Ultrasound treatment: effect on physicochemical, microbial and antioxidant properties of cherry (*Prunus avium*)

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Abstract The cherry was treated with ultrasonic waves (33 kHz, 60 W) at different time intervals (10, 20, 30, 40, 60 min) and study was carried out to analyze the change in physico-chemical properties (TSS, pH, color, acidity and firmness), antioxidant potential and microbial load of the fruit during the storage period of 15 days at 4 °C. It was observed that ultrasound treatment (US) between 30 and 40 min showed better retention of color of the fruit during the storage period. The antioxidant assays (DPPH, ABTS and TPC) also increased significantly ($P \leq 0.05$) up to 40 min, however the firmness of the fruit was affected and it showed a significant decrease beyond 20 min of US treatment. The sample with 40 min US treatment showed significantly less microbial load than other samples. The 20–40 min US treatment time (33 kHz, 60 W) was suggested for preservation of cherry during the storage at 4 °C.

Keywords *Prunus avium* · Ultrasound · Physiochemical · Antioxidant · Microbial analysis

Introduction

Fruits are considered important components of a healthy diet, because of the presence of phenolic compounds, fiber, anthocyanins and flavonols (Mulabagal et al. 2010;

Odriozola-Serrano et al. 2008). They possess anti-oxidant properties and contribute in various life processes through their interaction with bio-molecules like enzyme regulators and consequently maintaining good human health (Lee et al. 2007). However, the shelf life of the fruits is very limited and maintaining its quality during storage is a challenging task. Shelf life of fruits can be extended by canning, storage at lower temperature and use of various chemicals such as antimicrobials and fungicides. However the high cost, food safety concerns and environmental considerations limit their use (Lopez-Gomez et al. 2009). To overcome these problems, many emerging food preservation techniques (high pressure, Pulsed electric field, electrolyzed water, irradiation, ozone and Ultra sound treatment) have been widely studied and have offered promising results in food processing and preservation (Sampedro et al. 2010; Arzeni et al. 2012; Wang et al. 2015; Oancea et al. 2013; Ercan and Soysal 2013). Among these non thermal technologies, ultrasound is one of the most important green technologies used in food science for preservation (Chemat et al. 2011; Awad et al. 2012). A major advantage of ultrasound over other techniques in food industry is that sound waves are generally considered to be safe, non- toxic and cost effective at commercial level (Kentish and Ashok Kumar 2011). Use of ultrasound as a non thermal technology has also gained considerable importance because of its improvement in microbial safety and consequently the shelf life of the fruit.

Among various fruits, sweet cherry (*Prunus avium*) is one of the most perishable fruit with high market value. Attractive colour, sweetness, sourness, firmness, antioxidants and nutrients are its main characteristics (Ballistreri et al. 2013). Its narrow harvest season, soft texture, susceptibility to fungal rots, high transpiration rate and vulnerability to physiological disorders such as bruising,

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sugar-acid balance, fruit softening, desiccation and the browning or discoloration of the green stem limits its availability to the consumer in an optimum condition, over longer periods (Alique et al. 2005). The present study focuses on extending the shelf life and maintaining the quality and nutritional parameters of the sweet cherry by employing ultrasound treatment at different time intervals ((0, 10, 20, 30, 40 and 60 min)).

Materials and methods

Fruit and treatment

Cherries were harvested from local farms of Jammu & Kashmir, India and transported within an hour to our lab. The damaged fruits were sorted out and samples were randomly divided according to different treatments. Five hundred gram of fruit for each batch was directly immersed in a sonicator bath (Frequency 33 KHZ, Power 60 W, Jain scientific, India) and the treatment time (0, 10, 20, 30, 40 and 60 min) was varied for each batch. The level of water in the bath was kept constant for each experiment and the temperature of the water was maintained at 25 ± 1 °C. All fruits were then air-dried for approximately 20 min and then stored at 4 °C and were analyzed at an interval of 3-days for a period of 15 days from Day 1. On each experimental day, approximately 100 g of fruit was pulped, homogenized and centrifuged for 10 min at 5000 rpm and supernatant was collected and analyzed on the same day.

Total soluble solids (TSS), Total titratable acidity (TA) and pH

Total soluble solids (TSS) were determined at room temperature using a portable refractometer (Atago, Japan). Titratable acidity (TA) was determined by titrating 20 ml cherry pulp (supernatant) to pH 8.2 using 0.1 M NaOH and was calculated as reported by Gunness et al. (2009). The pH was measured using pH meter (HI 2215pH/ORP meter; Hanna Instruments Woonsocket RI USA).

Color analysis

Color of the Cherry fruit was determined using hunter lab colorimeter (D-25, Hunter Associates Laboratory, Ruston, USA) after being standardized using Hunter Lab color standards and their Hunter L (lightness), a (redness to greenness) and b (yellowness to blueness) values were measured. Total color difference (TCD) was considered for evaluation of colour changes. This parameter quantifies the overall colour difference of a given sample when compared

to a reference one (L_0 , a_0 , b_0), according to the expression reported by (Alexandre, Brandão and Silva 2012)

$$\Delta E^* = [(a - a_0) + (b - b_0) + (L - L_0)]^{1/2}$$

This parameter quantifies the overall color difference of a given sample when compared to a reference being the index “0” indicative of reference untreated samples (without ultrasound). Thirty samples were carried out for each treatment and storage time.

Antioxidant activity

On each experimental day, approximately 100 g of fruit was pulped and homogenized, the 2.5 g of sample for antioxidant analysis was taken from this pulp and homogenized with 2.5 ml of 95 % (V/V) cold ethanol and centrifuged at 10,000 rpm for 15 min, further 2.5 ml of 80 % (V/V) cold ethanol was used to extract the residue again. The supernatants were combined and the final volume was made to 10 ml. The ethanol extract was used for antioxidants analysis by DPPH (1,1-diphenyl-2-picrylhydrazyl) and ABTS (2, 2'-azino-bis-3-thiylbenz thiazoline-6-sulphonic acid) and total phenols.

Total phenolic content was measured using Folin-Ciocalteu's method described by Tezcan et al. (2009). The final results were expressed as milligram of gallic acid equivalents (mg of GAE/g of cherry fresh weight).

DPPH free radical scavenging activity was measured according to the method reported by Ahmad et al. (2015) with modifications. Briefly, 2.0 ml of 0.2 mM ethanolic DPPH solution was added in 2.0 ml pulp. This mixture was placed in dark at room temperature for 30 min. The absorbance was determined with spectrophotometer at 517 nm. The same procedure was revised for control by using ethanol instead of sample solution. Following equation was used to calculate the percent DPPH free radical scavenging activity:

$$\text{DPPH free radical scavenging activity (\%)} = (A_c - A_s/A_c) \times 100$$

where A_c is the absorbance of the control and A_s is the absorbance of the sample.

ABTS radical-scavenging assay was determined according to the method of Wang et al. (2010) with slight modification. All tests were performed in triplicates and means were calculated. The radical-scavenging activity of the samples was expressed as scavenging capacity (SC) % = $[(A_{\text{control}} - A_{\text{test}})/A_{\text{control}}] \times 100$ %, where A_{control} is the absorbance of the control (ABTS· + solution without test sample) and A_{test} is the absorbance of the test sample (ABTS· + solution plus extract).

Firmness

The firmness of ultrasound treated cherries (0, 10, 20, 30, 40, 60 min) was measured using a Texture Analyzer (TA.XT PLUS; Stable Micro Systems Ltd., UK) fitted with a cylinder plunger SMS-P/10 CYL Delrin probe (10 mm diameter). The probe was programmed to travel 5 mm distance after touching the surface of the fruit. The whole samples were placed on heavy duty platform under the probe along the transversal axis. Tests were performed in a compression mode (1.5 mm/s velocity). Firmness was recorded as a maximum force observed during penetration of sample. Test was done in replicates of 20 and the average peak force is recorded as the firmness.

Microbiological analysis

The microbial population of the fruit juice sample was determined by the FDA's standard method, mentioned in Bacteriological Analytical Manual (FDA 2001). All the analysis was carried out in triplicates.

Results and discussions

Total soluble solids (TSS), pH and Total titratable acidity (TA)

Ultrasound treatment for different duration significantly affected TSS content of the fruit during the storage period of 15 days (Table 1). The US treatment may have interfered with the fruit metabolism, which may have decreased the decomposition of organic acids and cell wall components. Further, ultrasound may have caused deactivation of enzymes that are involved in decomposition of organic acids to sugars (Sao-Jose et al. 2014). On contrary, US treatment for 60 min significantly increased TSS after day 1, which can be due to disruption of fruit cell structure and formation of microscopic channels that resulted in water loss (Fernandes et al. 2009).

US treatment significantly affected pH as well as acidity of the treated samples over 15 days of storage (Table 1). Lowest pH was seen at 30 min of US treatment (3.26, 3.28, 3.28, 3.31, 3.34, and 3.40 on day 1, 3, 6, 9, 12 and 15

Table 1 Physico-chemical properties of ultrasound treated Cherries

Treatment time (Min.)	Day 1	Day 3	Day 6	Day 9	Day 12	Day 15
pH						
0	3.32 ^{aA} ± 0.01	3.35 ^{aB} ± 0.01	3.38 ^{aC} ± 0.01	3.44 ^{aD} ± 0.01	3.48 ^{aE} ± 0.00	3.43 ^{aF} ± 0.01
10	3.28 ^{bA} ± 0.01	3.33 ^{bB} ± 0.01	3.35 ^{bC} ± 0.00	3.39 ^{bD} ± 0.02	3.41 ^{bE} ± 0.02	3.45 ^{bF} ± 0.01
20	3.28 ^{bA} ± 0.00	3.30 ^{bB} ± 0.00	3.32 ^{cC} ± 0.00	3.35 ^{cD} ± 0.01	3.37 ^{cE} ± 0.01	3.41 ^{cF} ± 0.00
30	3.26 ^{bA} ± 0.01	3.28 ^{dB} ± 0.02	3.28 ^{dB} ± 0.03	3.31 ^{dC} ± 0.01	3.34 ^{dD} ± 0.00	3.40 ^{dE} ± 0.01
40	3.27 ^{bA} ± 0.02	3.30 ^{EB} ± 0.01	3.32 ^{EC} ± 0.00	3.35 ^{ED} ± 0.00	3.38 ^{EE} ± 0.01	3.41 ^{EF} ± 0.02
60	3.39 ^{aA} ± 0.00	3.34 ^{FB} ± 0.01	3.40 ^{FC} ± 0.00	3.42 ^{FD} ± 0.01	3.50 ^{FE} ± 0.00	3.89 ^{FF} ± 0.02
TSS (%)						
0	10.17 ^{aA} ± 0.02	10.65 ^{aB} ± 0.08	10.95 ^{aC} ± 0.02	11.25 ^{aD} ± 0.05	11.76 ^{aE} ± 0.05	12.19 ^{aF} ± 0.05
10	10.13 ^{bA} ± 0.05	10.39 ^{bB} ± 0.05	10.76 ^{bC} ± 0.05	10.92 ^{bD} ± 0.08	11.34 ^{bE} ± 0.05	11.79 ^{bF} ± 0.05
20	10.10 ^{cA} ± 0.09	10.30 ^{EB} ± 0.05	10.55 ^{cC} ± 0.08	10.73 ^{cD} ± 0.05	11.13 ^{cE} ± 0.08	11.46 ^{cF} ± 0.05
30	10.07 ^{cA} ± 0.07	10.24 ^{dB} ± 0.08	10.33 ^{dC} ± 0.09	10.55 ^{dD} ± 0.05	10.84 ^{dE} ± 0.08	11.32 ^{dF} ± 0.05
40	10.09 ^{bA} ± 00.05	10.27 ^{dB} ± 0.08	10.56 ^{EC} ± 0.08	10.89 ^{ED} ± 0.05	11.26 ^{EE} ± 0.05	11.43 ^{EF} ± 0.01
60	10.56 ^{dA} ± 0.05	10.96 ^{EB} ± 0.08	11.32 ^{FC} ± 0.09	10.83 ^{FD} ± 0.05	8.24 ^{FE} ± 0.05	12.85 ^{FF} ± 0.01
TA (%)						
0	0.72 ^{aA} ± 0.02	0.66 ^{aB} ± 0.02	0.62 ^{aC} ± 0.01	0.59 ^{aD} ± 0.01	0.61 ^{aE} ± 0.01	0.52 ^{aF} ± 0.02
10	0.75 ^{bA} ± 0.01	0.73 ^{bB} ± 0.01	0.72 ^{bC} ± 0.00	0.70 ^{bC} ± 0.01	0.67 ^{bD} ± 0.01	0.64 ^{bE} ± 0.02
20	0.75 ^{bA} ± 0.01	0.75 ^{EB} ± 0.01	0.73 ^{cC} ± 0.01	0.71 ^{cD} ± 0.00	0.69 ^{cE} ± 0.00	0.67 ^{cF} ± 0.01
30	0.74 ^{bA} ± 0.00	0.76 ^{dA} ± 0.00	0.75 ^{dB} ± 0.01	0.72 ^{dC} ± 0.01	0.71 ^{cD} ± 0.01	0.69 ^{dE} ± 0.01
40	0.73 ^{cA} ± 0.01	0.72 ^{eA} ± 0.01	0.70 ^{EB} ± 0.01	0.68 ^{EC} ± 0.00	0.67 ^{dD} ± 0.01	0.67 ^{cE} ± 0.02
60	0.67 ^{dA} ± 0.01	0.65 ^{FB} ± 0.00	0.62 ^{aC} ± 0.01	0.62 ^{FD} ± 0.01	0.59 ^{aE} ± 0.01	0.54 ^{eF} ± 0.01

Values are mean ± standard deviations of three (n = 3) measurements with different small and capital superscripts in a row and column vary significantly ($p \leq 0.05$), respectively. TSS (Total soluble solids), TA (Titrable acidity)

respectively). Coinciding, with increase in pH a decrease in acidity was found (Table 1). Results showed that 30 min ultrasound treatment was effective in maintaining pH and total acidity of cherries during storage period. The US treatment can delay the degradation of organic acids (Almenar et al. 2007; Holcroft and Kader 1999) and thus can maintain the acidity and pH of fruits during storage periods. Cherries with Lower pH and higher TA are preferred for industrial use and have better consumer acceptability. However, lower intensities of ultrasound and processing times had no significant effect on pH of fruits according to different authors (Ugarte-Romero et al. 2006; Tiwari et al. 2008, 2009, 2010).

Color analysis

Color of some fruits is due to the presence of anthocyanins and it is considered an important quality attribute of the fresh produce, (Rodrigo et al. 2007). Color change was measured in terms of total color difference (TCD/ ΔE) that increased significantly with increase in ultrasound treatment time on day 1 (Table 2). Increase in ultrasound treatment time of fruit affects the anthocyanin stability by increase in cavitations and formation of hydroxyl radicals in fruit and hence affected the color of the fruit (Tiwari et al. 2010). The total color difference (ΔE) values increased significantly with storage at all treatment times; however least increase was seen at treatment time of 20 min. Thus, 20-min treatment with ultrasound was effective to preserve the bright red color of cherries when compared with control and other treatments during a storage period of 15 days (Bermudez-Aguirre and Barbosa-Canovas 2013). It is possible that ultrasound treatment for 20 min may have prevented degradation of anthocyanins

and can be considered an effective treatment for color preservation of cherries during storage.

Antioxidant properties

Total Phenolic content, DPPH and ABTS

Ultrasound treatment resulted in an increase in the total phenolic content of the samples on Day 1 (Fig. 1). As the US treatment time increased from 0 to 40 min, a significant increase (20.39–21.45 (mg GAE/g of sample) in TPC content was seen. Increase in total phenolic content of kasturi juice as result of sonication (25 kHz) has also been reported by Bhat et al. (2011). An increase of 6–35 and 30 % in total phenolic content, due to ultrasound treatment in comparison to conventional processing of some fruits was also reported by Vilku et al. (2008) and Pingret et al. (2013), respectively. The increase in total phenolic content may be due to the better extraction of polyphenols due to greater disruption of cell wall material. The hydroxylation of flavanols by ultrasound treatment has a positive effect on antioxidant activity to Soria and Villamiel (2010). Golmohamadi et al. (2013) also reported an increase in total phenolic content in red raspberry by increasing ultrasound power (20–1000 kHz) that was attributed to increase in temperature that would have facilitated release of bioactive components into the extraction solvent (Table 3).

Total phenolic content increased in both untreated as well as treated samples (except 60 min.) with a storage time of 15 days at 4 °C (Table 3). This increase was seen up to day 12 and after that total phenolic content decreased. However, when ultrasound treatment was provided to fruit for 60 min, the total phenolic content decreased, this may be due its longer exposure to ultrasonic waves which may

Fig. 1 Showing total phenolic content of cherries treated with ultrasound (US)

Table 2 Color analysis of US treated Cherries fruit stored for a period for 15 days 4 °C

US treatment (minutes)	Total color difference (ΔE^*) during storage at 4 °C					
	Day 1	Day 3	Day 6	Day 9	Day 12	Day 15
0	59.31 ^{aA} $\pm$ 0.2	62.47 ^{aB} $\pm$ 0.2	66.29 ^{aC} $\pm$ 0.2	70.58 ^{aD} $\pm$ 0.2	75.34 ^{aE} $\pm$ 0.2	78.85 ^{aF} $\pm$ 0.2
10	62.42 ^{bA} $\pm$ 0.2	62.57 ^{aA} $\pm$ 0.1	63.43 ^{bB} $\pm$ 0.2	65.31 ^{bC} $\pm$ 0.2	67.43 ^{bD} $\pm$ 0.2	70.12 ^{bE} $\pm$ 0.2
20	62.47 ^{bA} $\pm$ 0.2	62.67 ^{bA} $\pm$ 0.2	63.84 ^{bB} $\pm$ 0.2	64.37 ^{cC} $\pm$ 0.2	66.29 ^{cD} $\pm$ 0.2	68.25 ^{cE} $\pm$ 0.2
30	63.53 ^{bA} $\pm$ 0.2	65.49 ^{cB} $\pm$ 0.2	68.49 ^{cC} $\pm$ 0.2	70.67 ^{dD} $\pm$ 0.2	72.25 ^{dE} $\pm$ 0.2	74.35 ^{dF} $\pm$ 0.2
40	64.86 ^{cA} $\pm$ 0.2	68.84 ^{dB} $\pm$ 0.2	70.64 ^{dC} $\pm$ 0.2	72.38 ^{eD} $\pm$ 0.2	77.72 ^{eE} $\pm$ 0.2	79.46 ^{eF} $\pm$ 0.2
60	67.58 ^{dA} $\pm$ 0.2	69.79 ^{eB} $\pm$ 0.2	71.30 ^{eC} $\pm$ 0.2	76.83 ^{fD} $\pm$ 0.2	79.13 ^{fE} $\pm$ 0.2	81.94 ^{fF} $\pm$ 0.2

Values are mean $\pm$ standard deviations of three ($n = 3$) measurements with different small and capital superscripts in a row and a column vary significantly ($p \leq 0.05$)

Table 3 Antioxidant activity of Ultrasound treated Cherries

US Treatment (Min.)	Day 1	Day 3	Day 6	Day 9	Day 12	Day 15
DPPH (% inhibition)						
0	23.75 ^{aA} $\pm$ 1.2	27.34 ^{aB} $\pm$ 1.2	28.34 ^{aC} $\pm$ 1.3	32.46 ^{aD} $\pm$ 1.1	35.53 ^{aE} $\pm$ 1.3	34.32 ^{aE} $\pm$ 1.2
10	28.84 ^{bA} $\pm$ 1.1	32.08 ^{bB} $\pm$ 1.3	35.43 ^{bC} $\pm$ 1.2	39.26 ^{bD} $\pm$ 1.2	41.82 ^{bE} $\pm$ 1.2	35.36 ^{bF} $\pm$ 1.1
20	34.09 ^{cA} $\pm$ 1.2	37.58 ^{cB} $\pm$ 1.2	37.67 ^{cC} $\pm$ 1.1	41.86 ^{cD} $\pm$ 1.1	42.43 ^{cE} $\pm$ 1.3	37.78 ^{cF} $\pm$ 1.1
30	39.98 ^{dA} $\pm$ 1.1	43.23 ^{dB} $\pm$ 1.1	47.37 ^{dC} $\pm$ 1.1	51.03 ^{dD} $\pm$ 1.2	51.63 ^{dD} $\pm$ 1.1	44.03 ^{dE} $\pm$ 1.2
40	44.35 ^{eA} $\pm$ 1.1	48.87 ^{eB} $\pm$ 1.2	51.39 ^{eC} $\pm$ 1.2	55.92 ^{eD} $\pm$ 1.1	56.06 ^{eD} $\pm$ 1.1	48.73 ^{eE} $\pm$ 1.1
60	26.92 ^{fA} $\pm$ 1.2	29.46 ^{fA} $\pm$ 1.1	31.70 ^{fB} $\pm$ 1.1	33.48 ^{fC} $\pm$ 1.1	34.18 ^{fC} $\pm$ 1.2	32.26 ^{fD} $\pm$ 1.3
ABTS (% scavenging capacity)						
0	11.38 ^{aA} $\pm$ 1.05	13.21 ^{aA} $\pm$ 1.05	15.19 ^{aC} $\pm$ 1.05	17.01 ^{aD} $\pm$ 1.05	18.94 ^{aE} $\pm$ 1.05	18.14 ^{aE} $\pm$ 1.05
10	17.73 ^{bA} $\pm$ 1.05	21.49 ^{bA} $\pm$ 1.05	19.15 ^{bC} $\pm$ 1.05	20.84 ^{bD} $\pm$ 1.05	21.79 ^{bE} $\pm$ 1.05	19.83 ^{bF} $\pm$ 1.05
20	20.14 ^{cA} $\pm$ 1.05	19.92 ^{cB} $\pm$ 1.05	21.14 ^{cC} $\pm$ 1.05	22.29 ^{cD} $\pm$ 1.05	21.98 ^{cE} $\pm$ 1.05	18.48 ^{cF} $\pm$ 1.05
30	23.21 ^{dA} $\pm$ 1.04	22.35 ^{dB} $\pm$ 1.05	24.81 ^{dC} $\pm$ 1.05	26.01 ^{dD} $\pm$ 1.05	26.47 ^{dD} $\pm$ 1.05	23.79 ^{dF} $\pm$ 1.05
40	27.19 ^{eA} $\pm$ 1.05	26.63 ^{eB} $\pm$ 1.05	29.25 ^{eC} $\pm$ 1.05	29.92 ^{eD} $\pm$ 1.05	31.22 ^{eD} $\pm$ 1.05	26.67 ^{eF} $\pm$ 1.05
60	13.39 ^{fA} $\pm$ 1.05	14.92 ^{fA} $\pm$ 1.08	16.51 ^{fB} $\pm$ 1.05	19.95 ^{fC} $\pm$ 1.05	20.27 ^{fC} $\pm$ 1.05	17.88 ^{fD} $\pm$ 1.05

Values are mean $\pm$ standard deviations of three ($n = 3$) measurements with s small and capital superscripts in a row and a column vary significantly ($p \leq 0.05$)

have degraded the polyphenols. Jahouach-Rabai et al. (2008) also reported degradation of polyphenolic compounds due to excessive cavitations and cell disruption of the product.

Anti-oxidant activity of treated samples (by DPPH and ABTS) increased with increase in treatment time (Table 3). An abrupt increase was seen at 10 min treatment which was followed by a mild but significant increase with further increase in treatment time (Maximum value at 40 min). Higher antioxidant values than control were also seen in raspberry up to 30 min of ultrasound treatment according to Golmohamadi et al. (2013). Increased antioxidant activity can be associated with higher values of total phenolic content found in ultrasound treated samples. The greater retention of Vitamin C content by application of ultrasound on the fruit may have also improved its antioxidant activity. It has also been suggested that

generation of hydroxyl radicals by hydroxylation of food components by ultrasound can also increase its antioxidant activity (Ashokkumar et al. 2008). However, a treatment time of 60 min showed detrimental effect on antioxidant activity which might be due to excessive damage to cell structure leading to greater chances of oxidation as well as degradation of polyphenolic compounds.

Firmness

Fruit firmness is attributed to the physical anatomy of tissue, cell size, shape, cell wall strength and intercellular adhesion (Toivonen and Brummell 2008). Sonicated samples showed comparatively better retention of firmness than untreated samples over a storage period of 15 days, although the firmness of all samples decreased with increase in storage period as compared to day 1 (Table 4).

Table 4 Texture analysis of US treated strawberry fruit for a period for 15 days at 4 °C

US Treatment (minutes)	Firmness of fruit (g) during storage at 4 °C						
	Day 1	Day 3	Day 6	Day 9	Day 12	Day 15	Retention of fruit firmness %
0	863 ^{bA} ± 2.4	831 ^{cB} ± 3.1	787 ^{dC} ± 3.2	721 ^{dD} ± 1.8	625 ^{dE} ± 3.2	514 ^{eF} ± 1.2	53.77 ^e ± 2.5
10	862 ^{bA} ± 3.9	841 ^{bB} ± 2.1	815 ^{cC} ± 1.6	780 ^{cD} ± 2.3	715 ^{cE} ± 1.5	611 ^{dF} ± 3.5	66.19 ^d ± 2.3
20	871 ^{aA} ± 2.9	852 ^{aB} ± 1.8	831 ^{aC} ± 3.2	810 ^{aD} ± 1.5	771 ^{aE} ± 2.1	719 ^{aF} ± 1.5	80.08 ^a ± 3.5
30	872 ^{aA} ± 2.0	850 ^{aB} ± 1.9	830 ^{aC} ± 1.8	809 ^{aD} ± 3.6	772 ^{aE} ± 3.2	720 ^{aF} ± 4.1	80.10 ^a ± 1.9
40	869 ^{aA} ± 1.6	849 ^{aB} ± 1.1	830 ^{aC} ± 3.1	807 ^{aD} ± 2.1	767 ^{abE} ± 2.2	685 ^{bF} ± 1.5	75.88 ^b ± 3.1
60	868 ^{aA} ± 2.2	848 ^{aB} ± 2.1	823 ^{bC} ± 1.8	798 ^{bD} ± 1.5	763 ^{bE} ± 1.8	655 ^{cF} ± 3.5	72.17 ^c ± 1.9

Values are mean ± standard deviations of three (n = 3) measurements with different small and capital superscripts in a row and a column vary significantly ($p \leq 0.05$)

Table 5 Microbial analysis of US treated Cherries fruit stored for a period for 15 days 4 °C

Microbiology	Treatment	Day 1	Day 3	Day 6	Day 9	Day 12	Day 15
Bacterial (Log ₁₀ CFU g ⁻¹)	0	4.60 ^{aA} ± 0.11	5.1 ^{aB} ± 0.08	5.5 ^{aC} ± 0.11	6.14 ^{aD} ± 0.13	6.56 ^{aE} ± 0.15	6.91 ^{aF} ± 0.01
	10	4.01 ^{bA} ± 0.16	4.41 ^{bB} ± 0.27	4.81 ^{bC} ± 0.13	5.21 ^{bD} ± 0.07	5.81 ^{bE} ± 0.05	6.14 ^{cF} ± 0.11
	20	3.61 ^{cA} ± 0.08	3.90 ^{cB} ± 0.09	4.21 ^{cC} ± 0.03	4.34 ^{dD} ± 0.02	5.12 ^{dE} ± 0.11	5.42 ^{dF} ± 0.06
	30	3.32 ^{dA} ± 0.02	3.65 ^{dB} ± 0.07	4.01 ^{dC} ± 0.09	4.24 ^{dD} ± 0.05	4.82 ^{eE} ± 0.02	5.13 ^{eF} ± 0.01
	40	3.14 ^{eA} ± 0.01	3.42 ^{eB} ± 0.03	3.90 ^{eC} ± 0.03	4.11 ^{eD} ± 0.02	4.62 ^{eE} ± 0.01	4.91 ^{fF} ± 0.02
	60	3.01 ^{fA} ± 0.02	3.72 ^{dB} ± 0.04	4.21 ^{cC} ± 0.02	4.94 ^{cD} ± 0.01	5.72 ^{cE} ± 0.06	6.32 ^{bF} ± 0.01
Yeast and mold (Log ₁₀ CFU g ⁻¹)	0	4.52 ^{aA} ± 0.01	5.20 ^{aB} ± 0.03	4.46 ^{aC} ± 0.01	4.71 ^{aD} ± 0.01	5.64 ^{aE} ± 0.02	5.80 ^{aF} ± 0.03
	10	3.91 ^{bA} ± 0.02	5.09 ^{bB} ± 0.01	4.35 ^{bC} ± 0.01	4.62 ^{cD} ± 0.03	4.92 ^{cE} ± 0.02	5.11 ^{cF} ± 0.01
	20	3.41 ^{cA} ± 0.03	3.61 ^{cB} ± 0.05	3.94 ^{dC} ± 0.02	4.43 ^{dD} ± 0.04	4.41 ^{dE} ± 0.06	4.83 ^{dF} ± 0.01
	30	3.26 ^{dA} ± 0.02	3.43 ^{dB} ± 0.01	3.51 ^{eC} ± 0.01	4.04 ^{eD} ± 0.01	4.32 ^{eE} ± 0.01	4.67 ^{eF} ± 0.02
	40	3.24 ^{dA} ± 0.03	3.35 ^{eB} ± 0.02	3.42 ^{fC} ± 0.04	3.73 ^{fD} ± 0.06	4.03 ^{fE} ± 0.01	4.58 ^{fF} ± 0.03
	60	3.04 ^{eA} ± 0.05	3.65 ^{cB} ± 0.01	4.19 ^{cC} ± 0.03	4.59 ^{bD} ± 0.01	4.04 ^{bE} ± 0.02	5.31 ^{bF} ± 0.02

Values are mean ± standard deviations of three (n = 3) measurements with different small and capital superscripts in a row and column vary significantly ($p \leq 0.05$)

This may be due to inactivation of enzymes that are involved in breakdown of middle lamella and cell wall (Ketsa and Daengkanit 1999). Jackline et al. (2014) reported that Ultrasound induced the disruption of hydrogen bonding and van der Waals interactions in polypeptide chains that altered secondary and tertiary structure of the protein and the biological activities of enzymes were lost (Mason et al. 2005; Manas et al. 2006; Rawson et al. 2011). Retention of fruit firmness increased with increase in treatment time with maximum retention found during 20–30 min of ultrasound treatment. This can be due to destructive effect of prolonged exposure of ultrasound on stability of cell wall, which results in cell injury and loss of water (Gabaldon-Leyva et al. 2007).

Microbial analysis

Ultrasound treatment significantly affected microbial load of fruits. The total plate count (TPC) of bacteria, yeast and

mold growth of treated and untreated samples over a storage period of 15 days are shown in Table 5. Increase in US treatment time decreased the microbial load significantly ($P < 0.05$) from 4.60 ± 0.11 to 3.01 ± 0.02 for bacteria and 4.52 ± 0.01 to 3.04 ± 0.05 for yeast and mold on day 1. Birmpa et al. 2013 also found similar trend in case of lettuce and strawberries. Ultrasonic treatment of 20 kHz was found to enhance the inactivation of microorganisms due to the generation of hydroxyl radicals (Suslick 1989; Butz et al. 2002; Kadkhodae and Povey 2008). Another reason reported for inactivation/reduction of microbes by ultrasonication is cellular disruption. The shear forces generated during the movement of bubbles or sudden localized temperature and pressure changes caused by bubble collapse may be the responsible factor for cellular disruption (Ulusoy et al. 2007). Guerrero et al. (2005) also reported that ultrasound damages the cell wall and membrane functioning of moulds. After storage for 15 days at 4 °C, the microbial load of all treated samples

varied significantly ($P < 0.05$) but the lowest TPC (log CFU/ml) for bacteria (4.91 ± 0.02) and yeast and mold (4.58 ± 0.03) was observed at 40 min of US treatment time. Rivera et al. (2011) observed that ultrasound (35 kHz/10 min) promoted the elimination of 1 log CFUg⁻¹ of microorganisms. Similarly, Pagan et al. (1999) demonstrated the potential of Ultrasound (20 kHz) to inactivate pathogenic microorganism such as *L. monocytogenes*. However, the prolonged exposure (>40 min) of samples to ultrasound did not help in extending the shelf life of the fruit as the results showed comparatively higher microbial load than samples with treatment time less than 40 min. This may be due to that ultrasound increases temperature at a localized level causing injuries to the fruit (Gabaldon-Leyva et al. 2009) that can permit easy penetration of microbes into the fruit and thereby increasing microbial load of the sample.

Conclusion

The ultrasound treatment on cherry was found to be effective in maintaining and improving the color, pH, TSS and firmness of the fruit over the storage period of 15 days. The treatment not only enhanced the nutraceutical value, by increasing the antioxidant activity, but also enhanced the safety of the food from microbes and extended the shelf life of the product. The most desirable results were obtained between 20 and 40 min (33 kHz) of treatment time. However, some detrimental effects on antioxidant activity, color, firmness and microbial load of the fruit were observed during its longer exposure time (60 min) to ultrasonic waves (33 kHz). This suggests that efficiency of ultrasound depends on time of exposure of the food to ultrasonic waves. The future work should be carried out to explore its application on other perishable food products.

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References

- Ahmad M, Gani A, Baba WN, Wani TA, Gani A, Shah U, Wani SM, Masoodi FA (2015) Effect of green tea powder on thermal, rheological and functional properties of wheat flour and physical, nutraceutical and sensory analysis of cookies. *J Food Sci Technol*. doi:10.1007/s13197-014-1701-3
- Alexandre EMC, Brandão TRS, Silva LMC (2012) Efficacy of non-thermal technologies and sanitizer solutions on microbial load reduction and quality retention of strawberries. *J Food Eng* 108:417–426
- Alique R, Zamorano JP, Martinez ma, Alonso J (2005) Effect of heat and cold treatments on respiratory metabolism and shelf-life of sweet cherry, type picota cv. Ambrunes. *Postharvest Biol Technol* 35:153–165
- Almenar E, Del-Valle V, Hernandez-Munoz P, Lagaron JM, Catala R, Gavara R (2007) Equilibrium modified atmosphere packaging of wild strawberries. *J Sci Food Agric* 87:1931–1939
- Arzeni C, Perez OE, Pilosof AMR (2012) Functionality of egg white protein as affected by high intensity ultrasound. *Food Hydrocolloids* 29:308–316
- Ashokkumar M, Sunartio D, Kentish S, Mawson R, Simons L, Vilku K (2008) Modification of food ingredients by ultrasound to improve functionality, a preliminary study on a model system. *Innov Food Sci Emerg Technol* 9:155–160
- Awad T, Moharram H, Shaltout O, Asker D, Youssef M (2012) Application of Ultrasound in analysis, processing and quality control of food. A review. *Food Res Int* 48(2):410–427
- Ballistreri G, Continella A, Gentile A, Amenta M, Fabroni S, Rapisarda P (2013) Fruit quality and bioactive compounds relevant to human health of sweet cherry (*Prunus avium* L.) cultivars grown in Italy. *Food Chem* 140:630–638
- Bermudez-Aguirre D, Barbosa-Canovas GV (2013) Disinfection of selected vegetables under non-thermal treatments, chlorine, acid citric, ultraviolet light and ozone. *Food Control* 29:82–90
- Bhat R, Kamaruddin NSBC, Min-tze L, Karim AA (2011) Sonication improves kasturi lime (citrus microcarpa) juice quality. *Ultrason Sonochem* 18(6):1295–1300
- Birmipa A, Sfika V, Vantakar A (2013) Ultraviolet light and ultrasound as non-thermal treatments for inactivation of microorganisms in fresh read-to-eat foods. *Int J Food Microbiol* 16:96–102
- Butz P, FernandezGarcia A, Lindauer R, Dieterich S, Bongnar A, Tauscher B (2002) The influence of high pressure on the formation of diketopiperazine and pyroglutamate rings. *High Press Res* 22:697–700
- Chemat F, Zill-e-Huma, Khan MK (2011) Applications of ultrasound in food technology, processing, preservation and extraction. *Ultrason Sonochem* 18:813–835
- Ercan SŞ, Soysal Ç (2013) Use of ultrasound in food preservation. *Nat Sci* 5:8(A2): 5–13
- FDA (2001) U.S. Food and drug administration. In: Bacteriological analytical manual (8th edn). FDA Center for Food Safety and Applied Nutrition
- Fernandes FAN, Gallão MI, Rodrigues S (2009) Effect of osmosis and ultrasound on pineapple cell tissue structure during dehydration. *J Food Eng* 90(2):186–190
- Gabaldon-Leyva CA, Quintero Ramos A, Barnard J, Balandran RR, Talamas AR, Jimenez CJ (2007) Effect of ultrasound on the mass transfer and physical changes in brine bell pepper at different temperatures. *J Food Eng* 81:374–379
- Golmohamadi A, Moller G, Powers J, Nindo C (2013) Effect of ultrasound frequency on antioxidant activity, total phenolic and anthocyanin content of red raspberry puree. *Ultrason Sonochem* 20:1316–1323
- Guerrero S, Tognon M, Alzamora SM (2005) Response of *Saccharomyces cerevisiae* to the combined action of ultrasound and low weight chitosan. *Food Control* 16:131–139
- Gunness P, Kravchuk O, Nottingham SM, Arcy BR, Gidley MJ (2009) Sensory analysis of individual strawberry fruit and comparison with instrumental analysis. *Postharvest Biol Technol* 52:164–172
- Holcroft DM, Kader AA (1999) Carbon dioxide-induced changes in pH and organic acid metabolism may affect color of stored strawberry fruit. *Postharvest Biol Technol* 17:19–32. doi:10.4236/ns.2013.58A2002*
- Jackline F, de Brilhante SJ, Nélío JA, Fonso M, Ramos MC, Dantas V, Paulo CS, José BPC (2014) Decontamination by ultrasound application in fresh fruits and vegetables. *LWT - Food Science and Technology* 57:344–351

- Jahouach-Rabai W, Trabelsi M, Van Hoed V, Adams A, Verhe R, DeKimpe N, Frikha MH (2008) Influence of bleaching by ultrasound on fatty acids and minor compounds of olive oil. Qualitative and quantitative analysis of volatile compounds (by SPME coupled to GC/MS). *Ultrason Sonochem* 15(4):590–597
- Kadkhodae R, Povey MJ (2008) Ultrasonic inactivation of *Bacillus alpha-amylase*. I. Effect of gas content and emitting face of probe. *Ultrason Sonochem* 15(2):133–142
- Kentish S, Ashok Kumar M (2011) Frequency effects during acoustic. *J Phys Chem* 115(20):508–9–93
- Ketsa S, Daengkanit R (1999) Firmness and activities of polygalacturonase, pectinesterase, b-galactosidase and cellulose in ripening durian harvest at different stages of maturity. *Sci Hortic* 80:181–188
- Lee KH, Egleston PN, Brown WH, Barker AT, Gregory AN, Woodruff PWR (2007) The role of cerebellum in sub-second time perception. *J Cogn Neurosci* 19(1):147–157
- Lopez-Gomez PS, Palop FA, Periago PM, Martinez-Lopez A, Marin-Iniesta F, Barbosa-Canovas GV (2009) Food safety engineering: an emergent perspective. *Food Eng Rev* 1:84–104
- Manas B, Munoz D, Condon S (2006) Inactivation of lysozyme by ultrasonic waves under pressure at different temperatures. *Enzyme Microb Technol* 39:1177–1182
- Mason T, Riera E, Vercet A, Lopez-buesa P (2005) Application of ultrasound. In: Sun DW (ed) *Emerging technologies for food processing*, Elsevier Academic Press, California, pp 323–350
- Mulabagal V, Ngouajio M, Nair A, Zhang Y, Aditya L, Muraleedharan G, Nair G (2010) In vitro evaluation of red and green lettuce (*Lactuca sativa*) for functional food properties. *Food Chem* 118:300–306
- Oancea S, Grosu C, Ketney O, Stoia M (2013) Conventional and ultrasound-assisted extraction of anthocyanins from blackberry and sweet cherry cultivars. *Acta Chim Slov* 60(2):383–389
- Odriozola-Serrano I, Soliva-Fortuny R, Gimeno-Ano V, Martin-Belloso O (2008) Kinetic study of anthocyanins, vitamin C, and antioxidant capacity in strawberry juices treated by high-intensity pulsed electric fields. *J Agric Food Chem* 56(18):8387–8393
- Pagan R, Manas P, Alvarez I, Condon S (1999) Resistance of *Listeria monocytogenes* to ultrasound waves under pressure at sublethal (manosonication) and lethal (manothermosonication) temperatures. *Food Microbiol* 16:139–148
- Pingret D, Fabiano-Tixier AS, Chemat F (2013) Degradation during application of ultrasound in food processing: a review. *Food Control* 31:593–606
- Rawson A, Tiwari BK, Tuohy MG, O'Donnell CP, Brunton N (2011) Effect of ultrasound and blanching pretreatments on polyacetylene and carotenoid content of hot air and freeze dried carrots discs. *Ultrason Sonochem* 18:1172–1179
- Rivera CS, Venturini ME, Oria R, Blanco D (2011) Selection of a decontamination treatment for fresh *Tuber aestivum* and *Tuber melanosporum* truffles packaged in modified atmospheres. *Food Control* 22(3–4):626–632
- Rodrigo D, Jolie R, Van Loey A, Hendrickx M (2007) Thermal and high pressure stability of tomato lipoxygenase and hydroperoxide lyase. *J Food Eng* 79(2):423–429
- Sampedro F, Fan X, Rodrigo D (2010) High hydrostatic pressure processing of fruit juices and smoothies. Research and commercial application. Case Studies in Novel Food Processing Technologies: Innovations in Processing, Packaging, and Predictive Modelling, Elsevier Inc 34–72
- Sao-Jose JFBD, Andrade NJD, Ramos AM, Vanetti MCD, Stringheta PC, Chaves JBP (2014) Decontamination by ultrasound application in fresh fruits and vegetables. *Food Control* 45:36–50
- Soria AC, Villamiel M (2010) Effect of ultrasound on the technological properties and bioactivity of food: a review. *Trends Food Sci Technol* 21:323–331
- Suslick KS (1989) The chemical effects of ultrasound. *Sci Am* 260(2):80–86
- Tezcan F, Gultekin-Ozguven M, Diken TB, Erim FB (2009) Antioxidant activity and total phenolic, organic acid and sugar content in commercial pomegranate juices. *Food Chem* 115:873–877
- Tiwari BK, O'Donnell CP, Muthukumarappan K, Cullen PJ (2008) Effect of ultrasound processing on the quality and nutritional properties of fruit juices. *Stewart Postharvest Rev* 4(5):1–6
- Tiwari BK, O'Donnell CP, Muthukumarappan K, Cullen PJ (2009) Ascorbic acid degradation kinetics of sonicated orange juice during storage and comparison with thermally pasteurised juice. *LWT Food Sci Technol* 42(3):700–704
- Tiwari BK, O'Donnell CP, Patras A, Brunton NP, Cullen PJ (2010) Effect of ultrasound processing on anthocyanins and color of red grape juice. *Ultrason Sonochem* 17(3):598–604
- Toivonen PMA, Brummell DA (2008) Biochemical bases of appearance and texture changes in fresh-cut fruit and vegetables. *Postharvest Biol Technol* 48:1–14
- Ugarte-Romero E, Feng H, Martin SE, Cadwallader KR, Robinson SJ (2006) Inactivation of *Escherichia coli* with power ultrasound in apple cider. *J Food Sci* 71(2):102–108
- Ulusoy BH, Colak H, Hampikyan H (2007) The use of ultrasonic waves in Food Technology. *Res J Biol Sci* 2(4):491–497
- Vilkhu K, Mawson R, Simons L, Bates D (2008) Applications and opportunities for ultrasound assisted extraction in the food industry, a review. *Innov Food Sci Emerg Technol* 9:161–169
- Wang H, Gan D, Zhang X, Pan Y (2010) Antioxidant capacity of the extracts from pulp of *Osmanthus fragrans* and its components. *LWT Food Sci Technol* 43:319–325
- Wang W, Ma X, Zou M, Jiang P, Hu W, Li J, Zhi Z, Chen J, Li S, Ding T, Ye X, Liu D (2015) Effects of ultrasound on spoilage microorganisms, quality, and antioxidant capacity of postharvest cherry tomatoes. *J Food Sci* 80(10):117–126