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Effect of ultrasound treatment on physico-chemical, nutraceutical and microbial quality of strawberry

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ABSTRACT

Ultrasound (US) treatment (33 kHz, 60 W) was applied to freshly harvested strawberry for different time (0, 10, 20, 30, 40, 60, min) and analyzed for the storage period of 15 days at 4 °C. The pH, vitamin C, acidity & total soluble solids were better retained between 30 and 40 min, while as firmness and color of the fruit were found to be optimum between 20 and 30 min. At 40 min treatment time, the bacterial count decreased from 5.91 to 3.91 (Log_{10} CFU g^{-1}) and yeast and mold count decreased from 4.80 to 3.58 (Log_{10} CFU g^{-1}), while as the DPPH and ABTS % inhibition was increased by 14.41 and 8.53% from control, respectively on day 15. The 30–40 min US treated fruit was shelf stable for all the storage days. The higher exposure time (60 min) increased the microbial load, decreased antioxidant potential and changes the desirable quality of the fruit.

1. Introduction

Strawberry is a highly perishable fruit and known for its nutritional and organoleptic properties. It is a rich source of vitamins, minerals and potent bioactive components such as flavonoids, anthocyanins etc. (Aaby, Skrede, & Wrolstad, 2005) and its quality is determined by sensory attributes such as flavor, texture and color, the soluble sugars, organic acids, pectin and anthocynins are found to be responsible for these sensory attributes. Anthocyanins along with other phenolic compounds and secondary metabolites impart various health benefits such as effects on diabetic neuropathy (Anjaneyulu & Chopra, 2004), anti-inflammatory properties, antioxidant and anti-carcinogenic properties (Seeram, 2008; Zafra-Stone et al., 2007). However this fruit is quite delicate, its shelf-life is quite low and its survival is a challenging task which is further complicated by its susceptibility to fungal attacks and textural losses, for example *Grey mold rot* caused by *Botrytis cinerea* remains a major factor in limiting shelf-life of strawberries and causes up to 50% loss (Garcia, Aguilera, & Jimenez, 1996). However, the Shelf-life of strawberry can be extended to one week when stored at low temperature, fungicides and chemical sanitizers can also be used but food safety concerns and environmental considerations limit their use (Lopez-Gomez et al., 2009). Consumer awareness about pesticide residues on foods, pathogen resistance, and nutritional losses through traditional methods of storage has increased the need to develop new methods to control postharvest diseases (Yang, Cao, Cai, & Zheng, 2011).

Researchers are now looking for the most promising and result-oriented novel techniques that can be used without causing any loss of the nutrients. In recent years, interest has been developed from thermal food preservation methods towards non-thermal technologies. Therefore, several non-thermal technologies have been introduced such as ultrasound (US), high hydrostatic pressure (HHP), high pressure homogenization (HPH), Ultraviolet (UV) and pulse electric field (PEF). The non thermal technologies can improve the quality and shelf-life of fruits and has become a potential alternative to the conventional methods (Rupasinghe & Yu, 2012). Among newer non thermal technologies, application of ultra sound is gaining considerable importance as it increases the microbial safety and prolongs the shelf-life particularly in heat sensitive foods. Ultra sound waves have the advantage of being eco-friendly and non-toxic unlike other techniques employed in food industry for enhancing shelf-life of fruits and vegetables (Kentish & Ashokkumar, 2011). Some research has been carried out on

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ultrasound application, on chemical and physical properties of strawberry (Cao et al., 2010). However its effect on antioxidant potential of the fruit has not been reported to the best of our knowledge. Thus study aims at studying the effect of ultrasound for different time periods on physico-chemical, microbial load and antioxidant potential of the fruit during a period of 15 days stored at 4 °C. This study was designed to suggest the most effective treatment that can retain the quality parameters and maximizes the shelf-life of the fruit.

2. Material and methods

2.1. Materials

All the chemicals and reagents used were of analytical grade and purchased from Sigma Aldrich and Hi–media laboratories.

2.2. Sample procurement and ultrasound treatment

Ripened strawberry (*chandler*, cultivar) were harvested from local farms of Kashmir province, India and transported within an hour to our lab. The average length and breadth of the cultivar was 4.2 cm and 3.6 cm, respectively. They were sorted to eliminate damaged and selected for color and then randomly divided according to treatment. 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 surface of water (distilled) in the bath was kept at the same level during each experiment and temperature (25 ± 1 °C) of the water was kept constant using oven thermometer, whenever a slight change in the temperature was observed, fresh water was circulated to stabilize the temperature of ultrasound water bath at 25 °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 taken for analysis; some fruits were tested for color and firmness. The remaining sample (50–60 g approx.) was pulped, homogenized and centrifuged for 10 min at 5000 rpm and supernatant was collected and analyzed on the same day for measuring titratable acidity, pH, Vitamin C, total soluble solids and antioxidant activity of the fruit. The analysis was carried out in triplicates.

2.3. Physico-chemical analysis

2.3.1. Total titratable acidity (TA) and pH

Titratable acidity (TA) was determined by titrating 20 mL strawberry pulp (supernatant) to pH 8.2 using 0.1 M NaOH and was calculated as reported by Gunness, Kravchuk, Nottingham, Arcy, and Gidley (2009). The pH was measured using HI 2215pH/ORP meter (Hanna Instruments Woonsocket RI USA).

2.3.2. Total soluble solids & vitamin C

Total soluble solids (TSS) of the supernatant were determined at room temperature using a portable refractometer (Atago, Japan). Vitamin C content of the strawberries was measured using 2, 6-dichloro-indophenol titration as described by Jones and Hughes (1983). Fresh supernatant of pulp (10 mL) was mixed with 10 mL of 3% (V/V) metaphosphoric acid. The extract was made up to a volume of 100 ml and centrifuged at 3000 g for 15 min at 25 °C. The 10 mL supernatant was titrated against standard 2, 6- dichoro-indophenol, which had already been standardized against standard ascorbic acid and the results were expressed in mg/100 g fresh weight (FW).

2.3.3. Color

Color of the whole Strawberry fruit was determined using Color Flex Spectrocolorimeter (Hunter Lab Colorimeter D-25, Hunter Associates Laboratory, Ruston, USA) after being standardized using Hunter Lab color standards and their Hunter L*, a* and b* values were measured. The total color difference (TCD) parameter was considered for evaluation of color changes. 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 (0 min US treatment), according to the expression (DrLange, 1994)

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

2.4. Antioxidant activity

To prepare the fruit extracts, 2.5 g of fresh pulp was homogenized with 2.5 ml of 95% (V/V) cold ethanol and centrifuged at 10,000 rpm for 15 min, the 2.5 ml of 80% (V/V) cold ethanol was used to extract the residue. The supernatants were combined and the final volume made to 10 ml with ethanol. The ethanol extract was used for analysis of DPPH, ABTS and Total Phenols. All tests were performed in triplicate and means were calculated.

Total phenolic content was measured using Folin–Ciocalteu method described by Tezcan, Gultekin-Ozguven, Diken, Ozçelik, and Erim (2009). The final result was expressed as milligram of Gallic acid equivalents per 100 mg of Strawberry fresh weight.

DPPH (1, 1-dihpenyl-2-picrylhydrazyl) free radical scavenging activity was measured according to the method reported by Shah et al., (2015) with modifications. Briefly, 2.0 mL of 0.2 mM ethanolic DPPH solution was added in 2.0 mL juice sample. This mixture was placed in dark at room temperature for 30 min. The absorbance was determined with spectrophotometer (Hitachi) 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 inhibition of DPPH.

$$\text{Inhibition } (\%) = (A_c - A_s/A_c) \times 100 \quad (2)$$

Where A_c is the absorbance of the control and A_s is the absorbance of the juice sample.

ABTS (2, 2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)) radical-scavenging assay was determined according to the method of Wang, Gan, Zhang, and Pan (2010) with slight modification. The radical-scavenging activity of the samples was expressed as scavenging capacity (SC %)

$$(SC)\ \% = [(A_{control} - A_{test})/A_{control}] \times 100 \quad (3)$$

Where, $A_{control}$ is the absorbance of the control ($ABTS^{\bullet+}$ solution without test sample) and A_{test} is the absorbance of the test sample ($ABTS^{\bullet+}$ solution plus extract).

2.5. Firmness

The firmness of each ultrasound treated strawberry (0, 10, 20, 30, 40, 60 min) was carried out by the method of Alexandre, Brandao, and Silva (2012) with some modifications and it was measured using a TA.XT PLUS Texture Analyzer (Stable Micro Systems Ltd., UK) fitted with a cylinder plunger SMS-P/10 CYL Delrin probe (10 mm diameter). The whole strawberry 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 of strawberries is recorded as the maximum