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The impact of the ultrasound-assisted extraction process on the extracted solution from dried asparagus roots (*Asparagus officinalis* L.)

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Abstract

The root of *Asparagus officinalis* L., considered an herbaceous by-product, possesses substantial potential for the production of value-added food items due to its remarkable nutritional attributes and distinctive flavor and fragrance profile. This study specifically focused on identifying an impact of advanced extraction using ultrasonic technique to obtain the most nutrients content and other functional features. The study investigated the impact of different power level (140, 180, 220, and 260) and durations (10, 15, 20, and 25 minutes) during the extraction process on the quality of the extracted solution including bioactive compounds (vitamin C, phenolic, flavonoid, saponin) and antioxidant capacity of extracted solution from dried asparagus roots. Optimal parameters for the extraction under the assist of ultrasonic were 180 W for 20 minutes. Under this condition, the extracted solution from dried asparagus roots presented the highest amount of nutrients (saccharose, vitamin C, phenolic, flavonoid, and saponin) and antioxidant capacity (DPPH and FRAP) (per 100 g dry matter) with 1.50 g, 1.06 g, 0.63 g TAE, 0.15 g QE, 1.39 g SE, 58.15%, and 1.27 M Fe^{2+}, respectively. This study well illustrated the effects of ultrasonic technique on the extraction process of the dried crop product especially asparagus root. The results are able to provide useful outcomes with the aim to investigate the most appropriate parameters for the extraction process based on the content of nutrients and bioactivity from the obtained solution.

Keywords: Antioxidant capacity, bioactive compounds, temperature, time, ultrasound-assisted

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1. Introduction

Asparagus (Asparagus officinalis L.) holds a global reputation as one of the top 20 valuable vegetable crops, primarily due to its outstanding nutritional profile. The characteristic flavor and fragrance of asparagus are attributed to a variety of volatile compounds, including pyrazines and sulfur-containing elements [1]. Recognized as a nutritious choice, asparagus boasts low calories, high fiber, and a spectrum of phytochemicals, including fructans, flavonoids, vitamins, saponins, and cinnamic acids [2,3]. These constituents exhibit diverse effects, ranging from anti-cancer and antitumor to antioxidant, immunomodulatory, hypoglycemic, anti-hypertensive, and anti-epileptic actions, enhancing the overall value of the crop [1,4]. However, despite its nutritional advantages, the production and processing of asparagus result in substantial agri-food by-products, including discarded aerial parts (approximately 6 Tm/ha, comprising stems, leaves, and fruits) and underground parts (over 30 Tm/ha, including roots) [5,6]. Managing this waste poses significant challenges for producers in agronomic, economic, and environmental aspects [6]. In recent decades, it has become evident that asparagus by-products, particularly the roots, serve as valuable sources of dietary fiber and are rich in fructans. These components, often associated with immunomodulatory and antioxidant activities due to their connection to saponins and phenolic compounds [3,5,7], remain largely undigested during transit through the digestive system [8]. Consequently, there is considerable potential for utilizing these by-products as food ingredients with notable nutritional value and health-promoting properties [3,5,6]. Therefore, these non-edible parts should be regarded not merely as cultivation waste but rather as food by-products with relevance to various industrial sectors, contributing to sustainable agriculture.

The extraction process is essential for obtaining desired natural products from raw materials, employing various methods such as solvent extraction, distillation, pressing, and sublimation, all grounded in extraction principles. Ultrasound-assisted extraction (UAE) involves applying high-intensity ultrasonic waves to food samples, offering a straightforward and cost-effective alternative to traditional extraction methods [9]. Ultrasonic technique is known to have the ability to alter the physical and chemical properties of plant materials. The generation of ultrasound in

a liquid medium produces significant energy, causing a phenomenon known as cavitation, which increases the release of intracellular substances into the solution [10-11]. The introduction of high-frequency waves disrupts the solute-solvent mixture, breaking cell walls and facilitating solvent diffusion [12]. The advantages of ultrasound extraction lie in its shorter residence time between particles and solvent, minimal material usage, low solvent requirements (minimum 100 mL), and improved overall polyphenolic extraction yields [13,14]. It proves particularly valuable for swiftly isolating bioactive compounds. This study investigated the impact of the extraction's parameters including the ultrasonic power level and duration on the nutritional composition and antioxidant capacity of the extracted solution from dried asparagus roots in order to achieve high-quality dried asparagus root.

2. Materials and Methods

2.1 Materials and Equipment

The roots of *Asparagus officinalis* L. with excellent quality and no signs of physical damage or infestation were harvested in My Thoi Ward, Long Xuyen City, An Giang Province, Vietnam. The heat-resistant variety UC 157 F2 supplied by Walker Brothers (USA) is used. The green asparagus shoots are harvested when they reach 25-30 cm [15]. The roots are cut from the shoots, with a length approximately one-third of the shoot's length.

2.2 Experimental design

Every sample comprised 2 kg of green asparagus roots, sliced into approximately 1 cm pieces. The roots underwent sorting and washing, followed by a preliminary blanching treatment involving immersion in hot water at 85°C for 2 minutes. Following this, the blanched samples were subjected to drying in a Forced Convection Oven (ESCO, OFA-110-8, Indonesia) at 70°C with an airflow velocity of 1 m/s. After the drying process, the samples were finely crushed and sifted through a mesh with a 1 mm diameter [16]. The ultrasonic treatment was repeated three times. Each sample, containing 100 g of finely ground dried material, underwent extraction in a triangular flask with a water-to-material ratio of 20:1. Ultrasound-assisted extraction was performed using UP400St Ultrasonicator (Hielscher, Germany) at the power level ranging from 140, 180, 220, and 260 W for durations of 10, 15, 20 and 25 minutes. The resulting extracted solution was then collected for subsequent analysis and evaluation.

2.3 Determination of color

Color assessment was conducted by analyzing L, a, b values using a colorimeter (Konica Minolta CR400, Japan) to evaluate the extracted solution's color.

2.4 Determination of vitamin C

Vitamin C content was assessed using a modified version of the 2,4-dinitrophenyl hydrazine colorimetric method as outlined by Sharaa and Mussa [17]. About 1 gram of sample was mixed with 5 mL of a solution comprising 3% meta-phosphoric acid (w/v) and 8% glacial acetic acid (v/v) in a 15 mL centrifuge tube. This mixture was then placed on a Reciprocating shaker (Stuart, UK) for 1 hour. Following centrifugation, 1 mL of the supernatant was combined with 0.5 mL of 3% bromine, 0.25 mL of 10% thiourea, and 0.25 mL of 2,4-dinitrophenyl hydrazine. The resulting mixture was incubated for 3 hours at 37°C. Subsequently, 10 mL of 85% H_2SO_4 was added to the tube, resulting in the formation of a red complex. After cooling the solution to room temperature, its absorbance was measured at 520 nm. The concentration of vitamin C was determined using a standard ascorbic acid graph, where the absorbance (y) was calculated using the equation $y = 0.2253x + 0.0024$ ($R^2 = 0.9999$), with x representing the concentration of the solution in the tube.

2.5 Determination of total phenolics

The phenolics contents (g TAE/ 100 g of dry matter) were determined using the Folin-Ciocalteu method as described by Sumaiyah *et al*. [18]. In brief, 0.15 mL of the sample was combined with 1.2 mL of distilled water and 0.45 mL of 5% (w/v) Na_2CO_3 in a test tube. This mixture was then mixed with 0.1 mL of Folin-Ciocalteu reagent and allowed to react at room temperature for 90 minutes. Phenolic compounds in the extract reacted with the Folin-Ciocalteu reagent, resulting in the formation of a phosphomolybdenum complex with a blue color in an alkaline environment. The absorbance of the solution was measured at 750 nm, using a UV-visible spectrophotometer (V730, Jasco, Japan). The concentration of total phenolics was determined using a standard tannic acid graph (TAE), where the absorbance (y) was calculated using the equation $y = 0.0021x + 0.0064$ ($R^2 = 0.9999$), with x representing the concentration of the solution in the tube.

2.6 Determination of total flavonoids

The flavonoids contents were assessed using a colorimetric reaction with aluminum chloride, with modifications to the method outlined by Sumaiyah *et al*. [18]. This reaction forms a stable acid complex between $AlCl_3$ and the C-4 keto groups, as well as the hydroxyl groups at C-3 or C-5 of the flavonoids and flavonols. In brief, 0.1 mL of the sample was mixed with 1.2 mL of distilled water and 30 µL of 5% (w/v) $NaNO_2$. After 5 minutes, the mixture was supplemented with 60 µL of 10% (w/v) $AlCl_3.H_2O$; 0.2 mL of 1 M NaOH, and 0.11 mL of distilled water. The resulting solution was then measured at 510 nm. The total flavonoid concentration was determined using a standard quercetin graph (QE), where the absorbance (y) was calculated using the equation $y = 8.2634x + 0.0182$ ($R^2 = 0.9999$), with x representing the concentration of the solution in the tube.

2.7 Determination of saponins

The determination of saponins contents were conducted using the vanillin-sulfuric acid method, as described by Le *et al*. [19]. This method relies on a red-violet color reaction resulting from the oxidation of triterpene saponins by sulfuric acid and vanillin. Approximately 0.25 mL of the sample was mixed with 0.25 mL of 8% (w/v) vanillin in 96% ethanol and 2.5 mL of 72% H_2SO_4. The mixture was then incubated for 30 minutes at 60°C and subsequently cooled to room temperature. The absorbance of the solution was measured at 560 nm. The concentration of saponin was determined using a standard saponin graph (SE), where the absorbance (y) was calculated using the equation $y = 0.1348x + 0.0075$ ($R_2 = 0.9999$), with x representing the concentration of the solution in the tube.

2.8 Determination of DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity

The antioxidant activity of the sample was assessed by its ability to scavenge free radicals using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. Following the protocol outlined by Molyneux [20], the DPPH assay was conducted with minor adjustments. Specifically, a mixture of the sample (1.5 mL) and DPPH solution in a 1:1 ratio (v/v) was prepared. This assay relies on electron transfer, resulting in the formation of a purple solution in ethanol, which was then analyzed spectrophotometrically at 517 nm. The inhibition of DPPH free radicals was determined using Equation 1.

Inhibition of DPPH radical (%) = $100 \times (A_c - A_s)/A_c$ (1)

Where: A_c is the absorbance of the control and A_s is the absorbance of the sample.

2.9 Determination of ferric reducing antioxidant power (FRAP)

The FRAP assay (mM of $FeSO_4$/g dry matter) was conducted following the method outlined by Sudha *et al*. [21] with some adjustments. This method relies on the reduction of the tripyridyltriazine complex Fe $(TPTZ)^{3+}$ to the blue-colored Fe $(TPTZ)^{2+}$ by antioxidants in an acidic medium. The FRAP reagent consisted of 100 mL of 200 mM acetate buffer (pH 3.6), 10 mL of 20 mM $FeCl_3.6H_2O$, and 10 mL of 10 mM TPTZ in 40 mM HCl. A volume of 0.05 mL of the sample was mixed with 1.5 mL of the FRAP reagent and 0.15 mL of distilled water. The mixture was then incubated at 37°C for 8 minutes and subsequently analyzed for absorbance at 593 nm.

2.10 Data analysis

The statistical analysis of the extraction study was performed utilizing the Statgraphics Centurion XVI software (U.S.A.).

3. Results and discussion

The influences of ultrasonic power and extraction time on the extracted solution from dried asparagus roots including of the nutritional content and antioxidant capacity are illustrated in Figures 1 and 2.

The results indicate that ultrasonic power and extraction time significantly affected the chemical composition, bioactive components, and antioxidant capacity of dried asparagus roots' extraction. However, there was no significant impact on the color of the extracted solution (via L, a, b values), except for the extraction time affecting the b value. As ultrasonic power increased, the values of Brix, sucrose, vitamin C, phenolic, flavonoid, saponin, and DPPH increased up to the peak and then gradually decreased. Specifically, when ultrasonic power increased from 140 to 260 W (corresponding to 35-65% of the maximum power), Brix increased from 2.14 to 2.28 (1.064 times), with no statistically significant difference at 220 and 260 W. Vitamin C content increased by 1.04 times (from 0.99 to 1.03 g/100 g dry matter) when ultrasonic power enhanced from 140 to 220 W, with no statistically significant difference compared to the sample extracted at 180 W. The content of sucrose, phenolic, flavonoid, saponin, and DPPH increased from 1.04 to 1.15 times when ultrasonic power increased from 140 to 180 W. The iron reduction ability (FRAP) increased by 1.93 times (from 0.87 to 1.67 M Fe^{2+}/100 g dry matter) when ultrasonic power rose from 140 to 260 W. Ultrasonic power creates chaos by forming vortices, leading to the disruption of cell structures, making it easier for nutrients to escape from cells. With higher ultrasonic power, greater gas penetration and pressure can generate hydroxyl radicals, affecting bioactive compounds, reducing their content, and scavenging free radicals. Similar patterns of ultrasonic power impacting the extraction of nutrients from plants have been observed in previous studies [22,23].

Similarly, the Brix level, the content of vitamin C, flavonoid, saponin, and antioxidant capacity (DPPH and FRAP) increased to an optimum value and then gradually fell down with the longer extraction time aided by ultrasonic power. Specifically, the highest values for Brix, phenolic, flavonoid, and FRAP of the extracted solution were 2.27, 0.59 g TAE, 0.11 g QE, and 1.38 M Fe^{2+}/100 g dry matter, respectively, when the ultrasonic-assisted extraction time is 20 minutes. Meanwhile, the highest levels of vitamin C, saponin, and DPPH are 1.05 g, 1.32 g SE/100 g dry matter, and 55.44% at 15 minutes of the extraction process; however, there is no statistically significant difference compared to the extraction at 20 minutes ($p>0.05$).

The results also indicate that the sucrose and phenolic content increased with the prolonged extraction time, reaching the highest values at 25 minutes. During ultrasonic-assisted extraction, soluble compounds come into contact with the solvent, and thus, the interaction time between the two phases significantly influences the extraction efficiency [24]. With longer ultrasonic-assisted extraction times, the transformations induced by ultrasonic power become more profound, resulting in higher cell disruption rates and increased extraction efficiency [24,25]. Moreover, upon reaching equilibrium and a certain power level, extended extraction times lead to a substantial reduction in the nutrient content [24,26]. Consequently, the antioxidant activity of the extracted solution decreases [27]. This trend aligns with the findings of Sun *et al*. [28] in ultrasonic-assisted saponin extraction from *Zizyphus jujuba* Mill var. spinosa leaves, where saponin levels peaked after 50 minutes of extraction, but continued extraction up to 60 and 70 minutes led to a decline. Similarly, the study by Tiên *et al*. [29] on saponin extraction from *Abelmoschus sagittifolius* revealed that a 10-minute extraction yielded the highest saponin content, and further increasing the extraction time to 15 and 20 minutes did not significantly alter the saponin levels compared to the 10-minute treatment. Studies by [27, 30, 31] also observed a similar trend when extending ultrasonic-assisted extraction times.

The graph illustrating the regression coefficients in Figure 3 has confirmed the extent of influence of independent variables as well as interactions affecting the chemical composition and bioactive compounds of dried asparagus roots solution. The results indicate that Brix was significantly influenced by ultrasonic power; the content of sucrose and saponin was greatly affected by the interaction between ultrasonic power and extraction time; the levels of phenolic compounds, flavonoids, and DPPH were strongly influenced by the quadratic term of ultrasonic power, and the vitamin C content was significantly influenced by the quadratic term of extraction time.

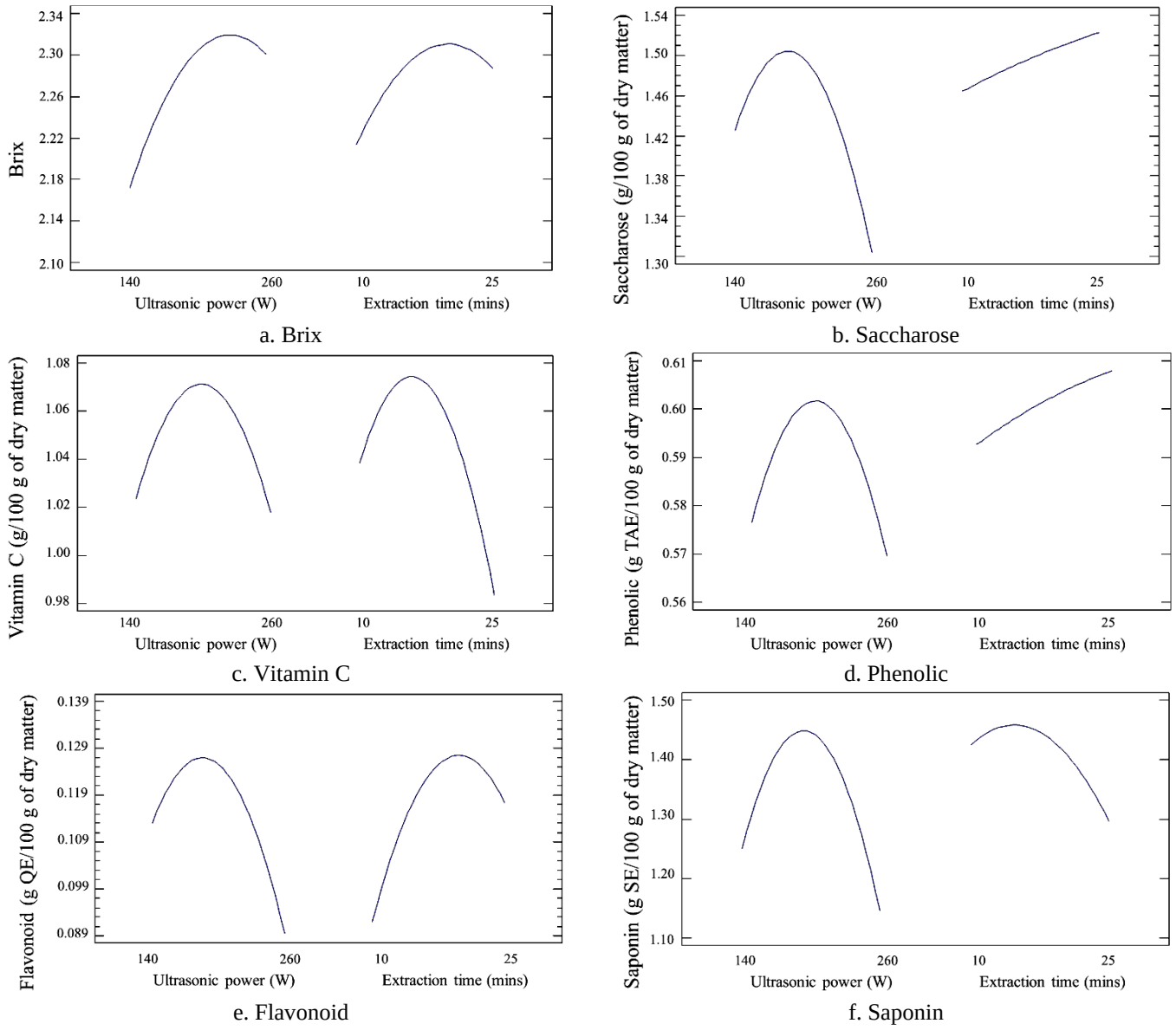

Figure 1: The correlation between ultrasonic power and extraction time with the ultrasonic assistance on the chemical composition of extracted solution from dried asparagus roots (a) Brix, (b) Saccharose (c) Vitamin C, (d) Phenolic, (e) Flavonoid amd (f) Saponin

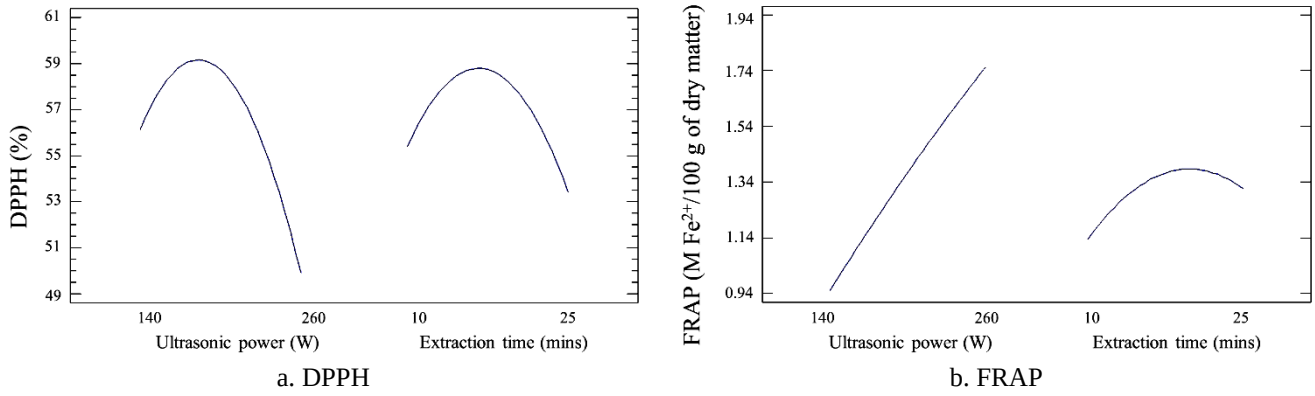

Figure 2: correlation between ultrasonic power and extraction time with the ultrasonic assistance on the antioxidant capacity of extracted solution from dried asparagus roots (a) DPPH and (b) FRAP

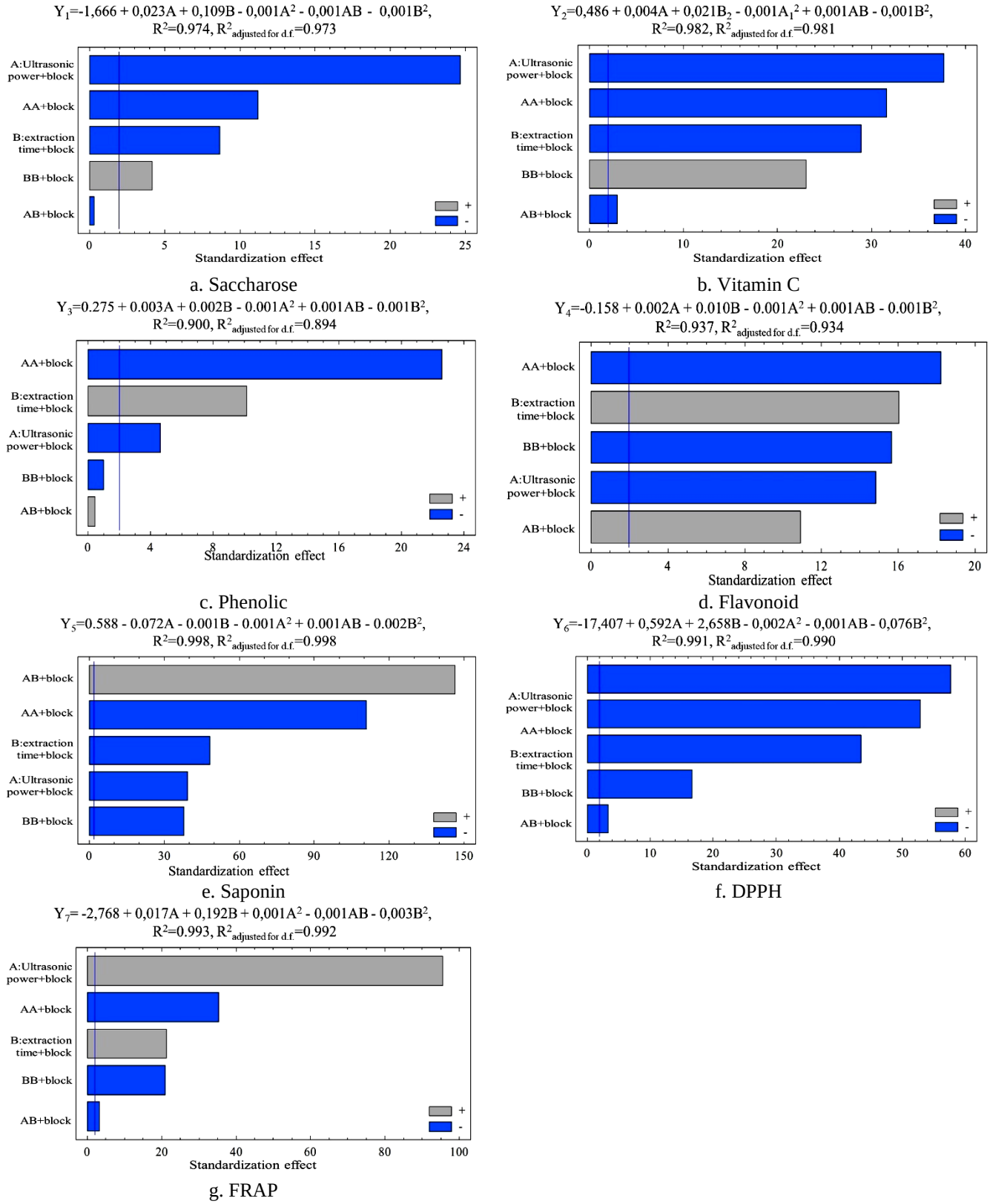

Figure 3: The level of influence of ultrasonic power, extraction time, and the interaction between ultrasonic power and extraction time on (a) Saccharose, (b) Vitamin C, (c) Phenolic, (d) Flavonoid, (e) Saponin, (f) DPPH and (g) FRAP

The study also developed regression equations to predict the levels of phenolic compounds, flavonoids, saponins, vitamin C, sucrose, and antioxidant capacity (DPPH and FRAP) in the extracted solution based on different ultrasonic power and extraction time (Figure 3). The obtained equations had correlation coefficients R^2 and R^2_{adj} greater than 0.89, indicating a high degree of correlation. Therefore, these equations can be used to predict the variations in the levels of bioactive and chemical compounds, as well as the antioxidant capacity of the extracted solution based on different ultrasonic power and extraction time as investigated in the study.

4. Conclusions

The study selected an ultrasonic power of 180 W for 20 minutes as the optimal parameters for this extraction process. This finding could be considered as a reference for ultrasound-assisted extraction methods to enhance the extraction efficiency of recovering high-nutrient-value compounds applied in the food and beverage industries.

Conflict of interest

The authors declare no conflict of interest.

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