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Sonication enhances polyphenolic compounds, sugars, carotenoids and mineral elements of apple juice

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ABSTRACT

A study was initiated with the objective of evaluating the effects of sonication treatment on quality characteristics of apple juice such as polyphenolic compounds (chlorogenic acid, caffeic acid, catechin, epicatechin and phloridzin), sugars (fructose, glucose and sucrose), mineral elements (Na, K, Ca, P, Mg, Cu and Zn), total carotenoids, total anthocyanins, viscosity and electrical conductivity. The fresh apple juice samples were sonicated for 0, 30 and 60 min at 20 °C (frequency 25 kHz and amplitude 70%), respectively. As results, the contents of polyphenolic compounds and sugars significantly increased ($P < 0.05$) but the increases were more pronounced in juice samples sonicated for 30 min whereas, total carotenoids, mineral elements (Na, K and Ca) and viscosity significantly increased ($P < 0.05$) in samples treated for 60 min sonication. Losses of some mineral elements (P, Mg and Cu) also occurred. Total anthocyanins, Zn and electrical conductivity did not undergo any change in the sonicated samples. Findings of the present study suggest that sonication technique may be applied to improve phytonutrients present naturally in apple juice.

1. Introduction

Apple (*Malus domestica*), one of the most widely cultivated tree fruits in many regions of the world, is the most important fruit and is liked by all classes of people due to its pleasant taste, established nutritional and economical value. It is a rich source of antioxidant compounds, carbohydrates, essential minerals and dietary fibers [1–3]. At present, consumption of apple and apple juice is of considerable importance to reduce the risk of many diseases owing to their functional components such as flavonoids, phenolic acids, tannins, minerals and vitamins [1,4,5].

Processing methods play an important role in determining product quality, safety and shelf life. It is well established that thermal processing technology assures the safety of food products with extended shelf life but it also causes losses in the beneficial nutrients [6]. These facts inspired the researchers all over the world to find more reliable and simpler technique that could enhance nutrients in the fruit juices [7,8]. Ultrasound treatment is an innovative emerging food processing technique that could effectively improve the health related compounds and other quality parameters of fruit juices [8,9]. Additionally, this technique is regarded as more beneficial due to its less energy consumption, reduced processing time and being environmental friendly [10,11]. It creates a comprehensive balance between processor and consumer expectations by tangible improvement of quality and safety at affordable cost along with environmental sustainability.

A recent study depicted that sonication treatment significantly enhanced the phenolic compounds, ascorbic acid, cloud value, DPPH free radical scavenging activity and total antioxidant capacity besides significant reduction in microbial population of apple juice [12]. However, no report is available about the effects of sonication technique on the individual polyphenolic compounds, sugars, total carotenoids and essential minerals of apple juice. Therefore, the specific objective of this study was to evaluate the effects of sonication treatments on the selected polyphenolic compounds (chlorogenic acid, caffeic acid, catechin, epicatechin and phloridzin), sugars (sucrose, glucose and fructose), total carotenoids, total anthocyanins, essential minerals (Na, K, Ca, P, Mg, Cu and Zn), viscosity and electrical conductivity of apple juice.

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2. Materials and methods

2.1. Chemicals

Formic acid, acetonitrile and sodium acetate were obtained from Sinopharm Chemical Regent Co., Ltd. (Shanghai, China). HPLC grade methanol was purchased from Hanbon Science and Technology (Nanjing, China). Catechin and epicatechin were purchased from Funakoshi Co., Ltd (Tokyo, Japan). Phloridzin was purchased from Aladdin Chemistry Co., Ltd. (Shanghai, China). Chlorogenic acid, caffeic acid, glucose, fructose and β-carotene were purchased from Sigma–Aldrich Chemical Co. (St. Louis, Mo, USA). Sucrose was purchased from Fluka Chemie GmbH (Buchs, Switzerland). Chloroform and acetone were purchased from Ling Feng Chemical Reagent Co., Ltd. (Shanghai, China). Nitric acid, *n*-hexane, hydrogen peroxide and petroleum ether were purchased from Nanjing Chemical Reagent Co., Ltd. (Nanjing, China). All other chemicals and reagents used were of analytical grade.

2.2. Preparation of apple juice

Fresh apple fruits (*M. domestica* cv. Fuji) were purchased from a local fruit market of Nanjing, China. The fruits were washed with tap water to remove adhered dirt and dust particles, dried with paper towels, cut into four pieces with stainless steel knife. Seeds, stems and over-ripened portions were removed. Apple juice was then obtained by using electrical juice extractor (MJ-M176P, Panasonic Manufacturing Berhad, Malaysia) and filtered through double layered sterilized muslin cloth to remove coarse particles and impurities from the juice. After vortex mixing, the juice was divided into three different parts as control fresh sample and working samples subjected to sonication treatments.

2.3. Ultrasound treatment

An ultrasonic bath cleaner (SB-500 DTY, Ningbo Scientz Biotechnology Co., Ltd., Ningbo, China) was used to treat the apple juice samples. Sonication of working juice samples (60 mL in a 100 mL jacketed vessel for 30 and 60 min, respectively) was done at 70% amplitude and 25 kHz frequency. The ultrasonic intensity measured by using HI 9063 thermocouple (Hanna Instruments Ltd., UK) was 2 W/cm^2. Constant temperature of 20 °C was maintained by circulating the cold water through jacketed vessel and all the treatments were performed in darkness to avoid any interference of light with samples. All the samples were stored in sterilized air tight media bottles of 100 mL for 24 h at 4 °C. All the sample preparations and treatments were carried out in triplicate. Fresh untreated apple juice was selected as control.

2.4. Analysis of polyphenolic compounds

The polyphenolic compounds were determined by the method described by Kahle et al. [13] with slight modifications. An Agilent 1100 series HPLC (Agilent Technologies, USA) consisted of a model G1379A degasser, a model G1311A pump, a model G1316A column oven and model G1315B diode array detection (DAD) system was used. Agilent Zorbax Eclipse XDB-C18 column (4.6 × 150 mm, 5 μm particle size, USA) was used. The mobile phase consisted of aqueous 0.1% (v/v) formic acid (A) and methanol (B). The gradient applied was 10–90% B in 40 min at a flow rate of 1.0 mL/min, and 20 μL injection volumes were used. Before injection into the column, sample was filtered using a syringe filter of 0.45 μm diameter. The peaks were identified by comparison of retention time and UV spectra (200–600 nm) with that of authentic reference substance. Catechin, epicatechin and phloridzin were determined at 280 nm, while chlorogenic acid and caffeic acid were determined at 320 nm. Calibration curves were prepared using different concentrations of standard solutions of catechin, epicatechin, phloridzin, chlorogenic acid and caffeic acid and the results were expressed as mg of catechin, epicatechin, phloridzin, chlorogenic acid and caffeic acid equivalent per liter of sample.

2.5. Analysis of fructose, glucose and sucrose

The sugars were determined by the method described by Hurst et al. [14]. An Agilent 1100 series HPLC with a G1315B refractive index detector (RID) was used. Cosmosil Sugar-D column (4.6 × 250 mm) was used, and the mobile phase consisted of 75% acetonitrile. The flow rate of 1.0 mL/min and 20 μL injection volumes were used. The peaks were identified by comparison of retention times with those of authentic reference substances. Sucrose, glucose and fructose were used as standards and the results were expressed as g of sucrose, glucose and fructose equivalent per liter of sample.

2.6. Determination of total carotenoids

The content of total carotenoids was determined by the method stated by Liao et al. [15] with some modifications, by measuring the absorbance at 450 nm using spectrophotometer at ambient temperature. Mixing 25 mL of juice sample with 80 mL of *n*-hexane/acetone (1:1, v/v) in a separation funnel and shaking well, the organic phase after separation was taken out. Again by using 15 mL of n-hexane/acetone (1:1, v/v), the aqueous phase was repeatedly extracted until it was colorless. The organic phase was dehydrated using anhydrous sodium sulfate. Different concentrations of standard β-carotene solution were prepared for making a standard curve. The results were expressed as μg β-carotene equivalent per mL of sample.

2.7. Determination of total anthocyanins

The content of total anthocyanins was measured by pH-differential method reported by Lee et al. [16] with slight modifications, using two buffer systems: potassium chloride buffer (0.025 M, pH 1.0) and sodium acetate buffer (0.4 M, pH 4.5). In short, 1 mL of juice sample was mixed with 9 mL of each buffer solution and the absorbance (Abs) was measured by a 722S Visible Spectrophotometer (Shnghai Jinghua Science & Technology Instruments Co., Ltd., Shanghai, China) at 520 and 700 nm, respectively. The total anthocyanins content was calculated using the following equation:

$$\text{Total anthocyanins } (\text{mg/L}) = \text{Abs} \times \text{MW} \times \text{DF} \times 1000/(\varepsilon \times 1)$$

where $\text{Abs} = (\text{Abs}_{520}-\text{Abs}_{700})_{\text{pH = 1.0}} - (\text{Abs}_{520}-\text{Abs}_{700})_{\text{pH = 4.5}}$, MW = molecular weight, DF = dilution factor, 1 = path length (1 cm), pigment contents were calculated as malvidin-3-*O*-glucoside using an extinction coefficient ε of 28,000 L/mol/cm and a molecular weight of 493.2 g/mol, 1000 = conversion from g to mg.

2.8. Determination of mineral elements

The cleaning procedure for microwave vessel, sample containers, glassware for the detection of mineral elements by inductively coupled plasma-optical emission spectrometer (ICP-OES) was carried out as recommended by American Public Health Association [17] with slight modifications. In brief, the required glassware was dipped in 10% HNO_3 solution for 4 h, washed several times with distilled water and then oven dried prior to use. One milliliter of juice sample was put into Teflon vessel and digested with 7 mL of 65% HNO_3 and 1 mL of 30% H_2O_2 on the microwave work station. The acid digested samples were diluted with pure water in 50 mL

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