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Development of Shelf Stable Ready to Serve Green Coconut Water with Chemical Preservatives at Different Storage Condition

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Abstract:*The study was conducted at twelve different samples of pasteurized coconut water termed as coconut water with preservative SB 100 mg (CW-1 and CW-8), with preservative SB 20 mg (CW-2 and CW-9) with preservative SB 50 mg (CW-3 and CW-10), with preservative CMC 20 mg (CW-4 and CW-11) and with preservative KMS 20 mg (CW-5 and CW-12) and non-pasteurized without preservative coconut water was termed as CW-7 were made and stored for 21 days at room and freezing temperature. Coconut water was analyzed for physico-chemical, microbiological and sensory qualities. Result found that the moisture, ash, TSS, pH , acidity, Carbohydrate of fresh coconut water were 95.25%, 0.6%, 4% , 4.95, 0.08% and 3.45% respectively. After 21 days the TSS of all samples stored at room temperature were increased to 5.8 percent and the TSS of refrizerated samples were increased to 5.3 for most of the samples. On the other hand the pH of sample CW-1, CW-2, CW-3 ,CW-4, CW-5, CW-6 and CW-7 were reduced to 3, 3.25, 3.22,3, 3.15, 3.18 and 3.35 respectively at room temperature and for samples CW-8, CW-9, CW-10 , CW-11and CW-12 the pH were reduced to 4.2, 3.9, 4, 3.8 and 3.9 respectively at freezing temperature . After 6 days at room temperature the maximum bacterial load was found in CW-7(14.5×10^3) In case of fungal load the maximum fungal load was found in CW-6(2.8×10^3) and maximum E.coli load was found in CW-7(0.4×10^3). As the microbial load was high and also the sensory qualities of water were very poor, the preserved coconut water stored at room temperature was unacceptable for consumption after 6 days. After 18 days in case of total viable count the maximum bacterial load was found in CW-10(9.7×10^3) and in case of total fungal count the maximum fungal load was found in CW-9, CW-10 and CW-11(0.9×10^3). Also in case of total coliform count the maximum was in CW-9, CW-12 (0.2×10^3). After 18 days the microbial load was within limit. It ensured that preserved juice stored under freezing temperature was acceptable for consumption.*

Key Words:*Coconut Water, Preservatives, Storage Condition, microbial load, Ready to serve*

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I. Introduction

Coconut (*Cocosnucifera*) popularly known as "Tree of Life" is one of the most extensively grown palmtrees worldwide. Coconut wateris the liquid endosperm that fills the central cavity enclosed by a solid endosperm protected by the hard cell and husk. Coconut water is fat free and rich in minerals like potassium, sodium and magnesium but low in calories. It contains significant amount of vitamin C and sugars and rich in natural polyphenol.Sugars like sucrose, sorbitol, glucose and fructose are the main fraction of soluble solids in coconut water[1]. Coconut water is a good sports drink as it is a high-electrolyte beverage with a low glycemic index (54 ± 4). It is also called as isotonic beverage due to the presence of balanced electrolytes like Potassium and Sodium[2]. Traditionally, coconut water prescribed in Aurvedic medicine during urination, gastritis, dysuria, indigestion, burning pain in eyes or even expelling of retained placenta[3].The tender coconuts areoften consumed immediately after harvestor sold on local or international market. The fresh coconut water has a very short shelf lifewhose transport can beexpensivethus limiting exports. Coconut water is sterilein the inner cavity ofthe nut but as soon as the nut is opened, its biochemicalcomposition and physical appearancechange. When it comes in contacts with oxygen, their peroxides (POD) and polyphenoloxidase (PPO) cause undesirable changes that affect the color and flavor[4].The use of adequate processes likethermal and non-thermal treatments,sometimes combined with additives or preservative for conserving coconut water make possible to extend its shelf-life and, consequently, to optimize the fruit use; it can also contribute to formalize its commercialization and reduce the intermediate actors that make the product more expensive[5]. Some studies have been made to evaluate the effect of pasteurization conditions on the inactivation of oxidative enzymes, peroxides and polyphenoloxidase in coconut water as it has already been verified the activity of thermal resistant enzymes in such product[4]. Thus

this study was aimed to expand shelf life of tender coconut water by using different preservative in a hygienic way and preserved at Normal and freezing temperature.

II. Materials And Methods

Sample collection

Fresh green coconuts were collected from Santosh Bazar Tangail region and were selected for experiment. Chemical and solvents used in the study were analytical/reagent grade and water was glass distilled unless specified otherwise. Bottle jar were used for packaging and storage of samples. Other ingredients were used from laboratory stocks.

Processing of green coconut water

Fresh coconut water was collected from freshly harvested coconut and filtered. The filtered water was prepared by pasteurization at predetermined temperature for a definite period of time and then cooled. The potassium metabisulfite ($K_2S_2O_5$), Sodium Benzoate and Carboxymethyle Cellulose was used as preservative in different concentrations. For room temperature the sample were identified as CW-1 to CW-7 and for freezing temperature the sample were CW-8 to CW-12.

Bottling of green coconut water

The processed water was filled into clean glass bottle keeping about 6 mm head space (0.25 inch). The bottles were exhausted in boiling water for 20 minutes and immediately closed with pp caps and/or crown caps. The bottles were then sterilized at 121°C for 15 minute. The heat processed bottles were immediately cooled under running water to 40°C. The bottles were then labeled and stored at ambient temperature and refrigerated temperature (4 to 10°C).

Storage studies

Processed green coconut water were stored at room temperature and refrigerated temperature (4 to 10°C) and shelf-life of the water was assessed by objective and subjective tests at different time intervals. The acidity, color, flavor and microbial load were observed initially for 21 days at an interval of three days up to twenty one days.

Proximate analysis of green coconut water

The pasteurized and strained green coconut water was analyzed for moisturebyoven drying method, ash content was done by incineration method, vitamin-C was determined by the method of Ranganna[6], β carotene content was determined by following Srivastava and Kumar[7] method, pH by using pH meter, titrable acidity, carbohydrate (Anthrone method), total soluble solid was determined by following AOAC[8] method.

Microbial Analysis

Microbiological tests;Total Viable Count (TVC), Total Coliform Count (TCC) and Total Fungal Count (TFC) were conducted for coconut water. Microbial test were performed in third and sixth days at room temperature whereas in freezing condition it was analysed up-to 21 days with three days interval.

Preparation of Media

In this study, Nutrient Agar from OXOID, CM0003 Hampshire, and England was employed. Ingredients of recommended quantities were weighted by electric balance and were dissolved in prescribed amount of distilled water. Prepare 500 ml nutrient agar media in a conical flask then the mixture was boiled to mixture the ingredients thoroughly. All glass wares like plate, pipette were sterilized in a hot oven (Model No.980435, made in Germany) at 175 ^{0}C for 2 hours. All kinds of media, solutions and glass bottles were sterilized at 15 P.S.I (Pounds per square inch) for 20 minutes at 121 ^{0}C in an autoclave (Model No. Mc-40 w. Made in Tokyo, Japan).

Preparation of sample

1 ml of the coconut water was diluted with 9 ml of distilled water and mixed well (10^{-1} dilution). Serial dilutions were prepared and spread plate technique was used on solid media. Serial dilutions of samples were made up to 10^{-3} with sterile distilled water of each dilution was evenly spread on the nutrient agar medium and incubated. Plates were screened for the presence of discrete colonies after incubation period and the actual numbers of bacteria were estimated as colony forming unit per ml (cfu/ml).

Incubation and Colony Count

After solidification of agar, the plates were inverted and placed in an incubator operated at 37 ^{0}C for 24-48 hours. Colonies were counted with the aid of a Garber colony counter. The numbers of colonies were multiplied by the dilution and the total viable count per ml of sample was recorded. In this study, two petri plates were used for each sample to obtained duplicate data.

Data analysis

All analyses were performed in triplicate. The data was evaluated by analysis of variance (ANOVA) and Duncun's Multiple Range Test (DMRT) procedures of the statistical analysis system using SPSS (Statistical Package for the Social Science) software package version 16.0 (SPSS Inc., Chicago, IL, USA).

III. Results And Discussion

Compositional analysis of fresh coconut water

Proximate composition of fresh cocunut water are showed in Table 1.The amount of Moisture, Ash, P^H , TSS, Acidity, Vitamin-C, and Carbohydrate of fresh coconut water were 95.5%, 0.6%, 4.95, 4, 0.08%, 2 mg per 100gm and 3.45% respectively. The study found after 3 days the TSS of CW-1, CW-2, CW-3, CW-4, CW-5, CW-6, CW-7 were 4, 4.2, 4.2, 4.2, 4.2, 4.2 and 4.3 percent respectively at room temperature and 4, 4, 4, 4 and 4 percent respectively at freezing temperrature. And finally after 21 days the TSS of CW-1, CW-2, CW-3, CW-4,CW-5, CW-6, CW-7 were increased to 5.8 percent for all sample at room temperature and 5.4.The reult found after 3 days the P^H of CW-1, CW-2, CW-3, CW-4, CW-5, CW-6, CW-7 were 4.20, 4.50, 4.20,4.50, 4.60, 4.10 and 4.85 respectively at room temperature and 4.90, 4.90, 4.90, 4.70 and 4.80 . At 6 days the P^H of all samples were reduced. Then from 9 to 21 days the P^H were reduced both at room temperature and freezing temperature.

Table 1: Compositional analysis of fresh coconut water

Component	Amount
Moisture	95.5%
Ash	0.6%
P^H	4.95
Total soluble solids (TSS)	4
Acidity	0.08%
Vitamin-C	2 mg per 100 gm
Carbohydrate	3.45%
β-Carotene (Identification)	Not found

Microbial Analysis of coconut water in several storage conditions

Total viable count (TVC) of coconut water in several storage conditions

Table 2 represents that total viable count of preserved coconut water at room temperature at 3 days found in CW-1, CW-2,CW-3,CW-4,CW-5,CW-6 and CW-7 were 4.5×10^3, 4.8×10^3,5.2×10^3, 6.2×10^3, 7.3×10^3, 10.4×10^3,13.5×10^3 and at freezing temperature 2×10^3, 2.2×10^3, 2.7×10^3, 3×10^3 and 3.4×10^3 respectively. After 6 days the maximum bacterial load was found in CW-7(14.5×10^3) and minimum was in CW-1(10.5×10^3) at room temperature and and table 3 shows that at freezing temperature after 6 days the maximum bacterial load was found in CW-10(5.2×10^3) and minimum was in CW-11(3.4×10^3). At freezing temperature after 9 days the maximum bacterial load was found in CW-3(5.7×10^3) and minimum bacterial load was found in CW-11(4×10^3).After 12 days the maximum bacterial load was found in CW-10(7.2×10^3) and minimum was in CW-11(5×10^3).After 15 days the maximum bacterial load was found in CW-12(8.8×10^3) and minimum bacterial load was found in CW-8(7.2×10^3).After 18 days the maximum bacterial load was found in CW-10(9.7×10^3) and minimum bacterial load was found in CW-8 and in CW-11(9.2×10^3).After 21 days the maximum bacterial load was found in CW-10(11.3×10^3) and minimum bacterial load was found in CW-11(10.3×10^3).

Total fungal count of coconut water in several storage conditions

Total Fungal count of preserved coconut water at room temperature at 3 days found in CW-1, CW-2, CW-3, CW-4, CW-5, CW-6 and CW-7 total fungal recorded in 3rd diluted samples were 0.3×10^3, 0.5×10^3, 0.7×10^3,0.4×10^3, 0.6×10^3, 1.2×10^3 and 1.4×10^3 respectively. After 6 days the maximum fungal load was found in CW-6(2.8×10^3) and minimum was in CW-1 (1.3×10^3).Total fungal count of preserved coconut water at freezing temperature at 3 days found in CW-8, CW-9, CW-10, CW-11 and CW-12 total fungal recorded in 3rd diluted samples were 0.1×10^3, 0.2×10^3, 0.2×10^3, 0.1×10^3 and 0.2×10^3 respectively. After 6 days the maximum fungal load was found in CW-10(0.6×10^3) and minimum was in CW-9(0.1×10^3). After 9 days the maximum fungal load was found in CW-10(0.7×10^3) and minimum bacterial load was found in CW-8(0.4×10^3).After 12 days the maximum fungal load was found in CW-10(0.8×10^3) and minimum was in CW-8(0.5×10^3). After 15 days the maximum bacterial load was found in CW-12(8.8×10^3) and minimum bacterial load was found in CW-8(7.2×10^3). After 18 days the maximum fungal load was found in CW-9, CW-10 and CW-11(0.9×10^3) and minimum fungal load was found in CW-8 and in CW-8(0.8×10^3). After 21 days the maximum fungal load was found in CW-12(1.5×10^3) and minimum fungal load was found in CW-11(1.1×10^3).

Total Coliform count of coconut water in several storage conditions

Total coliform count of preserved coconut water at room temperature after 6 days found nil in CW-1,CW-2,CW-3,CW-4,CW-5,CW-6 and CW-7 sample.

At freezing temperature after 3 days found in CW-8, CW-9, CW-10, CW-11 and CW-12 total coliform recorded in 3rd diluted samples were 0 respectively. After 6 days the TCC found 0 for every sample, After 9 days the E.coli load was found in CW-9 and CW-12 (0.1×10^3) .The E.coli count was increased day by day. And finally after 21 days the maximum load was found in CW-9 and CW-12 (0.0098×10^4) and the minimum count was in CW-11(0.1×10^3).

Table 2: TVC, TFC and TCC of coconut water at Room Temperature

Days		Samples						
		CW-1	**CW-2**	**CW-3**	**CW-4**	**CW-5**	**CW-6**	**CW-7**
3	TVC	4.5×10^3	4.8×10^3	5.2×10^3	$62.\times 10^3$	7.3×10^3	10.4×10^3	13.5×10^3
	TFC	0.3×10^3	0.5×10^3	0.7×10^3	0.4×10^3	0.6×10^3	1.2×10^3	1.4×10^3
	TCC	0	0	0	0	0	0	0
6	TVC	10.5×10^3	11.6×10^3	12.7×10^3	13×10^3	13.5×10^3	14×10^3	14.5×10^3
	TFC	1.3×10^3	1.6×10^3	2×10^3	2.5×10^3	2.2×10^3	2.6×10^3	2.8×10^3
	TCC	0	0	0.1×10^3	0	0	0	0

Table 3: Maximum and Minimum TVC, TFC, TCC of coconut water at Freezing Temperature

		Samples				
Days	**Microbial count**	**CW-8**	**CW-9**	**CW-10**	**CW-11**	**CW-12**
3	TVC	2×10^3	2.2×10^3	2.7×10^3	3×10^3	3.4×10^3
	TFC	0.1×10^3	0.2×10^3	0.2×10^3	0.1×10^3	0.2×10^3
	TCC	0	0	0	0	0
6	TVC	4×10^3	4.8×10^3	5.2×10^3	3.4×10^3	3.6×10^3
	TFC	0.3×10^3	0.1×10^3	0.6×10^3	0.3×10^3	0.4×10^3
	TCC	0	0	0	0	0
9	TVC	4.5×10^3	5×10^3	5.7×10^3	4×10^3	4.8×10^3
	TFC	0.4×10^3	0.5×10^3	0.7×10^3	0.6×10^3	0.5×10^3
	TCC	0	0.1×10^3	0	0	0.1×10^3
12	TVC	6.3×10^3	6.9×10^3	7.2×10^3	5×10^3	5.8×10^3
	TFC	0.5×10^3	0.6×10^3	0.8×10^3	0.7×10^3	0.6×10^3
	TCC	0.1×10^3	0.1×10^3	0	0	0.1×10^3
15	TVC	7.2×10^3	7.2×10^3	8×10^3	7.5×10^3	8.8×10^3
	TFC	0.7×10^3	0.8×10^3	0.8×10^3	0.8×10^3	0.3×10^3
	TCC	0.1×10^3	0.2×10^3	0	0	0.1×10^3
18	TVC	9.2×10^3	9.5×10^3	9.7×10^3	9.2×10^3	9.5×10^3
	TFC	0.8×10^3	0.9×10^3	0.9×10^3	0.9×10^3	0.8×10^3
	TCC	0.1×10^3	0.2×10^3	0.1×10^3	0.1×10^3	0.2×10^3
21	TVC	10.5×10^3	10.7×10^3	11.3×10^3	10.3×10^3	10.5×10^3
	TFC	1.2×10^3	1.3×10^3	1.4×10^3	1.1×10^3	1.5×10^3
	TCC	0.2×10^3	0.3×10^3	0.2×10^3	0.1×10^3	0.3×10^3

Comparison of TVC, TFC and TCC of preserved coconut water between room and freezing temperature:

Table 2 mentioned that CW-1, CW-2,CW-3,CW-4,CW-5,CW-6 and CW-7 stored in room temperature were crossed the maximum bacterial permitted count and totally spoiled. But on the other hand, these samples stored in freezing temperature were in the maximum bacterial permitted count and these can be consumed. Table 2 mentioned that CW-1, CW-2, CW-3, CW-4, CW-5, CW-6 and CW-7 stored in room temperature were crossed the maximum fungal permitted count and totally spoiled. (The maximum fungal count permitted is 1000).So that these coconut water cannot be consumed. But on the other hand, these samples stored in freezing temperature showed in table-3 were in the maximum fungal permitted count and these can be consumed. Table 2 mentioned that CW-1,CW-2,CW-3,CW-4,CW-5,CW-6 and CW-7 stored in room temperature were crossed the maximum coliform permitted count and totally spoiled. (The maximum coliform count permitted is 100). But on the other hand, these samples stored in freezing temperature were in the maximum coliform permitted count and these can be consumed.

Overall Sensory Evaluation and acceptability of coconut water

Based on Hedonic Scale the sensory evaluation and acceptance of preserved coconut water (in room temperature) of CW-1,CW-3,and CW-4,were disliked slightly.CW-2 and CW-5 werw neither liked nor disliked by panelist But CW-6 and CW-7 were unacceptable by panelists.And at freezing temperature overall taste of CW-8, CW-9, CW-10,CW-11 and CW-12,were liked slightly by panelists.

IV. Conclusion

In this study results indicated that at room temperature changes occurred in all physico-chemical parameters such as moisture content, ash content, TSS and pH was significant. In case of sensory evaluationa

significant change in appearance,color, flavor, texture and overall taste was noticed in preserved coconut water than fresh coconut water. It was noticed that the overall taste of preserved coconut water at freezing temperature was better than coconut water preserved in room temperature.The microbial parameter of the preserved coconut waterwith preservative was of superior quality up-to 18 days at freezing temperature and as there be no perceptible microbial contamination or growth during the preserved period. So the coconutwaterwith preservative and also withoutpreservative stored in freezing temperature can be considered safe for consumption up-to 18 days. Finally it can be concluded that, the shelf life of preserved coconut water at room temperature was 3 days and in freezing temperature was 18 days. So the coconut water stored in freezing condition can be a refreshing, sweet and wonderful substitute for consumption.

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