Ultrasonics Sonochemistry 24 (2015) 247–255

Contents lists available at ScienceDirect

Ultrasonics Sonochemistry

journal homepage: www.elsevier.com/locate/ultson

Effects of ultrasonic treatments on the polyphenol and antioxidant content of spinach extracts

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ARTICLE INFO

Article history:
Received 20 February 2014
Received in revised form 1 July 2014
Accepted 10 October 2014
Available online 18 November 2014

Keywords:
Ultrasonic extraction
Spinach
DPPH
Flavonoids
Antioxidant activity

ABSTRACT

The objective was to test ultrasound treatments on spinach leaves during extraction, and conventional extraction was used as a control. The effects of different combinations of the ultrasonic water bath factors tested on phenolic compound yields included frequency (37 and 80 kHz), exposure time (5, 10, 15, 20, 25, and 30 min), temperature (30, 40, and 50 °C), and ultrasonic power (30%, 50%, and 70%). The best conditions for extraction yields were ultrasonic frequency of 37 kHz, extraction time of 30 min, reaction temperature of 40 °C, and ultrasonic power of 50%. The mean yield (mg/100 g), total phenol (mg gallic acid/g DW), flavonoids (mg/g DW), % DPPH free-radical scavenging activity, and % ferric reducing antioxidant power were all high (64.88 ± 21.84, 33.96 ± 11.30, 27.37 ± 11.85, 64.18 ± 16.69 and 70.25 ± 9.68). Treatments were significantly different. The interaction among the ultrasonic parameters was significant. Temperature and power had significant effects on all other dependent variables.

1. Introduction

Consumption of vegetables was associated with reduced risks of many diseases (such as cancer and cardiovascular disease) in epidemiological studies [1]. Numerous studies have attempted to screen commonly eaten vegetables (carrots, potatoes, sweet potatoes, red beets, cabbage, Brussels sprouts, broccoli, lettuce, and spinach) for bioactive compounds and their antioxidant activities using different assays [2]. Advanced extraction methods have paved the way for rapid extraction of bioactive compounds [3]. Despite assays to show the activity of vegetables' bioactive compounds, little is known about the activity of antioxidant components that can be isolated from these vegetables. Researchers have tended to focus on developing advanced methods to isolate, identify, and measure the activity of natural antioxidant compounds such as flavonoids, phenolic acids, tocopherols, carotenoids, and ascorbic acid [4].

Spinach (*Spinacea oleracea* L.) is one of the most popular vegetables in the world [5]. The number of people in the United States who consume spinach increased in the past decades. According to analytical chemists, spinach is a good source of violaxanthin and neoxanthin because these kinds of compounds are not commercially available as supplements [6]. Generally, in green vegetables such as spinach, only the green chlorophylls are seen by the consumer because they mask the bright colors of carotenoids. Carotene, lutein, violaxanthin, and neoxanthin are the major carotenoids in raw spinach [7]. The health benefits of spinach are partly due to the photoprotective function of carotenoids. Some of the carotenoids contain provitamins such as carotene which can be converted to vitamin A inside the human body through metabolism. In addition, scientists have confirmed that carotenoids have the ability to protect against certain forms of cancers, eye diseases such as age-related macular degeneration, and cardiovascular diseases [8].

Consumption of spinach is important in both developed and developing countries. Spinach in developed countries is mostly consumed either fresh or blanched, and sometimes after being frozen or canned. Dehydrated spinach is used in many developing countries due to extended shelf life [5,6]. Isolated polyphenols and antioxidants from spinach may be obtained by an extraction and separation process for potential use in functional foods or nutraceuticals. Higashio et al. [9] used methanol to extract and identify phenolic compounds from spinach leaves. Approximately 15 peaks were successfully extracted and separated by high-pressure liquid chromatography (HPLC), but only quercetin was identified. Chemat et al. [10] have pointed to the possibility of using ultrasound in food processing to extend the shelf life, decrease process energy and processing time, and to enhance food

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http://dx.doi.org/10.1016/j.ultsonch.2014.10.023
1350-4177/

quality. Other studies reported use of ultrasound to enhance extraction by disrupting cell tissue, such as extracting anthocyanin from grape by-products [11] and phenolics from cranberry products [12]. Recently, Albu et al. [13] used ultrasound to extract phenolic compounds from rosemary. They compared ultrasonic bath, ultrasonic probe, and shaking water bath extraction methods at diverse temperatures and with different solvents to find the most efficient. In all situations, the operation time was decreased by using an ultrasonic bath or probe system. Similar behavior was reported by Luque-Garcia et al. [14] who used ultrasound due to its positive effects in extraction processes for capsaicinoids of hot peppers. Both mechanical and thermal effects of ultrasound were studied on plant cells and tissues. The thermal effects of ultrasound occurred when ultrasonic waves were converted to heat and absorbed by plant tissue while the mechanical effects of ultrasound caused acoustic cavitation thereby causing a bubble to grow resulting in cell disruption for improved extraction [15,16].

Ultrasonic treatments have not been reported for extraction of antioxidants from spinach, but may prove improve yield over traditional solvent extraction methods. The objectives of this study were to (1) compare phytochemicals extracted from ultrasound and a traditional solvent extraction method; (2) compare ultrasonic treatment at different frequencies, temperatures, power settings, and exposure times on the yield of total phenol, total flavonoids and antioxidant activity; and (3) compare yield of spinach polyphenols between the highest yielding ultrasonic treatment and the traditional extraction method.

2. Materials and methods

2.1. Raw material

Spinach leaves were provided by Dr. Alan Walters of the Department of Plant, Soil and Agricultural Systems, College of Agricultural Sciences, Southern Illinois University, USA. Raised beds with vermicompost for fertilizer on bare soil were used for organic production. Spinach (cv. 'Tyee') was planted in double rows (7–10 cm spacing) on the raised beds. Spinach leaves were harvested from several randomly selected plants. Leaves were harvested from several randomly selected plants. The leaves were cleaned, sliced, and crushed in a blender; and then sealed and stored in plastic bags at −18 °C for five days before freeze–drying.

2.2. Ultrasonic extraction

An ultrasonic bath with indirect ultrasonic contact through flasks was used for this study to minimize damage compared to a direct ultrasonic probe, as the chemical structure of the antioxidants could be changed by heat generated from a direct probe [16]. An Elmasonic P30 (P30) ultrasonic cleaner (Elma Hans Schmidbauer GMBH, Singen, Germany) with heated bath was used for treatments. User adjustable controls included frequency (37 and 80 kHz), heated bath temperature, and power setting as a percentage of full power (30–100%). The standard ultrasonic mode was used. Temperature settings used for this study were 40, 50, and 60 °C and power settings were 30%, 50%, and 70%. The manufacturer rated the P30 with an ultrasonic peak power of 480 W and an effective power rating of 120 W. The P30 had a proprietary algorithm to adjust power based on the impedance of the system. For a specific power setting, samples experienced the same degree of cavitation regardless of the load in the bath. For all treatments, the bath of the P30 contained 1.7 L of water before the treatment containers were added. Ultrasonic power was expressed as W/cm^2, based on the power setting as a percentage of rated power and the volume of the bath solution prior to the addition of treatment containers. Ultrasonic peak powers for the 30%, 50%, and 70% power settings were 85, 141, 198 W/cm^2, respectively and effective powers were 21, 35, 49 W/cm^2 respectively.

2.3. Preparation of crude extracts

The solvent extraction technique of Chang et al. [17] was used with slight adjustments. Ten grams of lyophilized spinach were weighed and placed in a 200 mL glass flask. Then 100 mL of methanol was added to the flask. The solution was transferred to a 116 mL polypropylene container with cylindrical shape and screw-on lid before insertion in the P30. For the traditional method, the mixture was placed in the P30 water bath for 30 min at 50 °C without ultrasound to solubilize bioactive compounds from spinach.

For each ultrasonic temperature–power treatment, the Elmasonic P30 was set to the desired temperature and power and the water bath was allowed to reach the set temperature. Then 6 identical samples, each in separate polypropylene containers, were placed in the ultrasonic bath and the ultrasonic treatment was initiated for 30 min. At each 5 min interval (5, 10, 15, 20, 25, and 30 min), one of the samples was randomly selected and removed from the ultrasonic bath. The remaining samples were immediately clustered together at one end of the ultrasonic bath.

All ultrasonic treatments were conducted in a systematic order from lowest to highest temperature (30, 40 and 50 °C). Within each temperature setting, power settings were adjusted from low to high (30%, 50% and 70%). Each treatment setting was repeated 3 times before changing to the next setting. The procedure was completed for 37 kHz frequency and duplicated for 80 kHz frequency.

After treatment, ultrasound and traditional extraction samples were filtered (Whatman No. 1, Whatman International Ltd, Maidstone, United Kingdom). The solids of the lower layer were re-extracted with 100 mL of methanol at room temperature to ensure all soluble bioactive compounds were recovered. The filtered liquids were placed into a rotary evaporator (BUCHI, Labortechnik AG, Flawil, Switzerland) under vacuum at 40 °C to reduce solvent volumes to 10 mL.

2.4. Phytochemical tests

Seven assays were used to identify phytochemical compounds of alkaloids, saponins, glycosides, tannins, phenols, flavonoids, and triterpenoids in each sample according to the methods of Harbone [18]. Three samples of the traditional extract method were analyzed. For the ultrasonic method, one sample of each combination of frequency, temperature, and power setting was analyzed.

2.4.1. Alkaloids

Mayer's reagent was prepared by mixing 13.5 g of mercuric chloride and 50 g of potassium iodide with 100 mL distilled water into 100 mL flask. The 50 mg of crude extracts were treated with 1–2 mL of hydrochloric acid (2 N) and then 1–3 drops of newly prepared Mayer's reagent were added. The appearance of red residue in the test liquid indicated alkaloids in the sample.

2.4.2. Saponins

Exactly 25 mL of distilled water were added to 2 mL of the spinach samples with manual shaking for 15–20 min. The appearance of a steady foam indicated the presence of saponins.

2.4.3. Glycosides

Hydrochloric acid, 5 mL of 70% (v/v) was added to 1 g spinach for hydrolysis in water bath at 100 °C. Afterward spinach extracts were treated with chloroform, and then 5 mL of dilute ammonia