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Study on packaging and storage of tender coconut water preservation by ultrasonication

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Abstract
Tender coconut water (TCW) is incredible healthy drink and the best one to hydrate the body. TCW is the sweet, clear, watery component of the young green coconut. TCW begins to degrade when exposed to air and stored at room temperature due to microbial contamination and oxidation processes, which induce changes in its sensory qualities. The preservation of the TCW's pristine natural property remains a struggle. Therefore nonthermal processing techniques treated tender coconut water (ultrasonication) and packed in 200 ml glass(S_1) and PET(S_2) bottles under refrigerated condition (4±2 ℃), and withdrawn a weekly interval to checked the quality and stability of TCW. The effect of ultrasonication treatment time (10, 20, 30 min) on different characteristics of TCW. Ultrasonication treated TCW was found at 20 min treatment time and packed in glass and PET bottle under refrigerated condition, which gave the experimental values of TSS 5.17 and 4.93ºBrix, TA 0.08 and 0.10%, pH 4.87 and 4.68, EC 5.24 and 5.15 mS/cm, TDS 4.03 and 4.62 ppm, TS 4.37 and 4.63%, TPC 2.43 and 1.77 (mg GAE/ml), Total plate count 1.718 and 2.418 log (CFU/ml), Yeast and Mould count 2.579 and 1.384 log (CFU/ml), POD 0.014 and 0.021, PPO 0.017 and 0.021(Δ O.D./min/ml) and Overall acceptability 8.13 for after 4th week of storage period.

Keywords: Tender coconut water, ultrasonication, glass bottle, PET bottle

Introduction
Coconut [*Cocos nucifera* (*C. nucifera*) L.] is an important multipurpose perennial horticultural crop in the world, providing food for millions of people, especially in the tropical and subtropical regions and with its many uses it is often called the “tree of life”.
The endosperm tissue is the edible component of the coconut fruit (coconut flesh and coconut water). Endosperm tissues develop in one of three ways: nuclear, cellular, or helobial, with coconut endosperm development falling under the nuclear category. The endosperm begins as a liquid containing free nuclei produced by a process in which the main endosperm nucleus divides without cytokinesis (the process by which the cytoplasm of a single eukaryotic cell is divided to form two daughter cells). Cytokinesis then proceeds, advancing from the periphery to the core, resulting in the formation of the cellular endosperm layer. (Lopes and Larkins, 1993) [11].
Coconut water (CW), often known as coconut juice, is a sweet and refreshing drink made from the inner section of coconut fruits. Coconut water is also thought to be an essential alternative for oral rehydration and even intravenous hydration of patients in rural areas. Coconut water may also help to prevent myocardial infarction. Interestingly, one study found that drinking coconut water on a daily basis can help reduce hypertension. Furthermore, micronutrients (nutrients required in small amounts) in coconut water, such as inorganic ions and vitamins, play an important function in assisting the human body's antioxidant system. (Alleyne *et al*., 2005) [4].
Coconut water has long been utilised as a medicinal food all throughout the world. Because of its distinct chemical composition, it is more effective in treating conditions such as heat stroke and severe dehydration induced by vomiting and diarrhoea. It relieves stomach pain and vomiting while also preventing heat rashes, smallpox, measles, and chickenpox. It is beneficial for kidney diseases such as kidney stones and aids in the treatment of urinary tract infections.
Coconut water’s rich enzyme systems include very effective and selective reductase, polyphenol oxidase (PPO) and peroxidase (POD). These are involved in its development of a brownish colour when it is exposed to air for a long time. (Awua *et al*., 2011) [8].
TCW is heat-sensitive because heating it changes its flavour and other quality parameters. As a result, thermal treatments like pasteurisation, sterilisation, etc., which can inactivate enzyme

activity and reduce microbes, have a negative impact on the quality of TCW by causing the degradation of heat-sensitive vitamins and minerals, volatile flavour compounds, alteration or denaturation of protein, and loss of other nutrients. Thermal treatment is adverse for TCW preservation due to the changes in organoleptic qualities, including colour, flavour, TSS, turbidity, and rheological characteristics. Consequently, it is crucial to look for non-thermal substitutes that do not negatively affect or do so in a way that is less harmful to the nutritional and sensory qualities of TCW than thermal methods. Hence, research is required to ascertain the effects of non-thermal treatment like ultrasound techniques on the storage life and quality of final produce of various types of packaging.

Materials and Methods

Experimental Location

The experiment was carried out at Department of Processing and Food Engineering, College of Agricultural Engineering and Technology, Junagadh Agricultural University, Junagadh during the year of 2022 – 2023.

Raw Materials

The tender coconuts was purchased from Instructional Farm, Jambuvadi, College of Horticulture, Junagadh Agricultural University, Junagadh, Gujarat, India. Coconut water samples were extracted from tender coconuts (*Cocos nucifera* L.) at the 7-9th maturation month. Coconuts were immersion in water for 20 min. The extraction was done manually with the aid of a sterile stainless steel knife. After extraction, the samples were homogenized and then filtered on standard muslin cloth.

Experimental design

The experiment was designed using Factorial Completely Randomized Design (FCRD). The experiment is planned giving nonthermal treatment as ultrasonication with different packaging material as PET and glass bottle stored at 4±2 ℃ temperature.

Biochemical properties of tender coconut water

Titratable acidity

Titratable acidity was estimated as reported by Kannangara *et al*. (2018) [10]. A TCW sample of 10 mL was dissolved in 100 mL of water. 10 mL of sample was pipetted into a 100 mL conical flask, and a few drops of phenolphthalein indicator were added. Titration was performed against 0.1 N NaOH until the pale pink end point was achieved. This method was continued until concordant readings were obtained. The titratable acidity was estimated in terms of citric acid equivalency using the formula shown below.

$$\text{Titratable acidity (\%)} = \frac{\text{Titre (ml)} \times \text{0.1N NaOH (ml)} \times \text{Volume made up (ml)} \times \text{Equivalent weight of cirtic acid}}{\text{Weight of sample (ml)} \times \text{Aliquot taken (ml)} \times 1000} \times 100$$

Total soluble solids (TSS)

Total soluble solids (TSS) of processed tender coconut water were determined following procedure described by Ali *et al.* (2010) [3]. A digital refractometer was used to measure TSS.

pH measurement

The pH is defined as the logarithm of the reciprocal of hydrogen ion concentration. The pH was measured using pH meter by dipping the electrodes of pH meter into the sample.

Total sugar

Total sugars were estimated by the modified method of Dubois *et al*. (1956). A 0.1 mL sample was combined with 10 mL of 2.5 N methanol. Then, a 0.1 ml aliquot was collected and 0.9 ml of distilled water was added to form a final volume of 1.0 ml. One by one, 1.0 mL of 5% phenol and 5.0 mL of 96% $H2SO_4$ were added. Then all samples were put in a water bath for 10-15 minutes. Spectrophotometer reading was taken at 490 nm wavelength.

$$\text{Total sugar (\%)} = \frac{\text{Sample O.D} \times \text{Standard O.D} \times \text{Total volume (ml)} \times \text{Dilution factor} \times 100}{\text{Aliquot taken (ml)} \times \text{Sample weight (ml)} \times 1000}$$

Electric conductivity

The conductivity meter was calibrated against standard buffer solutions. The samples were mixed well to homogenize and the conductivity was measured using the calibrated conductivity meter and expressed in mS/cm.

Total dissolved solids (TDS)

Total Dissolved Solids (TDS) is the total number of compounds dissolved in a liquid. The TDS of the samples was calculated from their electrical conductivity (EC). TDS is generally measured in parts per million (ppm) but can also be measured in mg/L.

Total phenol content

Phenol content was estimated by the method suggested by Mahayothee *et al*. (2016) [12]. In 10 ml of 80% ethanol, 0.1 g of material was extracted. The supernatant was collected after centrifugation. Then, 0.1, 0.2 of the working standard was pipetted into a series of test tubes, and the total volume of each was built up to 3 ml with distilled water. For the blank solution, 3 ml of distilled water was placed in a test tube. After that, 0.5 ml of Folin-Ciocalteau reagent was applied to each tube, including the blank. After three minutes, each received 2 ml of a 20% Na2CO3 solution. The solution was thoroughly mixed before being placed in a hot water bath for one minute. After cooling to room temperature, colour was read at 650 nm using UV visible spectrophotometer (Thermo Scientific, GENESYS 50). At last, percentage of total phenol was calculated by preparing standard curve of catechol. Following formula was used.

$$\text{Total Phenol content (\%)} = \frac{\text{Graph factor (µg)} \times \text{Optical density} \times \text{Total volume (ml)}}{\text{Sample aliquot (ml)} \times \text{weight of sample (ml)} \times 1000}$$

Enzyme activity

Assay of polyphenol oxidase (PPO)

Processed tender coconut water 10 ml of 100 mM sodium phosphate buffer having pH 6.5. The homogenate was centrifuged at 10,000 rpm for 15 min at 4 ℃ and the supernatant was used for enzyme assay.

The reaction mixture contained 2.9 ml of catechol (10 mM catechol in 10 mM phosphate buffer, pH 6.5) and reaction was initiated by the addition of 100µl of enzyme extract. The changes in the colour due to the oxidized catechol was read at 490nm for 15 minutes at an interval of 3 min. Blank was carried out without substrate. (Malick and Singh 1980) [13].

Assay of peroxidase (POD)

Processed tender coconut water (10 ml) was homogenized in a pre-chilled mortar with 2 ml of extraction buffer, containing 50 mM sodium phosphate buffer pH 7.0. The homogenates were centrifugal at 10,000 rpm for 15 minutes and the supernatant was used for the assay of antioxidant enzymes *viz.* peroxidase and catalase.

The reaction mixture contained 2.99 ml of 0.03% H_2O_2 in 0.1 M phosphate buffer (pH 6.0) containing 0.01% orthodianisidine dye (freshly prepared, dissolved in methanol). The reaction was initiated by the addition of 10 µl of enzyme extract. The change in colour of oxidized dye was read at 460 nm up to 1 minute at the interval of 15 seconds. Blank was run without the addition of enzyme (Malick and Singh (1980) [13].

Microbial analysis

Total yeast and mould count (CFU/ml)

One ml of suitable dilution from each sample prepared as described in a precious section was used for plating, and thereafter 15 ml of molten PDA agar was poured aseptically to plates. The samples were spread on petri plates and plates were cooled. The plates kept for incubation in an incubator to maintain temperature 27±0.5 ℃ for 72h. Number of colony forming units (CFU/ml) was recorded after every 24h. The total plate count in terms of log colony-forming unit per ml (CFU/ml) was calculated using the following formula (AOAC 2000b) [7]. The colonies of 10^{-3} and 10^{-7} were considered for calculations. All the procedure were carried out in aseptic condition and with proper precaution.

$$\text{YMC}\left(\frac{\text{CFU}}{\text{ml}}\right) = \log\left(\frac{\text{Mean Number of colony forming units}}{\text{Volume of samples} \times \text{Dilution factor}}\right) \times 100$$

Total plate count (CFU/ml)

One ml of suitable dilution from each sample spreading to prepared media petri plates. After that, the plates were kept in a incubator for incubation and maintained the temperature 37±0.5 ℃ for 48h. Number of colony forming units (CFU/ml) was recorded after every 24h. The total plate count in terms of log colony forming unit per ml (CFU/ml) was calculated using the following formula (AOAC 2002a) [6]. The colonies of 10^{-3} and 10^{-7} were considered for calculations.

$$\text{TPC}\left(\frac{\text{CFU}}{\text{ml}}\right) = \log\left(\frac{\text{Number of colony forming units}}{\text{Volume of samples} \times \text{Dilution factor}}\right) \times 100$$

Sensory analysis

Sensory evaluation was conducted for bread. A panel of 15 semi trained panel lists of faculty members and post graduate students of College of Agricultural Engineering and Technology, Junagadh Agricultural University, Junagadh were asked to assess the samples and mark them on a hedonic rating test in accordance with their opinion for colour, flavour, taste, and overall acceptability (Amerine *et al*. 1965). All tests were performed under uniform lighting conditions, and the subjects were not informed about the background of the study. The samples scoring an overall quality of 5 or above were considered acceptable and those scoring below 5 were considered unacceptable.

Storage analysis

In the last phase, storage stability was aimed to investigate the quality of processed tender coconut water stored in glass and PET bottles. The samples were kept for 4weeks storage at refrigerated condition at 4±2 ℃. At an interval of 1week, the bio-chemical and microbial analysis were carried out.

Statistical analysis

All the experiments in this study were conducted two times and the mean values were reported. Statistical analysis was done to study the effect of two different factors like treatment and packaging material on dependent parameters, i, e. pH, TSS, titratable acidity, EC, TDS, total sugar, total phenol content, microbial analysis and enzyme activities by Factorial Completely Randomized Design (FCRD by using Microsoft Office Excel 2013) (Pansee and Sukhatme, 1985) [14].

All the treatments were compared at 0.05% level of significance using the Critical Difference test (Microsoft Office Excel 2013). The Analysis of Variance (ANOVA), Standard Error of difference (SEd), Standard Error of mean (SEm) and Critical Difference (CD) for dependent parameter were tabulated and the level of significance was reported.

Results and Discussion

Effect of titratable acidity (TA), Total Soluble Solids (TSS), pH of treated tender coconut water

Titratable acidity (TA) was found ultrasonication treatment maximum U_2S_2 (0.10%) and minimum U_1S_1, U_1S_2, U_3S_1 (0.07%). Total Soluble Solids was found ultrasonication treatment maximum U_3S_2 (5.23ºBrix) and minimum U_2S_2 (4.93ºBrix). pH was found ultrasonication treatment maximum U_2S_1 (4.87) and minimum U_2S_2 (4.68) for 4th week of storage period. Similar behaviour has been reported for ultrasound processing of grape juice for various time intervals also showed no remarkable change in acidity, total soluble solids (TSS), pH during storage period. (Adil *et al*., 2015) [2]

Effect of Electrical Conductivity (EC), Total Dissolved Solids (TDS) of treated tender coconut water

Electrical conductivity (EC) was found ultrasonication treatment maximum U_3S_2 (5.83 mS/cm) and minimum U_2S_2 (5.15 mS/cm). Total Dissolved Solids (TDS) was found ultrasonication treatment maximum U_2S_2 (4.62 ppm) and minimum U_2S_1 (4.03 ppm) for 4th week of storage period.

These similar findings were reported by Kannangara *et al*., (2018) [10] in comparative analysis of coconut water in four different maturity stages.

Effect of Total Sugar (TS), Total Phenol Content (TPC) of treated tender coconut water

Total Sugar (TS) was found ultrasonication treatment maximum U_3S_2 (5.16%) and minimum U_3S_1 (3.72%) for 4th week of storage period. Similar reductions were reported for ultrasound treated carrot juice also (Zou and Jiang, 2016) [16]. A slight increase in total sugar (TS) content of TCW in the present study may be due to the higher release of sugars from tissues by ultrasound treatment.

Total Phenol Content (TPC) was found ultrasonication treatment maximum U_3S_2 (3.17 mg GAE/100 ml) and minimum U_2S_2 (1.77 mg GAE/100 ml) for 4th week of storage period. Adil *et al.* (2015) [2] also observed a siginificant decrease in total antioxidant capacity total phenols (approx. 2%) during 15 days storage of ultrasound treated grape juice.

Effect of Peroxidase activity (POD) and Poly Phenol Oxidase (PPO) of treated tender coconut water

Peroxidase (POD) was found ultrasonication treatment maximum U_2S_2 (0.021 ΔO.D./min/ml) and minimum U_3S_1 (0.012 ΔO.D./min/ml). Poly Phenol Oxidase (PPO) was found ultrasonication treatment maximum U_3S_2 (0.040 ΔO.D./min/ml) and minimum U_1S_1, U_2S_1 (0.017 ΔO.D./min/ml) for 4th week of storage period. These findings is similar to Rico, Martin- Diana, Frias, Gary and Barry-Ryan (2006) also reported the efficacy of ozone against PPO and POD enzymes in fresh cut lettuce.

Effect of microbiological parameters for stored tender coconut water

Total plate count of processed tender coconut water ranged from 0 log (CFU/ml) to 3.681 log (CFU/ml). The yeast and mould count of processed tender coconut water ranged from 0 log (CFU/ml) to 3.343 log (CFU/ml) for 4th week of storage period.Carrot juice treated by ultrasound for 20–60 min showed a significant increase in time-dependent reduction in total plate count as well as total yeast and mold count (Zou & Jiang, 2016) [16], and the similar results were also reported for apple juice (Abid *et al*., 2016) [1].

Sensory analysis of treated tender coconut water

The sensory characteristics of processed tender coconut water *viz.* taste, flavor, color and overall acceptability compared with control tender coconut water. The maximum score of overall acceptability was found in ultrasonication U_2S_1, U_2S_2 (8.13) tender coconut water stored at 4±2 ℃ for 4th week of storage period.

Conclusion

Based on above results, it may be concluded that, tender coconut water treated with 20 min ultrasonication treatment stored at 4±2 ℃ temperature in glass bottle for prolonging the shelf life up to 4 weeks for maintaining freshness and quality of tender coconut water with reduce the enzyme activities. The objective of this study is achieved because of reduce the enzyme activity and microbial count of tender coconut water.

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