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Influence of high-intensity ultrasound on bioactive compounds of strawberry juice: Profiles of ascorbic acid, phenolics, antioxidant activity and microstructure

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ABSTRACT

Strawberry is important fruit used for juicing due to its health-promoting bioactive compounds. The aim of the study was to evaluate the influence of ultrasound treatments (0, 4, 8, 12, and 16 min) on the color attributes, ascorbic acid, phenolic compounds content and antioxidant activity of strawberry juice. The microstructural properties of ultrasound treated strawberries were also evaluated. The results indicated 16-min ultrasound treatment significantly increased the color difference of samples ($p < 0.05$). The total phenols, flavonoids, ascorbic acid content and antioxidant activity of samples were highest in 12-min ultrasound treated among all. Microstructure analysis indicates the formation of irregular shapes, tearing and loss of tissue, distortion of cell walls, which explains why the ultrasound treatment enhances the extraction efficiency of phytochemicals. The current findings presented in this study provide a deeper understanding of ultrasound application in enhancing both of bioactivities compounds and antioxidant activity of strawberry juice.

1. Introduction

Strawberry (*Fragaria* × *ananassa*), one of most popular fruits is used for juicing due to its taste, rich essential nutrients and health-promoting bioactive compounds including vitamins, fiber, minerals, phenolic compounds and unknown biofactors. These bioactive compounds can delay aging, resist cancer and prevent infections (Battino et al., 2009). The USDA (United States Department of Agriculture) Nutrient Database shows that 100 g of fresh strawberry contain 7.68 g of carbohydrate, 2.0 g of fiber, 4.89 g of sugar, and 58.8 mg of vitamin C ("US Department of Agriculture," 2018). Strawberries are also rich in minerals including potassium (153 mg), phosphorus (24 mg), calcium (16 mg/100 g), magnesium (13 mg). Furthermore, strawberries are appreciated worldwide for its delicate flavor and color (Battino et al., 2009).

Conventionally, low transfer of nutrients during mechanical juicing and higher degradation of thermal sensitive bioactive compounds with pasteurization are two major challenges during fruit juice manufacture. Fruit juice is obtained through pressing machines like a juicer using mechanical processing which cannot provide a full breakdown of cells wall, thus leading to a reduced extraction rates of intracellular bioactive compounds. Furthermore, thermal processing of fruit juice before packaging is also a widespread practice. However, bioactive and sensitive nutrients present in juice are easily degraded due to extremely elevated temperature or pressure during processing (Gómez, Welti-Chanes, & Alzamora, 2011). It has been reported that thermal processing significantly results in the reduction of antioxidant capacity (18%), total phenol (22%), flavonoids (25%), and ascorbic acid content (36%) in blueberry juice (Leavens, 2007) and strawberry juice (Odriozola-Serrano, Soliva-Fortuny, & Martín-Belloso, 2008). In comparison, non-thermal processing treatments such as ultrasound treatment can be employed as an alternative which is inexpensive, reliable, and environmentally friendly (Tiwari, O'Donnell, & Cullen, 2009). It is considered as a potential alternative processing method due to its ability to maintain the original freshness, flavor, and color attributes of fruit juice (Cheng, Zhang, & Adhikari, 2014). Further, several studies reported that ultrasound processing is beneficial to enhance the bioactive compounds (e.g., total phenol, vitamin C) content and antioxidant activity in fruit juice. After 30-min ultrasound treatment, the total phenol content in gooseberry juice increased by 15% compared to the control group (Ordóñez-Santos, Martínez-Girón, & Arias-Jaramillo, 2017). Similarly, there was a significant increase in ascorbic acid, total phenolics, flavonoids content and antioxidant capacity present in lime juice under 60-min ultrasound treatment (Bhat, Kamaruddin, Min-Tze, &

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Karim, 2011). Additionally, ultrasound treatment enhanced the total phenolics, flavonoid, and antioxidant activity of carrot-grape juice significantly ($p < 0.05$) with increase in sonication time from 2 to 6 min. It was reported that this in turn can maintain better nutritional attributes during 90-day storage period compared to the control (Nadeem, Ubaid, Qureshi, Munir, & Mehmood, 2018).

However, the scientific reports are rare regarding the relationship between bioactive compounds and the microstructure changes of fruit through ultrasound techniques that increase the activity of these bioactive compounds. Therefore, the aim of this study is to evaluate the influence of ultrasound treatment (0, 4, 8, 12, and 16 min) on the physicochemical properties (protein, pH, Brix, and color attributes) and antioxidants (2,2-diphenyl-1- picrylhydrazyl (DPPH) radical scavenging activity, phenolic compounds, flavonoids, ascorbic acid, and antioxidant activity) of strawberry juice and changes in their microstructure properties due to ultrasonication.

2. Materials and methods

2.1. Plant material and treatments

A commercial strawberry variety 'Seascape' was used in this study. The fruits were harvested at their peak ripe stage from a farm in Notre-Dame-de-l'Île-Perrot, QC, Canada. Strawberries were smashed into double distilled water by the rate 1:2 (per gram/per milliliter) as experimental samples. 200 mL of freshly prepared strawberry juice was treated using an ultrasonic processer (Model 450 Sonifier, Branson, CT, USA) with a probe at 20 kHz frequency, 400 W. An ice bath was used to control the temperature during processing. The duty cycle of sonifier was set at 50% and a pulsed ultrasonic wave was applied for processing the juice. As shown in Table 1, the processing time was set for 0 min (as control), 4 min (US4), 8 min (US8), 12 min (US12) and 16min (US16), respectively. After the treatment, 100 mL of juice from each treatment was dried using a freeze drier (7420020, Labconco Corporation, Kansas City, USA) for 48 h, and the leftover juice samples were stored at 4°Cuntil further analysis. All treatments and analysis were performed in triplicates.

2.2. Chemicals and regents

Folin–Ciocalteau reagent, sodium carbonate, sodium nitrite, aluminium chloride, ferric chloride, sodium hydroxide, 2,4,6-Tripyridyl-S-triazine (TPTZ), 2,2-diphenyl-1- picrylhydrazyl (DPPH) and phenolic compound standards include catechin hydrate (≥98%), gallic acid (≥98%), ellagic acid (≥95%), ferulic acid (≥99%) and ascorbic acid (≥99%) were obtained from Sigma-Aldrich, Canada. HPLC grade of water, methanol, acetic acid and acetonitrile were purchased from Fisher Scientific, Canada.

2.3. Total protein, total soluble solids and pH value measurement

The total protein content of strawberry samples was analyzed using Pierce BCA protein assay kit from Thermo Fisher Scientific. The samples and controls were tested according to the protocol in the kit.

The total soluble solids (TSS) of treated strawberry juice samples were determined with a handheld refractometer (Cole-Parmer, QC, Canada) and results were expressed as °Brix at 25 °C. The pH values were determined using a digital pH meter (Fisher Scientific, USA) at room temperature.

Table 1
Parameters of ultrasound treatment on strawberry juice.

Sample	Treatment	Time (min)	Duty cycle (%)	Frequency and power
200 mL strawberry juice	Control	0	50	20 kHz, 400 W
	US4	4	50	20 kHz, 400 W
	US8	8	50	20 kHz, 400 W
	US12	12	50	20 kHz, 400 W
	US16	16	50	20 kHz, 400 W

2.4. Color determination

The color of the treated strawberry juice was recorded through a portable colorimeter (CR-300 Chroma, Minolta, Japan). The colorimeter was calibrated with a white tile (Y = 93.35; x = 0.3152; y = 0.3212) using the illuminant D65 and a 2° standard observer. The color changes were identified by three parameters: L* (brightness \darkness), a* (redness\greeness) and b*(yellowness\blueness) according to the Commission Internationale de l'Eclairage (CIE). The total color difference (TCD), chroma (C), hue angle (h), color index (CI) and yellow index (YI) were expressed according to the following equations (1)–(5) (Ordóñez-Santos et al., 2017):

$$TCD = \sqrt{(L* - L_0)^2 + (a* - a_0)^2 + (b* - b_0)^2} \quad (1)$$

$$C = \sqrt{a*^2 + b*^2} \quad (2)$$

$$h = \tan^{-1}(b*/a*) \quad (3)$$

$$CI = \frac{(180 - h)}{(L* - C)} \quad (4)$$

$$YI = (142.86b*)/(L*) \quad (5)$$

Where the values of parameters a_0, b_0 and L_0 were measured immediately after juicing and a*, b* and L* were determined after each ultrasonic processing.

2.5. Total phenols and flavonoids determination

For the extraction of antioxidants, the method described by previous researchers was used with slight modification (Larrauri, Rupérez, & Saura-Calixto, 1997). Strawberry juice samples were mixed with methanol at 1:1 (v/v) ratio and the mixture was extracted at 20 °C for 30 min. The mixture was centrifuged by a centrifuge (Sorvall Legend X1R, Thermo Fisher Scientific, Germany) at 5000 × g, 4 °C, for 15 min. And then, the supernatant was used to determine the total phenols and flavonoids content as well as antioxidant activity.

2.5.1. Total phenol

In this study, the Fourier Transform Infrared (FTIR) assay was used to examine the presence of total phenol in freeze-dried samples. It was equipped with deuterated triglycine sulphate (DTGS) detector and IR spectra analysis software package OMNIC (Thermo Nicolet Analytical Instruments, Madison, WI)). All spectra were recorded in triplicate with a range of 4000–500 cm^{-1} averaging 32 scans at a resolution of 4 cm^{-1}.

A Folin-Ciocalteau assay was used to quantify the total phenol present in ultrasound treated juice samples. 0.5 mL of strawberry juice extract was diluted with double deionized water by three times. And then, 0.25 mL of Folin–Ciocalteau reagent and 0.5 mL of 7.5% sodium carbonate solution were added (Krishnaswamy, Orsat, Gariépy, & Thangavel, 2013). The mixture was incubated at 20 °C for 30 min in the dark. A spectrophotometer (Ultrospec 2100 pro, Biochrom Ltd., Cambridge, England) was used to determine the absorbance changes at 765 nm. Gallic acid was used as the standard and the results was expressed as mg gallic acid equivalents (GAE) per 100 mL juice sample.

2.5.2. Total flavonoids

The total flavonoids content in ultrasound strawberry juice was analyzed through an aluminium chloride colorimetric method (Aadil, Zeng, Han, & Sun, 2013). 70 μL of sodium nitrite solution (5%) was added into 0.5 mL of juice extract. After 5-min incubation at room