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Effects of Ultrasound-Assisted Processing on the Extraction and Stability of Antioxidant-Rich Plant Compounds

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Abstract: ***Ultrasound treatment is an effective food processing method that improves the extraction and usability of plant-based antioxidants. This study examines how ultrasound affects the extraction of polyphenols, flavonoids and anthocyanins from vegetables and plant extracts, as well as their stability during storage. Results show that ultrasound-assisted processing increases the extraction efficiency of these antioxidants and enhances their activity. In addition, different ultrasound frequencies and power levels lead to varying rates of compound breakdown. These findings support the use of ultrasound in food processing and functional food development, highlighting its potential for health-focused applications.***

Keywords: Ultrasound treatment; Antioxidant activity; Plant compounds; Extraction efficiency; Stability.

1. INTRODUCTION

With the growing focus on health-conscious diets, foods rich in plant-based antioxidants have received increasing attention. Antioxidants help neutralize free radicals in the body and play a key role in reducing the risk of diseases linked to oxidative stress, such as cardiovascular conditions, cancer and neurodegenerative disorders [1]. Plants contain valuable antioxidants, including polyphenols, flavonoids, and anthocyanins, which show strong protective effects [2]. However, extracting these compounds efficiently while maintaining their stability during processing and storage remains a challenge in food science.

Traditional extraction methods, such as solvent extraction and thermal reflux, often require long processing times, consume large amounts of energy, and cause damage to heat-sensitive compounds [3,4,5]. In recent years, ultrasound-assisted extraction (UAE) has gained popularity due to its fast, efficient, and mild processing conditions [6]. Ultrasound waves create physical effects, including cavitation, mechanical stress, and localized heating, which help break plant cell walls and improve the release of target compounds into the extraction solvent [7,8].

Despite the advantages of ultrasound-assisted extraction, little research has been done on how different ultrasound conditions affect the extraction and stability of antioxidants from various plant sources. A better understanding of the relationship between ultrasound parameters and antioxidant preservation is important for improving food processing methods and developing high-quality functional foods [9,10]. This study systematically evaluates the impact of ultrasound-assisted processing on the extraction and stability of plant antioxidants, providing useful insights for its application in the food industry.

2. MATERIALS AND METHODS

2.1 Experimental Materials

This study selected spinach, blueberries, purple cabbage and ginkgo leaves as test materials, as they are rich in antioxidants. All samples were sourced from certified local markets or specialized farms to ensure freshness and consistency. The reagents used, including ethanol, methanol, acetone, Folin–Ciocalteu reagent, gallic acid standard, rutin standard, and cyanidin-3-glucoside standard, were of analytical grade and obtained from recognized suppliers to ensure data accuracy.

2.2 Ultrasound-Assisted Extraction Equipment

An ultrasound processor with adjustable power and frequency settings was used. The frequency was set between 20 and 100 kHz, and the power ranged from 100 to 1000 W [11,12]. A precise temperature control system was included to keep the reaction temperature within ±1°C of the target value, ensuring stable experimental conditions.

2.3 Extraction Procedure

The plant materials were washed, dried, and ground to a suitable particle size. A measured amount of plant powder was placed in a stoppered Erlenmeyer flask, and an ethanol–water mixture (pre-optimized for volume ratio) was added as the extraction solvent. The flask was immersed in the water bath of the ultrasound processor and processed under set frequency, power, and duration conditions. After extraction, the solution was cooled to room temperature and filtered to remove solid residues. The filtrate was used for further analysis.

2.4 Measurement of Antioxidant Compounds

The total polyphenol content was determined using the Folin–Ciocalteu method, with gallic acid as the standard. The extract was mixed with Folin–Ciocalteu reagent and sodium carbonate, and absorbance was measured at 765 nm to calculate polyphenol content (mg GAE/g sample). The total flavonoid content was measured using a sodium nitrite–aluminum nitrate–sodium hydroxide colorimetric method, with rutin as the reference. After sequential addition of reagents, absorbance was recorded at 510 nm to determine flavonoid concentration (mg RE/g sample). The anthocyanin content was determined using the pH differential method, where the extract was mixed with buffer solutions at pH 1.0 and 4.5, and absorbance was measured at specific wavelengths to calculate anthocyanin levels (mg CGE/g sample).

2.5 Antioxidant Activity Assay

The antioxidant activity of the extracts was evaluated using the DPPH radical scavenging assay, ABTS cation radical scavenging assay and FRAP ferric ion reducing power assay. Ascorbic acid was used as the standard. The DPPH and ABTS scavenging capacities and the FRAP value were calculated. Results were expressed as ascorbic acid equivalents (mg AAE/g sample).

2.6 Stability Assessment

The extracts were stored under different conditions, including varying durations (0–90 days), temperatures (4°C, 25°C, and 37°C), and light exposures (dark storage vs. natural light). Antioxidant content and activity were measured periodically to analyze the effects of these factors on compound degradation and stability.

2.7 Data Analysis

Data were analyzed using Origin software. Results were expressed as mean ± standard deviation (n = 3). One-way analysis of variance (ANOVA) and Duncan’s multiple range test were used to assess differences, with $P < 0.05$ considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 Ultrasound-Assisted Extraction Efficiency

3.1.1 Polyphenol Extraction

Table 1: Comparison of polyphenol yields in different plant materials (mg GAE/g sample)

Plant Material	Traditional Extraction	Ultrasound-Assisted Extraction	Increase (%)
Spinach	15.62 ± 0.85	28.45 ± 1.23	82.14
Blueberries	18.73 ± 1.02	30.56 ± 1.38	63.16
Purple cabbage	14.25 ± 0.91	25.89 ± 1.17	81.70
Ginkgo leaves	16.48 ± 0.88	27.65 ± 1.21	67.78

Compared with traditional extraction methods, ultrasound-assisted processing significantly improved the extraction efficiency of polyphenols from spinach, blueberries, purple cabbage, and ginkgo leaves ($P < 0.05$) [13,14]. Under optimized ultrasound conditions (40 kHz, 400 W, 30 min), the polyphenol yield in spinach increased from 15.62 ± 0.85 mg GAE/g (traditional extraction) to 28.45 ± 1.23 mg GAE/g, representing an 82.14% enhancement. A detailed comparison of polyphenol yields in different plant materials is presented in Table 1.

The significant improvement in extraction efficiency is mainly attributed to the cavitation effect of ultrasound. The rapid collapse of microbubbles in the liquid medium generates localized high pressure and high temperature, facilitating cell wall and membrane disruption [15]. This process accelerates the diffusion of polyphenols from plant cells into the solvent, leading to a higher extraction yield.

3.1.2 Flavonoid Extraction

Ultrasound-assisted processing also significantly enhanced the extraction of flavonoids across different plant materials. For instance, under optimal conditions (50 kHz, 350 W, 25 min), the flavonoid yield in blueberries increased from 12.36 ± 0.67 mg RE/g (traditional extraction) to 20.18 ± 0.95 mg RE/g, reflecting a 63.27% increase. The mechanical effect of ultrasound plays a key role in this enhancement. By breaking plant cell structures, ultrasound increases the contact area between flavonoids and the extraction solvent, facilitating the release of target compounds and improving extraction efficiency [16,17].

3.1.3 Anthocyanin Extraction

For anthocyanin-rich purple cabbage, ultrasound-assisted extraction demonstrated excellent efficiency. Under optimized conditions (60 kHz, 500 W, 20 min), the anthocyanin yield increased from 8.54 ± 0.52 mg CGE/g (traditional extraction) to 15.87 ± 1.05 mg CGE/g, marking an 85.83% improvement. The detailed comparison is presented in Table 2.

Table 2: Comparison of anthocyanin yields in purple cabbage (mg CGE/g sample)

Extraction Method	**Yield**	**Increase (%)**
Traditional Extraction	8.54 ± 0.52	-
Ultrasound-Assisted Extraction (60 kHz, 500 W, 20 min)	15.87 ± 1.05	85.83

Besides improving extraction efficiency, ultrasound treatment also reduced anthocyanin degradation. The rapid extraction process minimized exposure to high temperatures, reducing heat-induced degradation and allowing anthocyanins to be extracted with higher purity and yield.

3.2 Effect of Ultrasound-Assisted Processing on Antioxidant Capacity

3.2.1 DPPH Radical Scavenging Capacity

The DPPH radical scavenging capacity of ultrasound-extracted plant extracts was significantly enhanced ($P < 0.05$). For instance, in ginkgo leaf extract, the DPPH scavenging capacity, expressed as ascorbic acid equivalent, increased from 18.45 ± 1.12 mg AAE/g (traditional extraction) to 32.68 ± 1.56 mg AAE/g after ultrasound treatment. The variations in DPPH radical scavenging ability across different plant extracts are shown. This suggests that ultrasound not only enhances antioxidant compound extraction but may also modify their composition or structure, increasing their reactivity with DPPH radicals and thereby improving antioxidant capacity [18,19,20].

3.2.2 ABTS Cation Radical Scavenging Capacity

Table 3: Comparison of ABTS radical scavenging capacity in different plant extracts (mg AAE/g sample)

Plant Material	**Traditional Extraction**	**Ultrasound-Assisted Extraction**	**Increase (%)**
Spinach	20.12 ± 1.34	35.76 ± 1.89	77.73
Blueberries	23.45 ± 1.43	41.23 ± 2.01	75.82
Purple cabbage	21.03 ± 1.29	37.89 ± 1.78	80.17
Ginkgo leaves	20.87 ± 1.31	36.45 ± 1.69	74.65

Similar to the DPPH results, the ABTS cation radical scavenging capacity of ultrasound-assisted extracts was significantly higher than that of traditional extracts. For example, the ABTS scavenging capacity of spinach extract increased from 20.12 ± 1.34 mg AAE/g (traditional extraction) to 35.76 ± 1.89 mg AAE/g with ultrasound treatment. The results for different plant extracts are summarized in Table 3.

This confirms the role of ultrasound in enhancing antioxidant capacity, likely by promoting the extraction of highly active antioxidant compounds or inducing structural modifications that enhance their reactivity with ABTS radicals [21,22].

3.2.3 FRAP Ferric Ion Reducing Power

The FRAP assay results showed that ultrasound-treated plant extracts exhibited significantly higher ferric ion reducing power ($P < 0.05$). For example, in blueberry extract, the FRAP value increased from 22.35 ± 1.45 mg AAE/g (traditional extraction) to 38.97 ± 2.01 mg AAE/g after ultrasound treatment. The FRAP results for different plant extracts are illustrated. These findings indicate that ultrasound-extracted compounds had a greater ability to donate electrons, reducing Fe^{3+} to Fe^{2+} more effectively. This suggests that ultrasound treatment enhances antioxidant capacity by influencing the composition, concentration, and structural properties of antioxidant compounds [23,24].

3.3 Effect of Ultrasound Processing Conditions on Phytochemical Stability

3.3.1 Effect of Ultrasound Frequency

The stability of antioxidant compounds varied significantly with ultrasound frequency. As frequency increased, degradation rates initially decreased but then increased at higher frequencies [25,26]. For instance, in purple cabbage extract, anthocyanin retention after 90 days of storage was highest (78.56% ± 3.24%) at 40–60 kHz, whereas it dropped to 52.34% ± 4.12% at 100 kHz. .

3.3.2 Effect of Ultrasound Power

Ultrasound power also significantly affected phytochemical stability. Lower power levels helped maintain stability, while higher power levels accelerated degradation. In ginkgo leaf extract, flavonoid retention decreased from 85.43% ± 3.87% at 200 W to 61.25% ± 4.56% at 600 W after 90 days.

3.4 Effect of Storage Conditions on Phytochemical Stability

3.4.1 Effect of Temperature

Storage temperature strongly influenced antioxidant stability. At 4°C, polyphenol retention in blueberry extract remained at 83.25% ± 4.01% after 90 days, whereas at 37°C, it dropped to 48.67% ± 5.23%. Higher temperatures accelerated oxidation and hydrolysis, leading to faster degradation.

3.4.2 Effect of Light Exposure

Light exposure significantly impacted stability. After 90 days, anthocyanin retention in purple cabbage extract was 76.54% ± 3.65% under dark storage but dropped to 50.23% ± 4.87% under natural light. Light exposure induced photodegradation, reducing antioxidant activity. Thus, light protection is crucial for maintaining phytochemical stability after ultrasound processing [27,28].

4. CONCLUSION

This study examined the impact of ultrasound-assisted processing on the extraction and stability of antioxidant-rich plant compounds. The results confirmed that ultrasound treatment significantly increased the extraction efficiency of polyphenols, flavonoids, and anthocyanins from various plant sources while improving their antioxidant activity. In addition, ultrasound frequency and power played a key role in compound stability. Moderate ultrasound frequency and lower power levels helped preserve the stability of these compounds. During storage, temperature and light exposure were found to be major factors affecting stability. Storing extracts at low temperatures and in dark conditions effectively reduced degradation and maintained antioxidant activity. These findings provide practical insights for the food industry, supporting the use of ultrasound-assisted extraction to improve the yield and stability of plant-based antioxidants. The results also offer guidance for the development of functional foods with higher-quality ingredients. Future studies should further explore how ultrasound treatment affects the molecular structure and biological activity of plant compounds. Additionally, combining ultrasound with other extraction methods, such as enzyme-assisted or supercritical fluid extraction, may further improve efficiency and product quality. Expanding the application of ultrasound technology in health-related foods could offer broader benefits for food processing and human health.

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