



Preservation of tender coconut (*Cocos nucifera* L.) water by heat and UV-C treatments

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Abstract

Tender coconut water is a nutritious natural beverage which consists with many health benefits. This research reviews the preservation of tender coconut water using heat and UV-C treatments for selected combinations. After each treatment, physicochemical properties (Total Soluble Solids, pH, Conductivity and Titrable Acidity) and microbiological analysis were conducted. Finally sensory evaluation was conducted for both samples, to determine the most preferable sample. Results revealed that there is a significant difference ($p < 0.05$) in TSS, pH, electrical conductivity between the treated samples of all combinations in Tender coconut water during the period of three weeks. The total plate count and yeast and mould counts were in acceptable level (< 50 CFU/ml) in the samples treated at 85°C for 60s and 253.7 nm UV radiation for 30 minutes and the sample treated at 85°C for 60s. And also these tender coconut water samples were microbiologically safe for consumption when stored under refrigeration ($4 \pm 2^{\circ}\text{C}$). The tender coconut water samples, treated at 85°C for 60 minutes, without UVC and kept under refrigeration ($4 \pm 2^{\circ}\text{C}$) were found with high organoleptic qualities.

Keywords: heat treatment, preservation, tender coconut water, UV

1. Introduction

Tender coconut water is a mineral liquid, nutritious and used as a refreshing and rehydrating drink. It is rich in sugars, minerals, and amino acids. The calorific value of tender coconut water is 17.4 Kcal/100g^[1, 2, 3]. Sugars are the main fraction of soluble solids in coconut water^[4, 5]. The main sugars in mature coconut water are sucrose, sorbitol, glucose and fructose followed by minor sugars including galactose, xylose and mannose^[6, 7].

It has been reported that coconut water is an isotonic beverage due to the presence of balanced electrolytes namely; Potassium and Sodium that help restore losses of electrolytes through skin and urinary pathways^[8, 9].

In the Aurvedic medicine coconut water is used as a medicine^[10, 11]. It is traditionally prescribed for burning pain during urination, dysuria, gastritis, burning pain in eyes, indigestion, and hiccups or even expelling of retained placenta. Coconut water presents anti-carcinogenic properties and can be used as dehydrating solution administered orally or in intravenous form^[12, 13, 14].

There is a high export market potential for tender coconut water but the major problem with the export is the physicochemical, organoleptic and microbial changes that take place after harvest, during processing and transport up to the point of sale. The most challenging problem related to the stability of coconut water during its shelf life is the activity of the polyphenoloxidase (PPO) and peroxidase (POD) enzymes^[15]. Non-conventional technologies such as high pressure homogenization^[16], CO_2 in dense phase^[17], γ -irradiation^[18] and microwave heating^[19] have all been studied for processing coconut water. The use of high hydrostatic pressure technology has already been commercially adopted for this purpose. However, although these technologies are

effective for microbiological inactivation, they have resulted in undesirable changes and/or are ineffective at inactivating the PPO and POD enzymes^[20].

Therefore, this study was carried out to extend the shelf life of tender coconut water by applying heat, UV and a combination of heat and UV treatments with a view to determine the changes occurring on physicochemical, microbiological and sensory parameters of preserved tender coconut water.

2. Materials and Methodology

The tender coconuts (TC) obtained from a coconut plantation at Polgahawela, Sri Lanka, were in a sound condition at 9 months development stage were used for this research study.

2.1 Preparation of the Sample

The TC were washed with potable water and then soaked in a dilute bleach solution (1 tablespoon bleach per 4.5 liters of water) for 15 minutes and transferred to a clean surface (off the ground) and left for air drying. The water from TC was obtained within 24 hours of harvest. The extracted water was filtered through sanitized stainless steel strainer to remove fibers and endosperm (meat) fragments. All collected, coconut water was mixed thoroughly in a sterilized container. The tools, bottles and caps were washed with potable water and sanitized by soaking in a dilute bleach solution (0.1% NaOCl) for 15 minutes and allowed to air dry in the inverted position prior to filling them with TC water.

The TC water was put into a stainless steel saucepan and heated in a water bath at 85°C for 60s while stirring continuously.

2.2 UV treatment process

The polyester (PET) bottles containing TC water were placed

in a dark chamber and exposed to UV C radiation at 253.7 nanometer Ultraviolet light for 30 minutes (The light source: BIOBASE Fume Hood Model FH1200 A). The PET bottles were kept horizontally on a plastic tray allowing maximum exposure to the UV source maintaining a distance of 23.4 cm between the lamp and the PET bottles [21].

Table 1: Treatment Combination

Sample	Treatment
Sample A	Heated up to 85°C for 60 seconds and exposed to 253.7 nm UV radiation for 30 minutes and stored at 4±2°C.
Sample B	Exposed to 253.7 nm UV radiations for 30 minutes and stored at 4±2°C.
Sample C	Sample heated up to 85°C for 60 seconds and stored at 4±2°C.
Sample D	Untreated sample stored at 4±2°C.

All the TCW samples were analyzed at ambient temperature and the chemicals used for the analysis were in analytical grade.

2.4 Determination of Physicochemical properties

a) Total Soluble Solids (TSS)

The brix values (TSS) of samples were measured using a OPTICA HR-150 portable hand held refractometer at room temperature (30±2°C).

b) pH value

Samples were mixed well to get homogenize solution and the pH values were measured using the calibrated pH meter.

c) Electrical Conductivity

Samples were mixed well to get homogenize solution and the conductivity was measured using the calibrated sense ION™+ Portable Conductivity Meter.

d) Titratable Acidity (TA)

Titrate acidity, expressed as malic acid (%) was determined according to the AOAC method (Horwitz, W. & Latimer, G., 2005). A standard solution of 0.1N NaOH was prepared. Simultaneously 10 mL of coconut water was transferred to an Erlenmeyer flask without dilution few drops (nearly 1ml) of 1% phenolphthalein indicator was added. The sample was titrated against the standard NaOH solution to obtain an end point that gives a consistent pink color for 30 seconds.

The following equation was used to determine the Titratable Acidity (TA) as malic acid %. (coconut handbook.tetrapak.com/chapter/chemistry-coconut-water).

$$\text{Titrate Acidity as Malic acid (\%)} = \frac{V_1 \times N \times 67}{10 \times V_2}$$

Where,

V1 = Volume in ml of standard NaOH required for titration

N = Normality of the standard NaOH

V2 = Volume in ml of the coconut water sample taken for the test

67 = Mill equivalent weight of Malic acid

e) Sensory evaluation

Five point hedonic scale (5 = Like extremely, 1 = Extremely

2.3 Application of Heat and UV Treatments for Tender Coconut water

The Tender Coconut samples A, B, C, D were subjected to under mentioned heat and UV treatments while replicating them thrice.

dislike) with 30 semi trained panelists were asked to evaluate the sensory attributes namely Appearance, colour, smell, taste, after taste and overall acceptability of the treated samples along with a control (975). Final data were analyzed using the Kruskal-Wallis test, IBM SPSS Statistics 21.0 statistical software.

f) Microbiological Analysis

Microbiological analysis was done for total plate count and yeast and mould count according to AOAC (1995) method.

The peptone water (9ml) was added into test tubes and was sterilized in an autoclave at 121°C and 15 psi for 20 minutes. Sterile nutrient agar was used as the culture media to determine total plate count. Inoculations were done at 37°C for 24-48 hrs.

Sterile chloramphenicol yeast glucose agar was used as the culture media to determine yeasts and mould count. Agar was allowed to solidify and the solidified petri dishes were inverted and incubated quickly for 3-5 days at 25±1 °C.

A control plate was prepared to check the sterility. Colonies were counted and expressed as total colony forming units (C.F.U) per gram.

2.5 Statistical Analysis

Using IBM SPSS version 21.0 the Statistical Software package with a significance level of 0.05 was taken into contemplation of sensory attributes of the treated samples. One way ANOVA was done for physicochemical parameters to determine whether there is a significant variation of aforementioned characteristics during the period of storage followed by comparisons performed using the ANOVA test by the statistical software Minitab® 18. (CI = 95%).

Microsoft Excel computer software was used to obtain descriptive statistics (averages, standard error etc.), percentage changes (increases and decreases) and for the graphical representation of measured parameters.

3. Results and Discussion

3.1 Determination of Physicochemical Properties

Four physicochemical properties (TSS, pH, Electrical conductivity and Titratable acidity) of the samples of TC water were measured, starting from day 0, 3, and thereafter weekly for a period of 3 weeks, after exposure to heat at 85°C and UV as shown in the in the table 2.

Table 2: Physicochemical changes of TCW after 85°C heat and UV treatment.

Treatments	Storage Period	TSS (%)	pH	Titrateable Acidity (%)	Conductivity
A	Initial	6.0 ± 0 ⁿ	5.74 ± 0.01 ^a	0.08 ± 0.00 ^a	5.98 ± 0.01 ^a
B	Initial	5.5 ± 0 ^f	5.72 ± 0.01 ^{abc}	0.09 ± 0.00 ^a	5.77 ± 0.02 ^e
C	Initial	6.0 ± 0 ⁱ	5.73 ± 0.00 ^{abc}	0.08 ± 0.00 ^a	6.02 ± 0.02 ^{bc}
D	Initial	5.5 ± 0 ^j	5.72 ± 0.00 ^{abc}	0.09 ± 0.00 ^a	5.64 ± 0.01 ^h
A	Day 3	6.0 ± 0 ^m	5.73 ± 0.01 ^{ab}	0.08 ± 0.00 ^a	6.01 ± 0.01 ^b
B	Day 3	5.5 ± 0 ^a	5.72 ± 0.01 ^{abc}	0.35 ± 0.00 ^a	5.75 ± 0.01 ^{ef}
C	Day 3	6.0 ± 0 ^h	5.71 ± 0.01 ^c	0.13 ± 0.00 ^a	6.00 ± 0.01 ^e
D	Day 3	5.5 ± 0 ^p	5.72 ± 0.01 ^{bc}	0.08 ± 0.00 ^a	5.74 ± 0.01 ⁱ
A	Week 1	6.0 ± 0 ^l	5.72 ± 0.01 ^{abc}	0.08 ± 0.00 ^a	6.01 ± 0.00 ^b
B	Week 1	5.5 ± 0 ^f	5.65 ± 0.00 ^{cd}	0.15 ± 0.00 ^a	5.71 ± 0.01 ^{lm}
C	Week 1	6.0 ± 0 ^g	5.61 ± 0.01 ^c	0.11 ± 0.00 ^a	6.00 ± 0.01 ^a
D	Week 1	5.5 ± 0 ^o	5.66 ± 0.01 ^{cd}	0.10 ± 0.00 ^a	5.76 ± 0.01 ^a
A	Week 2	6.5 ± 0 ^d	5.72 ± 0.01 ^{abc}	0.08 ± 0.00 ^a	6.05 ± 0.01 ^a
B	Week 2	6.0 ± 0 ^k	5.71 ± 0.01 ^c	0.13 ± 0.00 ^a	5.75 ± 0.01 ^{ef}
C	Week 2	6.5 ± 0 ^b	5.73 ± 0.01 ^{abc}	0.11 ± 0.00 ^a	5.85 ± 0.00 ^{bc}
D	Week 2	6.0 ± 0 ^e	5.73 ± 0.01 ^{abc}	0.1 ± 0.00 ^a	5.76 ± 0.01 ^{ef}
A	Week 3	6.5 ± 0 ^c	5.24 ± 0.01 ⁱ	0.10 ± 0.00 ^a	6.01 ± 0.01 ^{bc}
B	Week 3	6.0 ± 0 ^j	5.44 ± 0.01 ^f	0.13 ± 0.00 ^a	5.94 ± 0.01 ^d
C	Week 3	6.5 ± 0 ^a	5.33 ± 0.01 ^g	0.09 ± 0.00 ^a	5.95 ± 0.01 ^d
D	Week 3	6.0 ± 0 ^e	5.26 ± 0.00 ^h	0.10 ± 0.00 ^a	5.57 ± 0.01 ^h

The brix value of a solution is its total soluble solid content. As given in the Table 2, TSS was significantly different ($p < 0.05$) among the treated samples of TCW¹. The initial Brix values of samples B and D are in the TSS range of natural TCW. The initial brix value of the thermally treated TCW (A and C) is slightly higher than that of other samples. By the first week the TSS of all the samples have increased and then remained constant during the rest of the period.

Compared to the raw juice, the brix value of the heat treated TCW was increased may be due to evaporation of water during the thermal treatment. The increase of TSS after the first week may be due to the breakdown of microbial cells in the samples.

According to previous research studies the moisture content of natural TCW is said to be 94.57% and the soluble solid content is 5.3⁰Brix. Therefore the solids present in TCW are mainly soluble solids such as sugars [22]. The UV light treatments have been shown no significant impact on the soluble solids content of juices [23].

Table 2 further shows that pH values are significantly different ($p < 0.05$) among the treated samples of TCW. In this study, the change in pH of sample A and C is negligible and remain in the acceptable pH range of heat treated TCW. The pH of the samples that were not subjected to thermal treatment

(sample B and D) has slightly increased during the second and third week.

According to the Brazilian regulation, the pH of pasteurized TCW shall be in between 4.30 and 4.50 and that the pH of sterilized TCW shall be in between 4.6 and 5.4. The pH of natural TCW is 4.78. According to previous researches, has shown that UV light processing has no significant impact on the pH of juices [23].

The titratable acidity of the samples was expressed as malic acid %. Acidity affects the flavour of TCW. Table 2 shows that TA values are not significantly different ($p > 0.05$) among the treated samples of TCW.

In this study, the change in pH of sample A and C is negligible and remain in the acceptable range of pH. According to previous research, as the coconut matures, the pH of coconut water increases in alkaline levels and become less acidic [24]. It couples with increasing sugar levels, which gives the coconut water a sweeter taste when it is seven to nine months old.

Conductivity is a measure of the concentration of ions in solution. The units are given in micro Siemens/cm (mS/cm) and milliSiemens/cm (mS/cm). Table 2 shows that electrical conductivity values are not significantly different ($p > 0.05$) among the treated samples of TCW.

¹TCW- Tender Coconut Water

Electrical conductivity of natural TCW is 5.9 ± 0.3 mS/cm and the conductivity of the study samples are observed more or less within that range [25].

3.2 Sensory evaluation of Tender Coconut water

According to statistical evaluation, there was a significant difference between in colour, appearance, aroma, taste, mouth feel and overall acceptability ($p > 0.05$) in all the TCW samples after one week of shelf life.

The mean values of colour and appearance of the thermally treated samples were lower than that of other samples due to formation of pink colour. Out of all, mean value for aroma, taste, mouth feel and overall acceptability was lowest and sensorial unacceptable by the sample that was subjected only to UV treatment. It can be due to the fact that although the thermal treatment was better processing option pertaining to enzyme inactivation, but ultraviolet treatment was found superior based on retention of nutritional attribute as observed by other research studies [26].

According to the results of sensory evaluation, sample that was processed at 85°C for 60 seconds scored the highest mean sensory scores. Therefore it is the most preferred sample throughout 3 weeks period compared to other TCW samples. The least preferred sample was the one treated with only UV radiation. The pink colour of thermally treated samples is due to the production of 5, 6 indole, quinine by oxidation of tyrosine [27].

Another previous research also suggests that the most important problem related to the stability of coconut water during its shelf life is related to the activity of the polyphenol oxidase (PPO) and peroxidase (POD) enzymes [28]. Such enzymes have relatively high thermal resistance and their activity leads to yellow, brown or even pink colouring during storage [15], even under refrigeration [18].

3.3 Microbial analysis of Tender Coconut water

Microbial analysis of TCW water was done by a serial dilution of the pour plate method described in AOAC. The number of colony forming units per ml was calculated. Results of the microbiological analysis to find the microbial stability of TCW samples under different treatment combinations are shown in Table 3 and 4.

Table 3: Total plate count values for TCW

Sample	CFU/ml		
	Week 1	Week 2	Week 3
A	ND	ND	25×10^2
B	35×10^3	40×10^3	50×10^4
C	ND	ND	2×10^2
D	32×10^3	40×10^4	140×10^4

Table 4: Yeast and mould count values for TCW

Sample	CFU/ml		
	Week 1	Week 2	Week 3
A	ND	ND	14×10^2
B	37×10^2	13×10^2	21×10^2
C	ND	ND	10
D	12×10^1	17×10^1	4×10^3

According to tables 3 and 4, no microbial growth within first two weeks was observed in samples that have undergone thermal treatment (A and C). The CFU values of sample A and C during the third week was within the acceptable level (less than 50 CFU in 1 ml).

The untreated TCW sample (D) and the TCW sample treated only with UV radiation (B) gave higher microbial counts in both TPC as well as in yeast and mould count which resulted in unacceptable organoleptic qualities.

The TCW exposed to thermal treatment at 85°C for 60s (A and C), have resulted in the pasteurization of the sample and reducing the microbial load.

Even though UV C rays have germicidal effect, highest microbial counts in sample B (treated only with UV) may be due to the improper exposure of the sample to the UV rays. As the sample bottles were placed horizontally under the UV rays, small parts of the TCW were covered by the cap of the bottles. The plastic cap of the PET bottle being opaque and may have prevented the passage of UV rays into TCW. Slight increase of the temperature during the UV treatment of the samples may have also resulted in increasing the microbial activity of the sample B.

As for TCW, it is initially sterile and remained aseptic as long as the fruit does not suffer any injuries that allow microorganisms to enter [29], [30]. Inappropriate storage and extraction conditions can also lead to microbial contamination of TCW especially during extraction and bottling of the TCW.

4. Conclusion

A significant difference ($p < 0.05$) was observed in TSS, pH, electrical conductivity between the treated samples of all combinations in Tender coconut water during the period of three weeks storage. No significant difference in titratable acidity was observed. The total plate count and yeast and mould counts were in acceptable level ($< 50\text{CFU/ml}$) in samples A (85°C for 60s and 253.7 nm UV radiation for 30 minutes) and sample C (85°C for 60s) of Tender coconut water, indicating those samples were microbiologically safe for consumption if stored under refrigeration ($4 \pm 2^{\circ}\text{C}$) after giving the treatments. The tender coconut water samples treated at 85°C for 60s, without UVC and kept under refrigeration ($4 \pm 2^{\circ}\text{C}$) were found with the highest organoleptic qualities.

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