percentage of the absorbance of the ABTS + control, obtained from the following equation: $\%\ \text{Activity} = [(A_{ABTS}{}^{\bullet+} - A_{sample})/A_{ABTS}{}^{\bullet+}] \times 100$, where $A_{ABTS}{}^{\bullet+}$ is the absorbance value of the ABTS control and A_{sample} is the absorbance value of the test solution. A calibration curve was plotted from the absorbance reduction and concentration of the Trolox (5–50 μg/mL, $R^2 = 0.998$). The results were expressed as microgram of Trolox equivalents per gram of fennel (μg TE/g).

2.6. Statistical Analysis

Minitab 18® software was used to verify the impact of the nominal power and working temperature on the extraction yield and functional properties of the fennel extracts obtained using thermosonication-assisted extraction and conventional solid–liquid extraction. Analyses of variance (ANOVA) at a significance level of 5% (p-value < 0.05) were performed.

3. Results and Discussion

3.1. Thermal History of Thermosonication Processing

Figure 1 exhibits the thermal histories of the thermosonication-assisted extraction processes of phytochemicals from fennel at different nominal power and temperature conditions. The thermal history describes temperature behavior according to the extraction time.

The initial temperature of the samples was standardized to 25 °C. Then, the samples were heated until the working temperature (40, 50, or 60 °C) was reached. The red dot line indicates the working temperature and the start of the extraction process assisted by thermosonication (Figure 1). The increase of nominal power and working temperature increased the maximum temperature reached by the thermosonicated sample. Thus, the combination between acoustic energy and an external heat source throughout the extraction process influenced the thermal history. The greater ΔT (maximum temperature-working temperature) values were observed for the extractions employing 400 W. For this nominal power, ΔT values of 35, 27, and 17 °C were observed for working temperatures of 40, 50, and 60 °C, respectively.

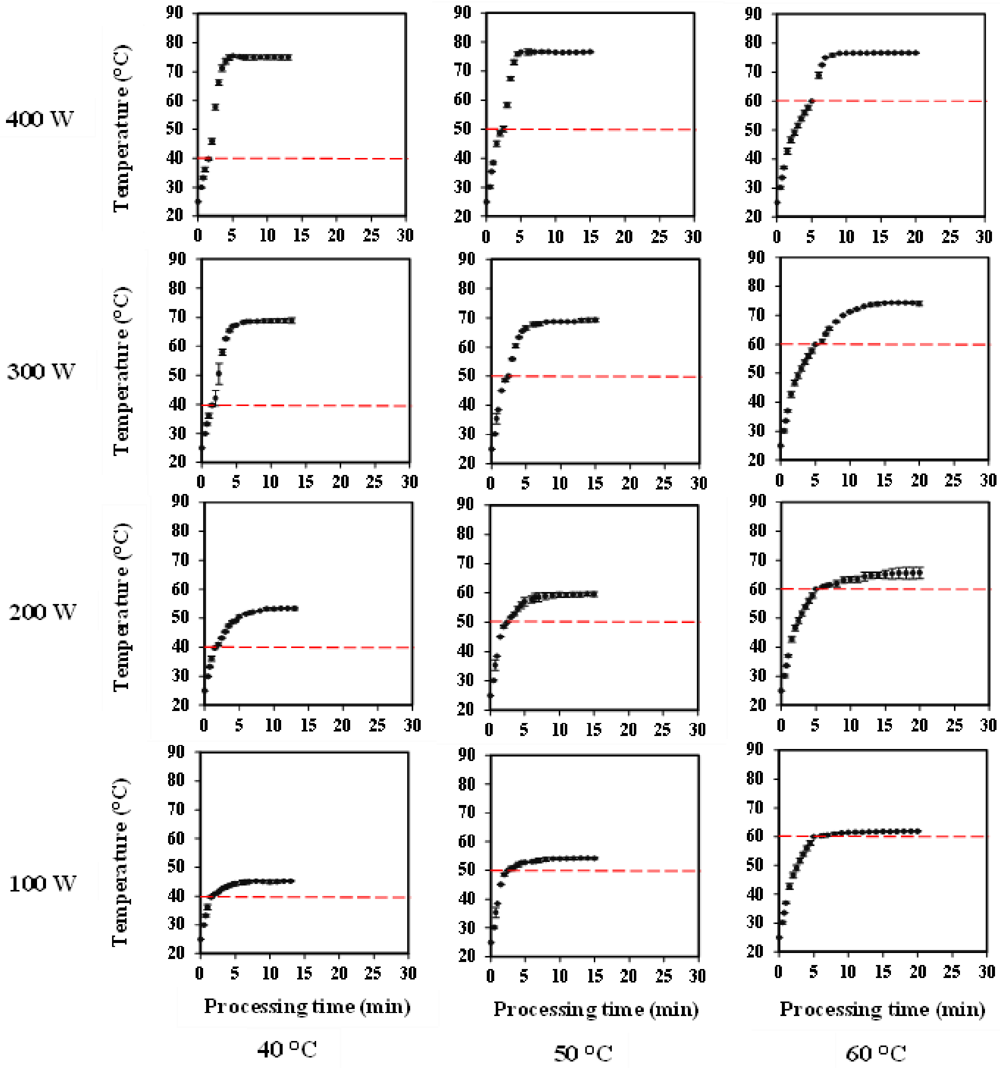

Figure 1. Thermal history of the processes of extraction by thermosonication.

The application of low-frequency (19 kHz) and high-power (100, 200, 300, and 400 W) ultrasound promotes the phenomenon of acoustic cavitation in the extraction medium. During the sonication treatment, cavitation bubbles are formed. These bubbles grow and explode violently, releasing mechanical and thermal energy in the sonicated liquid, increasing its temperature throughout processing [19]. The increase in nominal power from 100 W to 400 W intensified the acoustic cavitation effects resulting in greater ΔT values. Wu et al. [20] reported that increasing the ultrasonic power from 7.98 W to 32 W decreased the microbubble collapse time. Thus, the acoustic cavitation intensity was increased. Therefore, the nominal power and the working temperature affected the temperature of the thermosonication-assisted extraction processes. In this way, monitoring the temperature throughout the thermosonication is needed to prevent the fennel compounds' exposure to high temperatures, avoiding their bioactive compounds' thermal degradation.

On the other hand, Figure 1 shows that higher ΔT values are associated with lower working temperatures. In the extraction at 40 °C, ΔT values of 5, 13, 29, and 35 °C were observed for the nominal powers of 100, 200, 300, and 400 W, respectively. The thermosonication-assisted extractions carried out at 60 °C exhibited lower ΔT values of 2, 6, 14, and 17 °C for 100, 200, 300, and 400 W, respectively. The temperature can affect the physical properties of the solvent, such as saturation vapor pressure, surface tension, sound velocity, and viscosity. Our results demonstrated that the temperature rises gradually decreased the acoustic cavitation intensity since the nominal power increase did not cause the same response on maximum temperature for each working temperature evaluated.

3.2. Thermosonication Extraction Yields

Table 1 presents the impacts of the thermosonication process conditions on the global extraction yield of the fennel extracts. The nominal power and working temperature significantly affected the global extraction yield (p-value < 0.001). The extraction yield varied from 3.2 ± 0.2 to 5.8 ± 0.1 g dried extract/100 g ground fennel.

Table 1. Thermosonication extraction yields.

Nominal Power (W)	Temperature (°C)	Global Yield (g/100 g) [1]
100	40	3.2 ± 0.2
	50	3.7 ± 0.1
	60	4.2 ± 0.1
200	40	3.5 ± 0.2
	50	3.7 ± 0.2
	60	4.8 ± 0.1
300	40	5.3 ± 0.1
	50	5.5 ± 0.1
	60	5.5 ± 0.1
400	40	5.85 ± 0.01
	50	5.8 ± 0.1
	60	5.8 ± 0.1

[1] Global yield: g dried extract/100 g ground fennel.

The global extraction yield increased significantly when the temperature rose from 40 to 60 °C, except at 300 W and 400 W. The increase in temperature may favor the diffusion and solubility of the phytochemical compounds contained in the plant material. It can even favor the extraction of impurities. Thus, the increase in thermal energy contributes to the increase in extraction yield [8]. The nominal power rise from 100 W to 400 W also increased the extraction yield. At the nominal powers of 300 W and 400 W, the mechanical and thermal energies applied to the samples were intensified. The working temperature in these powers did not influence the extraction yield. Thus, the contribution of acoustic energy is greater for the extraction of solids by thermosonication than by external heat at high powers.

According to Figure 2, the global yield results were consistent with the respective fennel samples' visual appearance. Figure 2 shows the visual appearance of the fennel extracts right after the thermosonication-assisted extraction. The extracts produced with higher nominal powers of 300 W and 400 W presented a darker color. In addition, the working temperature increase from 40 to 60 °C produced darker extracts at 100 W and 200 W. This extract's darker color may be related to the extraction of more compounds (high global yields) besides its thermal degradation.

On the other hand, fennel contains proteins and carbohydrates [21,22]. Acoustic cavitation may have promoted the extraction of carbohydrates and proteins from plant material. Thus, thermosonication-assisted extraction may have promoted the Maillard reaction due to high temperatures, also contributing to the dark color of the extracts.

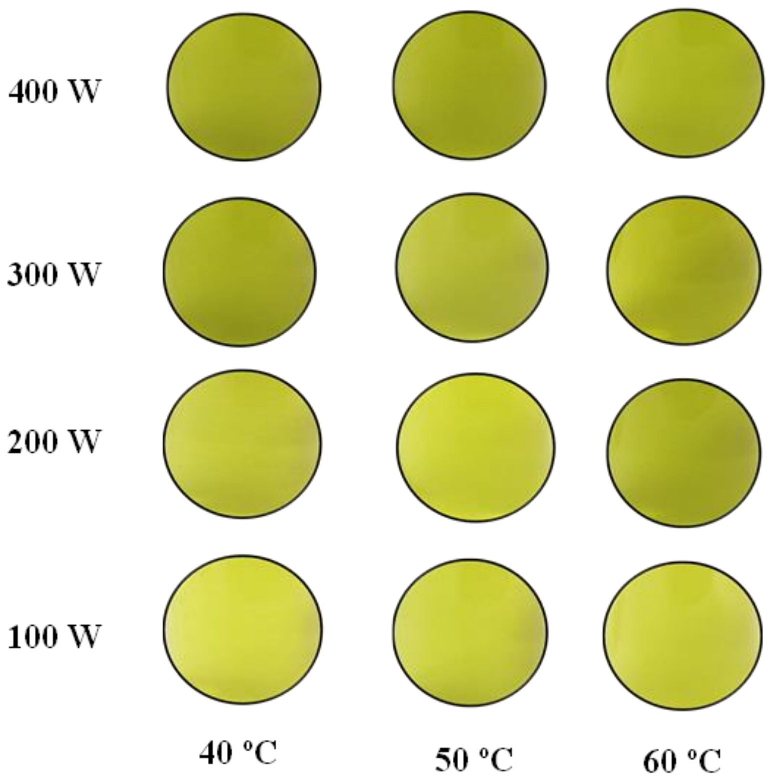

Figure 2. Thermosonication effects on the visual appearance of the fennel extracts.

3.3. Impact of Thermosonication on the Total Phenolic Content

Figure 3 presents the influence of the nominal power and the extraction temperature on the fennel extracts' total phenolic content (TPC). The increase in nominal power until 300 W and rise in temperature increased the TPC (p-value < 0.001). The TPC varied from 636 ± 4 to 3670 ± 67 μg GAE/g. The fennel extract produced at 60 °C and 300 W presented the highest phenolic content.

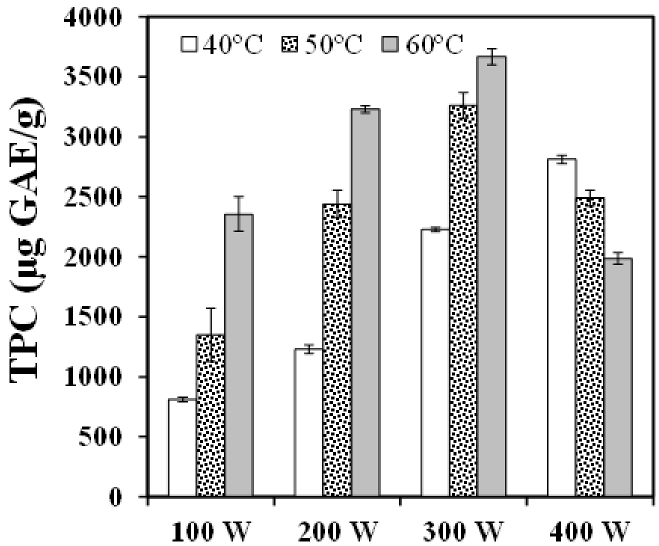

Figure 3. Thermosonication effects on the total phenolic content (TPC) of fennel extracts.

Acoustic cavitation is the phenomenon responsible for the extraction of interest compounds by ultrasound [14]. The mechanical and thermal effects provided by the acoustic cavitation can break the cell walls of the plant material, increasing the surface area of contact between the solid and liquid phases. The increase in contact area favors greater penetration of the solvent into plant cells, facilitating the diffusion and mass transfer of phytochemical compounds from the raw material to the solvent. The nominal power increase from 100 W to 300 W enhanced the extraction of the phenolic compounds from fennel. Tarone et al. [23] observed the same behavior increasing the ultrasound intensity from 1.1 W/cm^2 to 13 W/cm^2. This ultrasound intensity increase resulted in a high yield of anthocyanins recovered from the jabuticaba peel. The intensification of the acoustic cavitation provided by the increase in the ultrasound intensity favored the mass transfer rates. A similar study reported better efficiency in extracting phenolic compounds from orange peel, increasing the ultrasound nominal power from 50 to 150 W [24].

Otherwise, the fennel extract produced with 400 W and 60 °C presented a lower TPC than the extracts obtained with lower nominal power at the same working temperature. This result can be associated with the cushioning effect proportionate to high temperatures during the thermosonication process, since the extraction carried out at 60 °C reached up to 77 °C throughout the thermosonication performed at 400 W (Figure 1). The temperature rise can increase the vapor pressure and decrease the solvent's viscosity, favoring the formation of larger cavitation bubbles. In this case, the amount of steam inside the bubbles is greater, which dampens the implosion of the bubbles during cavitation and, consequently, reduces the acoustic cavitation performance on the extraction rate. In the thermosonication processes, there is an optimum temperature for maximum cavitation in liquid media at which the strongest effects of ultrasound emerge.

The global yield obtained at 400 W was higher than those obtained by the other nominal powers studied (Table 1), but the content of total phenolic compounds was lower (Figure 3). Thus, the high temperature achieved during the thermosonication-assisted extraction may also have degraded the extracted phenolic compounds. Moreover, the thermosonication treatment performed at 40 °C extracted more phenolic compounds than at the other temperatures evaluated. Adiamo et al. [25] also observed a decrease in phenolic compounds' content of carrot juice after increasing the working temperature of the thermosonication process (110 W, 40 kHz) from 40 to 60 °C. The authors stated that an increase in temperature might result in the degradation of phenolic compounds.

3.4. Impact of Thermosonication on the In Vitro Antioxidant Capacity

The results of antioxidant activity of the fennel extracts exhibited similar behavior to that observed for the total phenolic content (Figure 4a,b). Figure 4 presents the thermosonication effects on the fennel extracts' antioxidant activity. The nominal power (p-value < 0.001) and the working temperature (p-value = 0.001) promoted a significant effect on the antioxidant activity. Extracts obtained using higher nominal powers and higher working temperatures showed higher antioxidant activity. However, in the DPPH essays, the increase in nominal power from 300 W to 400 W at the working temperature of 60 °C did not increase the antioxidant activity of the fennel extract. The ABTS assays also demonstrated a reduction in the antioxidant activity of the extracts obtained by thermosonication performed at 400 W and 50 and 60 °C.

High working temperatures may also have promoted the cushioning effect in the extraction processes and, consequently, the reduction of the extraction efficiency of the antioxidant compounds of the fennel. Further, high temperatures can cause denaturation of antioxidant compounds, as observed in thermosonicated functional carrot juice [25]. Therefore, the best thermosonication condition to extract fennel compounds regarding phenolic compounds and antioxidant capacity was 300 W and 60 °C. In this thermosonication condition, the highest yield of TPC (3670 ± 67 µg GAE/g) and high antioxidant activity, measured by DPHH (1195 ± 16 µg TE/g) and ABTS (2543.12 ± 0.00 µg TE/g), were obtained.

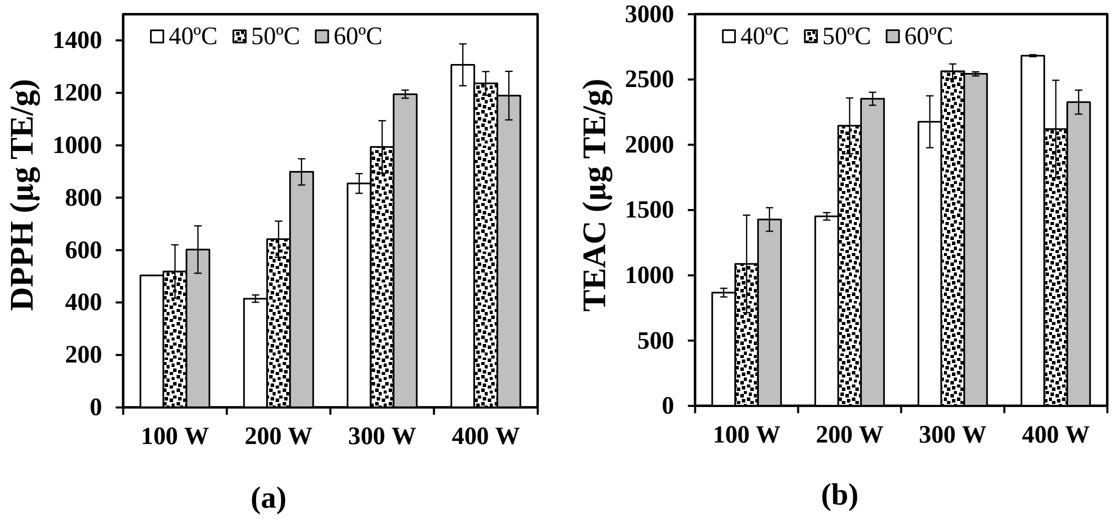

Figure 4. Thermosonication effects on the antioxidant activity of fennel extracts obtained by thermosonication using the (**a**) DPPH radical and (**b**) TEAC methods.

3.5. Comparison among the Soxhlet, Percolation, and Thermosonication Techniques

Fennel extracts obtained by the Soxhlet and percolation techniques were compared with the fennel extract produced with the best thermosonication condition (300 W and 60 °C). Figure 5 exhibits the visual appearance of each extract. Thermosonication and percolation produced visually clearer and greener fennel extracts. The extract obtained by Soxhlet had a darker, brown color. In the Soxhlet technique, the fennel sample was exposed at boiling temperature for 6 h [26]. Thus, this extraction condition may promote thermal degradation of fennel compounds producing a darker extract [13].

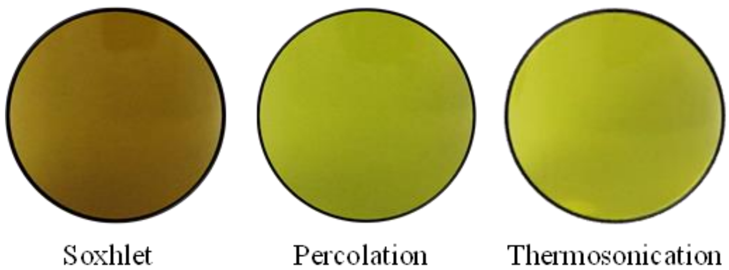

Figure 5. The effect of different extraction techniques on the visual appearance of fennel extracts.

Despite this, the fennel extract obtained by Soxhlet presented the highest global yield. Table 2 presents extraction yields acquired for each extraction technique.

Table 2. Global and extraction yields.

Extraction Technique	Global Yield (g/100 g)	Extraction Yield of Solids (g/100 g)
Soxhlet	14 ± 1 [a]	0.021 ± 0.001 [c]
Percolation	7.5 ± 0.3 [b]	0.98 ± 0.02 [a]
Thermosonication	5.5 ± 0.1 [c]	0.73 ± 0.02 [b]

Global yield: g dried extract/100 g ground fennel; solids extracted yield: g dried extract/100 g fennel ethanolic extract. Mean values ± standard deviation (n = 2). Values followed by different letters (a–c) in the same column show differences by Tukey's test at 95% significance (p-value ≤ 0.05).

The Soxhlet resulted in greater extraction of solids from the raw material. However, these solids were more diluted in the extract due to the greater amount of solvent used. Thus, this technique resulted in the highest global yield and the lowest solids' extraction yield (Table 2). The extraction by percolation produced an extract with higher global yield and extraction yield of solids than thermosonication. The higher global yields obtained by the Soxhlet and percolation techniques can be associated with the longer extraction times employed by them.

The Soxhlet technique also extracted more phenolic compounds from fennel than both the thermosonication and percolation techniques. Figure 6 presents the results of TPC recovery from fennel by each extraction technique.

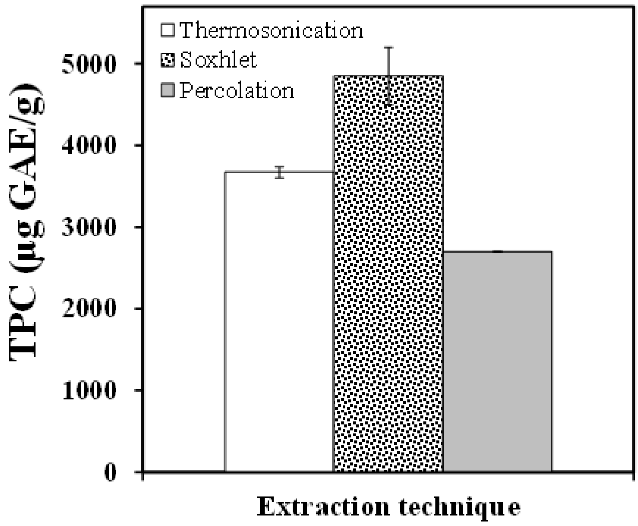

Figure 6. TPC yield acquired for each extraction technique.

The thermal energy provided by the Soxhlet and thermosonication extraction techniques may have favored the extraction of phenolic compounds since percolation uses the lowest and constant extraction temperature of 60 °C. Thermal energy favors the mass transfer by promoting an increase in the solubility of the solvent and mass transfer coefficients. Furthermore, Soxhlet employs a long extraction time with a longer contact time between solvent and solute. Despite this, the Soxhlet technique produced an extract with only 20% more phenolic compounds than thermosonication with just 20 min of extraction time. Gajic et al. [13] extracted the same content of phenolic compounds from black locust flowers using ultrasound and Soxhlet techniques. However, the ultrasound-assisted extraction was performed using an ultrasonic bath (40 kHz and 150 W) at 60 °C for 30 min. Thus, a longer extraction time by thermosonication could have produced an extract with a higher yield of phenolic compounds.

The results obtained for the antioxidant capacity of the fennel extracts corroborated with that observed for TPC. Figure 7 presents the extracts' antioxidant activity measured by DPPH and ABTS assays. The extract obtained by the Soxhlet technique presented the highest antioxidant capacity. The extract obtained by thermosonication also presented higher antioxidant activity than the extract acquired by percolation when measured by TEAC. However, an opposite behavior was verified by the DPPH results. Other studies have reported these discrepancies in antioxidant activity [27]. Despite this, the TEAC results seem to be more consistent in our study since the extract obtained by thermosonication showed a higher phenolic compounds' content than the acquired by percolation.

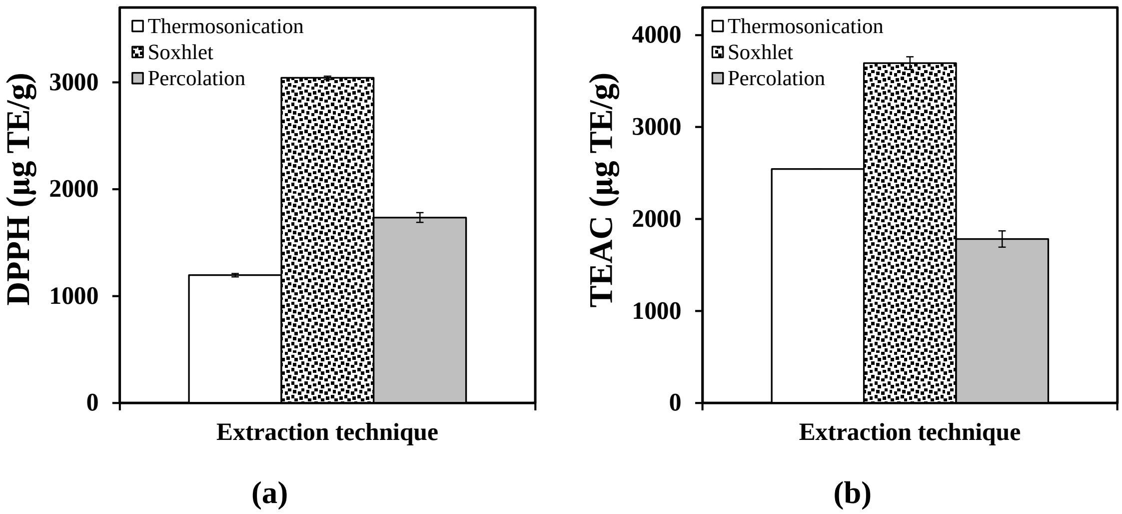

Figure 7. Antioxidant capacity of extracts acquired by each extraction method using (**a**) DPPH and (**b**) ABTS as chromophore radicals.

Although the Soxhlet technique produced an extract with the highest content of phenolic compounds and antioxidant capacity, it employed more electrical energy than percolation and thermosonication techniques. Table 3 presents the electricity consumption for obtaining fennel extracts by each extraction method.

Table 3. Electricity consumption to obtain fennel extract, dried fennel solids, and fennel total phenolics.

Extraction Parameters			Electricity Consumption (kWh)	Total Electricity Consumption (kWh/kg $_{extract}$)	Total Electricity Consumption (kWh/kg $_{dried\ solids\ extracted}$)	Total Electricity Consumption (kWh/kg $_{total\ phenolic\ extracted}$)
Technique	Equipment	Time (h)				
Soxhlet	Warming blanket (Fisatom, 330 W)	6	1.98	191	9096	353,094
	Water recirculation system (Marconi, 1800 W)	6	10.8			
Percolation	Shaker incubator with orbital shaking and heating (Marconi, 1200 W)	6	7.2	47	4817	156,241
Thermosonication (300 W, 60 °C)	Ultrasound (Unique, 300 W)	0.25	0.075	16	2127	42,276
	Circulating heating water bath (Marconi, 500 W)	0.32	0.16			

The cost of ethanol evaporation to obtain the dried solids extracted and total phenolic compounds was not considered.

The cost of electrical energy to carry out thermosonication was the lowest to obtain the ethanolic extract, dry solids, and phenolic compounds of fennel. After thermosonication, percolation required less electrical energy than the Soxhlet technique. The main difference in the energy cost for carrying out the extractions is the time used by each technique. Soxhlet and percolation extractions were performed for 6 h. In contrast, thermosonication was carried out in about 20 min. Additionally, the highest difference in energy expenditure was in total electricity consumption related to the extraction of phenolics compounds (Figure 8c). Figure 8 presents the electrical energy cost in dollars to obtain the extract, dried solids, and phenolic compounds of the fennel by each extraction method.

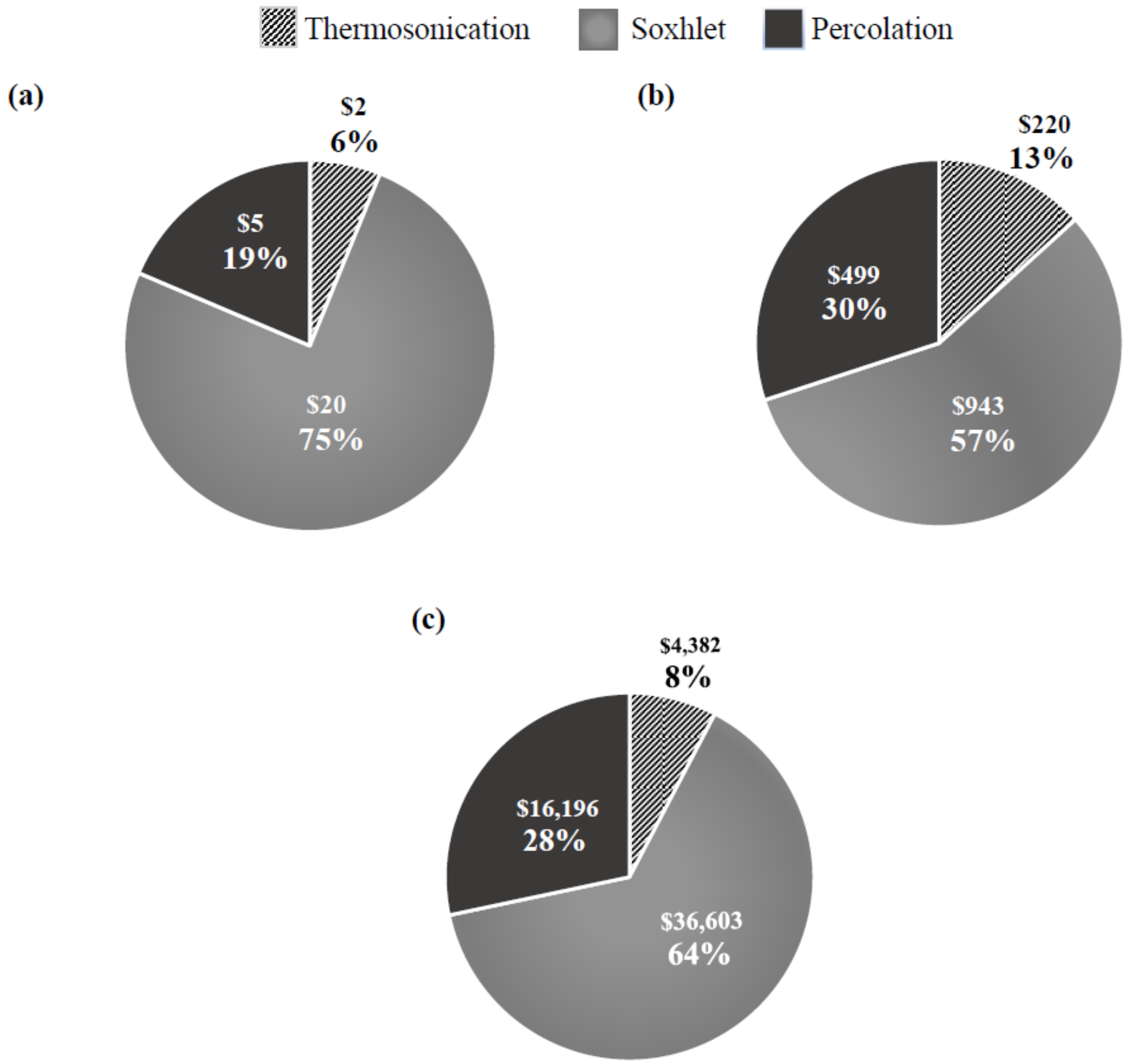

Figure 8. Electricity costs of each extraction technique to obtain: (**a**) 1 kg of fennel extract, (**b**) 1 kg of fennel dried solids, and (**c**) 1 kg of fennel phenolic compounds. Cost calculated considering the electrical energy price in Brazil per kWh (BRL 0.53) converted to dollars (USD 0.09). The cost of ethanol evaporation to obtain the dried solids and phenolic compounds was not considered.

The cost to extract the phenolic compounds from fennel by thermosonication was approximately 50 times lower than by percolation and Soxhlet processes. This great difference between total electricity consumption was due to the relationship between the mass of phenolic extracted and the mass of ethanol used in the extraction techniques. A greater mass of solvent was used by Soxhlet and percolation extraction techniques. Therefore, the phenols extracted were more diluted in the ethanol. Moreover, although the Soxhlet and percolation techniques extracted a similar amount of solids from the raw material, the electricity cost was directly related to the process's total electricity consumption (Table 3), representing a cost of four times and two times higher (Soxhlet and percolation, respectively) than thermosonication (Figure 8b). However, it also demonstrated that the thermosonication technique was more selective in extracting the interest compounds. In addition to being a faster technique, thermosonication was also economically most advantageous, since the extract obtained was more concentrated in phenolic compounds.

4. Conclusions

This paper examined the influence of process design of thermosonication on the recovery of phenolic compounds from *Foeniculum vulgare* using ethanol as solvent. Thermosonication-assisted extraction processes can reach high temperatures due to the temperature increase caused by acoustic cavitation and the external heat source. Higher ΔT values were observed for thermosonication performed at 400 W. A working temperature of 60 °C decreased the intensity of the acoustic cavitation and promoted a decrease in the ΔT values during thermosonication. The thermosonication carried out with 300 W and 60 °C extracted more phenolic compounds (3670 ± 67 µg GAE/g), and antioxidant compounds measured by DPPH (1195 ± 16 µg TE/g) and ABTS (2543.12 ± 0.00 µg TE/g). The thermosonication extraction technique showed advantages compared to longer extraction techniques of percolation and Soxhlet. The thermosonication (300 W and 60 °C) in 20 min of extraction achieved a greater recovery of TPC than percolation (2702 ± 4 µg GAE/g) and only 20% less than the Soxhlet (4849 ± 353 GAE/g) techniques. Moreover, thermosonication showed lower energy consumption due to the shorter extraction time and the lower volume of solvent used. Thus, thermosonication can be used as an innovative technique for the recovery and availability of bioactive compounds.

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