Stability Indicating and Evaluation of Preservers in Cocos Nucifera Water by means of HPTLC Techniques

Aditya Raghava Golakoti*1, Raju PSN2, Jaiganesh S3, Amrin S A4

1Department of Surgery, Konaseema Institute of Medical Sciences Research Foundation, Amalapuram, Andhra Pradesh-533201, India
2Department of OBG, Konaseema Institute of Medical Sciences Research Foundation, Amalapuram, Andhra Pradesh-533201, India
3Department of Radio Diagnosis, Meenakshi Academy of Higher Education and Research, Chennai 600078, Tamilnadu, India
4Department of OBG, Meenakshi Academy of Higher Education and Research, Chennai-600078, Tamilnadu, India

ABSTRACT

This examination manages the steadiness and successful utilization of additives in the coconut water. The new coconut water was separated in Aseptic zone through whatman channel paper and equivalent amounts of new coconut water are pressed in self-sealable spreads. Propylparaben and Benzoic corrosive are utilized as additives of various qualities. These examples are put away for around 1 to 12 weeks at room temperature and refrigerated condition at 25°C. Tests are tried by various diagnostic techniques like pH, Turbidimetry, Titrimetry, Flame photometry, and HPTLC the shading change of tests are likewise watched. The adjustment in the new coconut water and tests with the additives are estimated for pH at customary time stretches to such an extent that the pH is kept up in the middle of 5.5-6 and to decide the microbial development by utilizing Turbidimetry, % of magnesium and % of ascorbic corrosive by Titrimetry. Sodium Potassium particles are controlled by utilizing fire photometry and to decide the sugars by utilizing HPTLC. The expansion of the additives improved the straightforwardness of the examples and the impact of additives on the Magnesium, Sodium, Potassium, Ascorbic corrosive, sugars are less with the end goal that the put-away coconut water samples can be stuffed in a self-sealable spread.

*Corresponding Author
Name: Aditya Raghava Golakoti
Phone: 9493601361
Email: golakoti61@gmail.com

INTRODUCTION

The coconut palm (Cocos nucifera) an associate of the private are caceae (palm family). It’s the solitary recognized species within the genus Cocos (Subramaniam et al., 2012). Coconut L. is a crucial angiospermous tree within the world, providing nutrients to several individuals, significantly in the wet and semitropical provinces; because of its numerous usages, it’s frequently called the “tree of life” (Rukmini et al., 2017). The plant is local to tropical eastern areas and is likewise filled in Asian mainland (India, Ceylon, and Indonesia) and in Central and South America (Mexico, Brazil) (Sivakumar
et al., 2011). In Africa, the biggest delivering nations are Mozambique, Tanzania and Ghana (Akinpelu et al., 2015). Several investigations have been directed to distinguish the dynamic particles in coconut and their conceivable pharmacological and natural exercises (Rinaldi et al., 2009). Different concentrates, divisions and separated mixes from various pieces of the coconut natural product were tried, indicating various exercises, including anti hypertensive; absence of pain; vasodilation; insurance of kidney, heart and liver capacities; security against ulcers; and mitigating, hostile to oxidant, hostile to osteoporosis, anti diabetes, anti neoplastic, bactericidal, anti helminthic, antimalarial, leishmanicidal, anti fungal and antiviral exercises (Silva et al., 2013).

Figure 1: The redox reaction of ascorbic acid.

The principal goals of the current examination are to collect fresh coconut water and to estimate the nutrient values using different analytical techniques and to add preservatives into the coconut water at different concentrations and compare their physical properties of the fresh coconut water and the examples by preservers. To compare the % of metal ions and % of ascorbic acid in cocos nucifera water by flame photometry and titrimetry and to compare the sugars in cocos nucifera sample (Shantaram et al., 2011).

MATERIALS AND METHODS

The stability indicating and assessment of real usage of preservers in cocosnucifera water are determined by means of different analytical approaches like pH, Turbidimetry and Titrimetry and flame photometer (Thamban et al., 2007).

Instruments used
Elico pH meter, Elico Turbidimetry, Titrimetry, ElicoFlame photometer, Shimadzu 0.1mg Digital balance, Aetron HPTLC (Shantaram et al., 2014).

Chemicals used and sample
Coconut water, Propylparaben, benzoic acid, methanol, Acetonitrile, Pentanol, Water, sulphuric acid (Consignado et al., 1976).

Methodology
In the present investigation, we have determined the stability of coconut water by using a pH meter for about 1-12weeks. Firstly the fresh coconut water of equal quantities was taken in a self-sealing pack for the samples the pH is determined and the first sample was stored at room temperature and the other at refrigeration (4°C to 10°C) (Ranasinghe and Wimalasekara, 2006). The samples of fresh coconut water were observed for color, pH, clarity, turbidity and microbial load at an interval of 1-12 weeks. By observing the results of fresh coconut water samples it reveals that there is a change in color, pH, clarity, turbidity, and microbial load upon storage of 4 weeks at room temperature and refrigerated conditions to get better results preservatives like benzoic acid and propylparaben of different concentrations were added (Maciel et al., 1992). The fresh coconut water of equal quantities was taken in a self-sealing pack by adding the preservatives on one set of samples are store at room temperature and the other set of samples at refrigeration (4°C-10°C). Each set contains two benzoic acid concentrations i.e.; 10mg, 50mg and 3 propylparaben concentrations i.e.; 5 mg, 10 mg and 50mg.

Figure 2: Stability indicating HPTLC method.

Procedure for determination ascorbic acid
This method used to determine the amount of vitamin C in answer is by an oxidoreduction titration using Iodine. The blue-black starch- iodine complex is formed when all the ascorbic acid molecules are tarnished; the extra iodine is permitted to respond by a starch pointer. This is the endpoint of the titration (Walter et al., 2009) as shown in Figure 1.

Preparation of reagents
Iodine solutions (0.005 mol / L)
Weighed 2gms of potassium iodide and moved into a 100 ml measuring Bessel. At that point weighed 1.3 gm of iodine and included it into a similar measuring Bessel. At that point scarcely any ml of refined water was included and whirled for a couple of moments to disintegrate iodine. At that point, iodine arrangement was moved into a 1lit volumetric flask. Make up the volume up to the 1lit mark with distilled water (Assis et al., 2000).
Table 1: pH evaluation of coconut water for about 3 months.

| Day  | Propylparaben 5mg Room temp | Propylparaben 10mg Room temp | Propylparaben 50mg Room temp | Benzoic acid 5mg Room temp | Benzoic acid 10mg Room temp | Control Room temp |
|------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|---------------------|
|      |                             |                             |                             |                             |                             |                     |
| Day 1| 5.38                        | 5.47                        | 5.45                        | 5.46                        | 5.12                        | 4.90                |
|      |                             |                             |                             |                             |                             | 5.27                |
| Day 10| 5.25                       | 5.44                        | 5.44                        | 5.42                        | 5.05                        | 5.08                |
|      |                             |                             |                             |                             |                             | 4.57                |
| Day 20| 4.91                       | 5.40                        | 5.36                        | 5.30                        | 5.30                        | 4.91                |
|      |                             |                             |                             |                             |                             | 4.86                |
| Day 30| 4.90                       | 5.39                        | 5.30                        | 5.28                        | 5.28                        | 4.80                |
|      |                             |                             |                             |                             |                             | 4.76                |
| Day 40| 4.88                       | 5.37                        | 5.29                        | 5.24                        | 5.24                        | 4.75                |
|      |                             |                             |                             |                             |                             | 4.52                |
| Day 50| 4.85                       | 5.31                        | 5.25                        | 5.20                        | 5.22                        | 4.54                |
|      |                             |                             |                             |                             |                             | 4.49                |
| Day 60| 4.60                       | 5.20                        | 5.17                        | 5.01                        | 5.10                        | 4.44                |
|      |                             |                             |                             |                             |                             | 4.20                |
| Day 90| 4.20                       | 4.90                        | 5.00                        | 4.81                        | 4.98                        | 4.10                |
|      |                             |                             |                             |                             |                             | 3.99                |
|      |                             |                             |                             |                             |                             | 3.78                |
|      |                             |                             |                             |                             |                             | 3.28                |
|      |                             |                             |                             |                             |                             | 2.50                |
|      |                             |                             |                             |                             |                             | 3.70                |

Table 2: Physical evaluation of coconut water in presence of preservatives.

| Evaluation Parameter | Room temperature | Refrigeration |
|----------------------|------------------|---------------|
|                      | Benzoic acid 5mg | Propylparaben 5mg |
|                      | 5mg              | 10mg          |
| Colour               | Colour less      | Colour less   |
| Odour                | Odour less       | Odour less    |
| pH                   | 5.12             | 4.85          |
| Clarity              | Clear            | Clear         |
| Turbidity            | 17.0             | 16.8          |

Starch pointer answer (0.5%)  
0.25 gm of soluble starch is considered and 50 ml of roasting water is added in 100 ml tapering flask. Then it was stirred and cooled before using.

Procedure for willpower of ascorbic acid by titration technique

Determination of ascorbic acid by using Titrimetry method was done by weighing 20 mg of sample and then added 150 ml of water to it. It was titrated against 0.05M iodine solution, using starch as an indicator. The endpoint was determined by a change in color to dark blue-black. The titration is recurrent for additional aliquots of example answer until concuring consequences are obtained (Jarimopas and Kuson, 2007).

Equivalent weight of ascorbic acid ≈ 0.008806gm

Procedure for determination of magnesium by titration method

Determination of magnesium by using Titrimetry method was done by weighing 45 mg of sample and make up to 100 ml by the overhead solution. 50 ml is pipetted out into a conical flask and to that solution, 5 ml ammonia was added. The sample was titrated against 0.05 M EDTA, using erichrome black-T as an indicator. The endpoint was determined by the change in color to deep blue. The results were reported as percent magnesium (%w/v) in Table 1.

Equivalent weight of ascorbic acid ≈ 0.008806

Procedure for determination of sodium and potassium by flame photometry

Potassium is the main positive ion originated inside of cells. The usual blood potassium equal is 3.5-5.0
Table 3: Determination of % magnesium and ascorbic acid by titrimetry.

| Samples with preservatives | Magnesium | Ascorbic acid |
|---------------------------|-----------|---------------|
|                           | Mg       | %     | Mg    | %     |
| Fresh coconut water sample| 43mg     | 100   | 26mg  | 100   |
| Sample with Propylparaben(10mg) | 42mg     | 97.6  | 24mg  | 92.3  |
| Sample with Propylparaben (50mg) | 40mg     | 93    | 20mg  | 76.9  |
| Sample with benzoic acid (5mg) | 26mg     | 60    | 15mg  | 57.6  |

Table 4: Estimation of % sodium and potassium ions present in fresh.

| Samples with preservatives | Sodium | Potassium |
|---------------------------|--------|----------|
|                           | PPM    | %        | PPM   | %        |
| Fresh coconut water reference | 90     | 100      | 100   | 100      |
| Sample with Propylparaben (10mg) | 81     | 90       | 92    | 92       |
| Sample with Propylparaben (50mg) | 76     | 85       | 86    | 86       |
| Sample with benzoic acid (5mg) | 65     | 73       | 74    | 74       |

Table 5: Determination of sugars by using HPTLC.

| Sample with preservative | AU (Absorbance Unit) | Percentage |
|-------------------------|----------------------|------------|
| Standard sugar solution | 250                  | 100%       |
| Fresh coconut water     | 210                  | 84%        |
| Sample with Propylparaben (10mg) | 215     | 86%        |

The cationic answer of sodium and potassium was sprayed into the flame, this causes the solvent to evaporate and ions were changed into an atomic state. The small fractions of atoms were excited in the heat of flame in Table 2 (temp about 1800°C)(Na: 589nm; K: 766nm). The attentiveness of specific atoms in flame decides the strength of the released light. Flame photometer instrument is used to measure the concentration of Na and K (Jarimopas and Ruttanadat, 2007) in Figure 2.

The flame photometer was made to warm for 5-10 min. Then Distilled water was feed into the instrument. Then the reading was adjusted to 0 using the knobs. The maximum focused standard answer was aspirated and figures were adjusted to about 90 (on the upper most scale) using the big knobs. Then purified water was aspirated and the gadget read as 0. The standard solution number 1, 2, 3, test solution, and then values 4, 5, 6 were pronounced and the results were recorded in Table 3.

Stock solutions of Na + and K + con. = 1mg/ml

Preparation of CW–II solution
10 mg of propylparaben was weighed and transferred into a 10 ml volumetric flask and fresh cocos nucifera water was added and made up to the mark and shacked well (Wazir, 1997; Raju and Zahida, 2007).

Grounding of typical coconut water for HPTLC
300 mg of sample is considered and moved into a 10 ml volumetric flask and the capacity is completed up to the scratch by methanol and sonicated to dissolve in methanol. The solution was filtered into auto-injector violin Table 5.

Groundwork of typical sugar solution
10 mg of sugar is considered and dissolved in 10 ml of distilled water.

Chromatogram layers
TLC and HPTLC plates silica gel 60F 254 (20x10cm).

Sample application
Manually with disposable micro pipettes of 15 micro liter volume.

Spraying reagent
10% of H₂SO₄ spray reagent.

HPTLC Conditions
Mobile phase -Acetonitrile: pentanol : water (4:1:1)
Stationary phase - Silica gel 60F_{254} (20X10 cm)
Wavelength - 500nm
Diluent - Methanol Application volume - 15 \mu L
Spraying agent - 10%H_{2}SO_{4}

**CONCLUSIONS**

In the present study, the stability evaluation and indication of different preservatives in different concentrations in cocos nucifera water were done and compared between the two preservatives, i.e., Benzoic acid and propylparaben. Among these two propylparaben have shown better results. The pH, Metal ions like sodium, potassium, magnesium, ascorbic acid and sugars present in coconut water were determined by using pH meter, Titrimetry, HPTLC, Flame photometry are with in limits. Finally, it is concluded that the following observations can be helpful for commercialization of coconut water in the presence of propylparaben at 10 mg concentration. Thus we are planning for commercialization of coconut water using propylparaben preservative in tetra packs.

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**Conflict of Interest**
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