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Effect of Food Grade Preservatives on the Physicochemical and Microbiological Properties of Coconut Toddy during Fermentation

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Abstract

Toddy is a sugary sap obtained from young inflorescence of a coconut tree. It turns alcoholic and sour due to the uncontrolled rapid fermentation caused by the natural microbiota present in it. This ultimately leads to poor quality toddy of lesser shelf life. To control the rapid fermentation, "E-class" preservatives viz., sodium metabisulfite, sodium benzoate and calcium hydroxide were added at various levels to the toddy collection pots fastened to the spadix of the coconut trees prior to its collection. The effects of the preservatives on major physical, chemical and microbiological changes during natural fermentation were studied upon storage for 48 h.

The reductions in the pH of the samples to which sodium metabisulfite (9%) or calcium hydroxide (4%) were added appeared to be lesser than the sodium benzoate (13%) and control samples (14%). The titrable acidity (TA) of the sodium metabisulfite and sodium benzoate treated samples increased by 10, 45% respectively while the TA of the calcium hydroxide reduced by 37%. There was a maximum reduction of ºBrix in the control samples (54%) followed by sodium metabisulfite (28%), calcium hydroxide (19%) and sodium benzoate (13%) treated samples. There was an increase in ethanol content up to 9% in the sample to which sodium metabisulfite had been added. After 48 h of storage, spoilage bacteria such *Acetobacter*, *Enterobacter*, *Bacillus* and *Staphylococcus* species were developed.

Keywords: Coconut toddy; Fermentation; Food grade preservatives; Microbial activity; Physicochemical properties

Introduction

Toddy is a sugary sap obtained from the tender infloresence of various palmae family trees,which develops not more than 5% alcohol by volume. The sap obtained undergoes natural fermentation and becomes alcoholic and acidic due to microbial actions. This sap is largely consumed in Asian and African countries. It is referred as "tuba" in Philipines, "tuak" in Indonesia, "mibo" in cameroon and "Emu" in Nigeria.The methods of obtaining toddy from various palms have been described in detail by Gibbs [1] and Browning and Symons [2]. The young infloresecence is tightly bound with twigs and bruised with wooden stick, every morning and evening for 10 to 15 days.When the inflorescence begins to ooze sap, the tip is sliced 3 cm and the exudate is allowed to trickle into an earthern pot. The natural microflora as well as the microflora in the pot serve as source of organisms for fermentation of the sugary syrup. The major drawback during this process is uncontrolled fermentation while collecting the sap which leads to over fermentation of the sugars in the sap. Eventually the toddy becomes excessively acidic, sour with unacceptable off odour.

Palm wine has many nutritional properties and medical uses which have been reported to have enhanced the demand for this natural product [3-6]. Over fermentation of palm wine may cause diseases or infections such as diarrhea, hernia and headache [7] and hence it is important to classify and control the development of fermentation and the pathogenic microorganisms.

Various researchers have attempted to control the fermentation by the application of chemicals or antiferments including toluene, cupric sulphate, sodium metabisulfite, milk of lime, Benzoic acid, and Child have reported that chemicals can control the fermentative activity of microbes present in toddy [8-13].

Sodium metabisulfite is used successfully in wine industries to suppress the wild yeast to produce acceptable wines [14]. Sodium salt of benzoic acid may occur naturally in some foods and added as preservatives to foods as it works very well at killing bacteria, yeast and fungi. Calcium hydroxide is used in the making of pickles for providing firmness to the produce like cucumber [15]. While there are such evidences to infer it may be possible to maintain the quality of toddy by adding preservatives like sodium metabisulfite, sodium benzoate and calcium hydroxide, there were no literatures which have investigated the same. The Concentration as a preservative is limited by the FDA in the U.S. is 0.1% by weight. Sodium benzoate is also allowed as an animal food additive at up to 0.1%, according to AFCO [16].

Hence, this study was carried out in order to control or slow down the spontaneous fermentative changes in the freshly collected toddy using different E-Class preservatives viz., sodium metabisulfite, sodium benzoate and a saturated solution of calcium hydroxide and to study their effect on the physicochemical properties and microbiological properties during the natural fermentation process. Calcium hydroxide is not falling under the category of class-E preservative but play some role in traditional uses.

Materials and Methods

Collection of sap

Toddy was collected by tapping the unopened inflorescence of

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the coconut tree (*Cocos nucifera*) in the plantation of Indian Institute of Crop Processing Technology, Thanjavur (Tamil Nadu, India). The collected samples were filtered through filter paper to remove the foreign matters present in the sample and used for studies immediately.

In order to control or slow down the spontaneous fermentation in toddy class – E' food grade preservatives, sodium metabisulfite - E223 (150 ppm), sodium benzoate – E211 (150 ppm) and calcium hydroxide (450 ppm) were placed into seperate toddy collection pots prior to collection. (The concentrations of the preservatives were fixed by a preliminary study conducted for 10 days. Initially average volume of the sap collected in the pot ranged from 820 to 1010 ml everyday. The additives were freshly added into the collection pot daily after collecting the sap. Addition of 140 mg of preservative into each collection pots approximately gave an average concentration of about 150 ppm for sodium metabisufite and sodium benzoate. Traditionally 450 ppm of calcium hydroxide is used for the collection and processing of the non-alcoholic beverage called "Nira". Control sample was collected without adding any preservatives in a the earthern pots.). The samples were analysed after collection and after storage at ambient temperature (28 ± 2°C) for three days.

S.no.	Physico chemical properties	Sample type	Time (hours)		
			0	24	48
1	pH	A*	4.62**^{a}	4.5^{a***}	4.2^{a}
		B	4.56^{a}	4.02^{a}	3.96^{a}
		C	9.5^{a}	9.22^{a}	9.07^{a}
		D	4.2^{a}	3.8^{b}	3.6^{b}
2	Titrable acidity (g/100 ml)	A	1.7^{a}	1.85^{a}	1.87^{a}
		B	1.93^{a}	2.75^{b}	2.8^{b}
		C	0.27^{a}	0.17^{b}	0.17^{b}
		D	0.37^{a}	2.47^{b}	2.55^{b}
3	Brix (sucrose)%	A	14.17^{a}	12.07^{b,c}	10.15^{c}
		B	11.2^{a}	10.7^{a}	9.67^{a}
		C	17.82^{a}	17.52^{a}	14.42^{b}
		D	16.12^{a}	9.65^{b}	7.38^{c}
4	Ethanol%	A	9.4^{a}	9^{b}	8.96^{b}
		B	3.2^{a}	3^{b}	2.9^{b}
		C	0.5^{a}	0^{b}	0^{b}
		D	4.2^{a}	5^{b}	4.5^{c}

*A- sample treated with 150 ppm sodium metabisulfite; B- 150 ppm Sodium benzoate; C – 450 ppm Calcium hydroxide; D- Control

**Values are expressed as mean of three repetitions

***superscripts with same letters in a row are not significantly different at 95% confidence interval.

Table 1: Change in the physicochemical properties of the toddy.

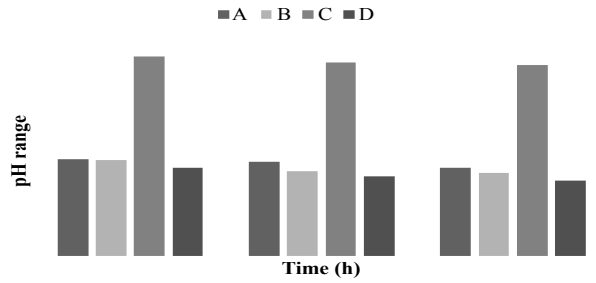

Figure 1: pH values of the toddy samples collected with different preservatives after storage. (A- sample treated with 150 ppm sodium metabisulfite; B- 150 ppm Sodium benzoate; C – 450 ppm Calcium hydroxide;D- Control).

Physicochemical analysis of toddy

The physicochemical properties analysed were pH, total acidity, Total Brix and Ethanol.

pH: Sample pH was measured using pH meter (pH Tutor, Eutech Instruments Pvt. Ltd., Singapore) at ambient temperature [17].

Total acidity: Total acidity of each sample was estimated by titrating against 0.1N sodium hydroxide using phenolphthalein as an indicator and expressed as a percentage of acetic acid [18].

Total Brix: Total brix values were determined using Otago refractometer (model HSR-500, Japan; 0-42 °Brix) [19].

Ethanol: Ethanol concentrations in the samples were estimated by potassium dichromate method [20].

Microbiological Analysis of Toddy

Total bacterial count and total yeast count

Total yeast population and total bacterial population in the coconut toddy collected with various preservatives were counted using a counting chamber. Serial dilutions of each sample were prepared using 0.1% sterile peptone water and inoculated on Nutrient agar (Hi-Media) for bacterial population and on Oxytetra glucose yeast extract agar (Hi-Media) supplemented with 100 mg Oxytetracycline (Hi-Media) for yeast population. Inoculated plates were incubated at 30 ± 2° C for 18-24 hours and 48-72 hours for the counting of total heterotrophic bacteria, and yeasts respectively. Discrete colonies in incubated plates were counted and expressed as colony forming units per ml (log cfu/ml) of toddy samples.

Isolation and identification of microorganisms

Yeasts were identified by the methods given by Lodder [21]. Bacteria were characterized and identified by descriptions given in Bergey's Manual of Determinative Bacteriology [22]. Duplicates were used for the isolation of microorganisms.

Statistical Analysis

All the experiments were carried out in triplicates and the data were represented as mean (Table1). MS Excel was used to plot the graphs. SPSS18.0 [23] was used to conduct analysis of variance (ANOVA) followed by Duncan's multiple range test to investigate the significant difference among treatments.

Results and Discussion

Changes in the physicochemical properties of toddy containing various preservatives during fermentation

The changes in the physicochemical properties of the dorry added with preservatives during fermentation is shown in Table 1.

pH: As can be seen from Table 1, the pH of the toddy samples treated with various preservatives viz., sodium metabisulfite, sodium benzoate and calcium hhydroxide were ranging from 3.96 to 9.5. Generally, a decresing trend was observed for all the samples (Figure 1) though the changes were not significant (Table 1).

It was noted that the pH of toddy was maintained at above 9.0 in all the periods in calcium hydroxide treated sample due to the alkaline nature of the calcium hydroxide itsef, whereas in sodium metabisulfite and sodium benzoate treated samples it was below 4.0 (Figure 1) but in the control sample the pH was reduced to 3.0 ± 0.02 after 48 h rs storage.

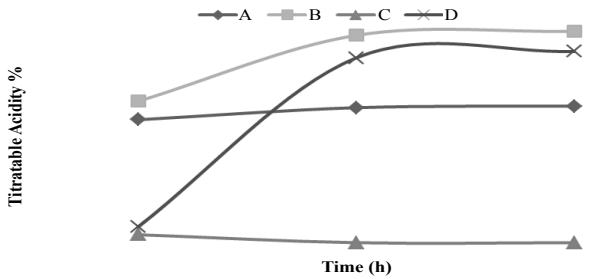

Figure 2: Evolution of titratable acidity value during storage of the coconut toddy. (A- sample treated with 150 ppm sodium metabisulfite; B- 150 ppm Sodium benzoate; C – 450 ppm Calcium hydroxide; D-Control).

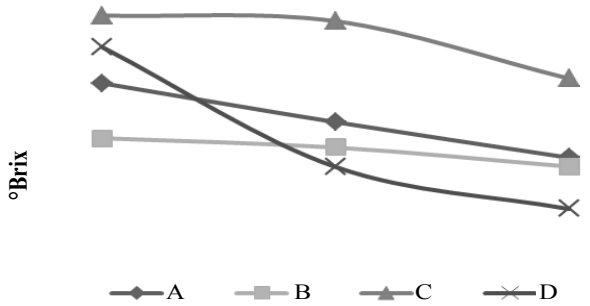

Figure 3: Evolution of Brix° value during storage of the coconut toddy. (A-sample treated with 150 ppm sodium metabisulfite; B- 150 ppm Sodium benzoate; C – 450 ppm Calcium hydroxide;D-Control).

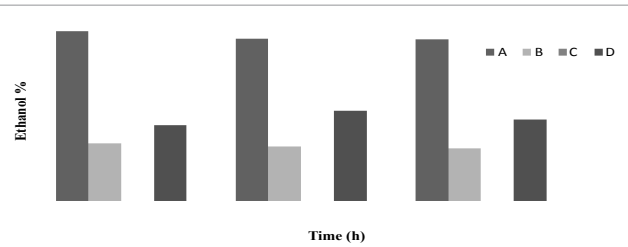

Figure 4: Ethanol concentration of toddy collected with various preservatives after storage. (A- sample treated with 150 ppm sodium metabisulfite; B- 150 ppm Sodium benzoate; C – 450 ppm Calcium hydroxide; D-Control).

This was in agreement with the study by Igyor et al. [24] recorded pH value between 3.36 and 4.86.

Titrable acidity: The titrable acidity slightly reduced from 0.27 ± 0.03 to 0.17 ± 0.02 on 48 hrs storage in calcium hydroxide treated sample. In the samples treated with the other two preservatives, the acidity was maintained at 1.85 ± 0.03 and 2.70 ± 0.04 respectively for sodium metabisulfite and sodium benzoate (Figure 2).

Brix: As the microbial proliferation was curtailed by the addition of calcium hydroxide, the brix percentage was maintained at 14.4 ± 2 the "Neera" (Unfermented form of toddy) samples. The other preservatives showed around 10.0 ± 0.85%. (Figure 3) after 48 hours storage at ambient temperature (28 ± 2°C). Which means the sap slowly turns into alcohol and vinegar due to the presence of natural yeast and bacteria.

Ethanol concentration: The rate of fermentation of the sugary sap was found to be lower in all the toddy samples collected from different pots treated with sodium metabisulfite, sodium benzoate and calcium hydroxide compared to control. However the effect of arresting the fermentation acivity was higher in calcium hydroxide treated sample. Toddy collected with pots containing sodium metabisulfite yielded higher alcohol content up to 9% upon collection. This agrees with the bactericidal activity of sodium metabisulfite which retards the growth of acetic acid bacteria and lactic acid bacteria without affecting the yeast growth in the pot which keep on fermenting the sugar and produces ethanol. Samrajeeva et al., [14] reported maximum ethanol yield concentration of 9.4% in the toddy samples treated with 188mg L^{-1} level of sodium metabisulfite. A maximum of 3.2% alcohol content was observed in the toddy collected from the pots containing sodium benzoate, since it retards the growh of predominant yeast but less inactivation of bacteria resulting in less alcohol production with more acidity. Yeast cells present in the pots, spadix and environments play a role in controlled fermentation and natural toddy will yield 5% of alcohol [25] by spontaneous natural fermentation. Whereas maximum retention of microbial activity was observed in the samples treated with calcium hydroxide and thus only 0.5% alcohol was observed (Figure 4). On storage of the samples at 28°C for 48 hours the reducing trend was observed due to the lower microbial activity.

Changes in the microbiological properties of toddy containing various preservatives during fermentation

The changes in the microbiological properties of the toddy added with preservatives during fermentation is shown in Table 2.

Total microbial population

Total cell count of the three different toddy samples were assessed (Figure 5) from initial sample to 48 hours during storage at ambient temperature (28 ± 2°C). The changes in microbial load in toddy tapped with each preservatives were compared using Total Plate Count method and Yeast Plate Count method. The toddy samples collected in pots containing sodium metabisulfite contained total bacterial load of 0.38 log cfu/ml on initial day which incresed after 24 hours to 1.593 log cfu/ml and reduced after 48 hours, whereas it shows high yeast population ranging between 3.020 to 3.482 log cfu/ml in second day which favours the sugar fermentation lead into increase in alcohol production. In a fermentation studies, Atputharajah et.al., [18], reported that yeast population of 10^4 cells/ ml was observed when adding sodium metabisulfite to the coconut palm sap in a storage studies conducted. Toddy samples collected in pots containing sodium benzoate has a bacterial count range from 1.550 to 1.742 log cfu/ml whereas yeast cells were present at only 0.528 to 0.801 logt/ml on storage. This leads to the formation of higher acidity in the product rather than the alcohol

S.no.	Microbiological properties	Sample type	Time (hours)		
			0	24	48
1	Total bacterial count	A*	0.386**[a]	1.593[b***]	1.482[c]
		B	1.742[a]	1.624[b]	1.55[c]
		C	0.495[a]	0.521[b]	0.508[c]
		D	6.281[a]	8.521[b]	8.241[c]
2	Total yeast count	A	3.452[a]	3.621[b]	3.02[c]
		B	0.528[a]	0.891[b]	0.801[c]
		C	0.256[a]	0.293[b]	0.203[c]
		D	7.852[a]	8.652[b]	8.621[b]

*A- sample treated with 150 ppm Sodium metabisulfite; B- 150 ppm Sodium benzoate; C – 450 ppm Calcium hydroxide; D- Control
**Values are expressed as mean of three repetitions
***superscripts with same letters in a row are not significantly different at 95% confidence interval.

Table 2: Change in the microbiological properties of the toddy.

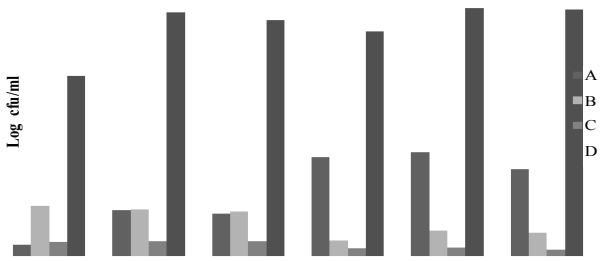

Figure 5: Changes in the microbial population of toddy treated with preservatives on storage:

(A) Sodium metabisulfite, (B) Sodium benzoate, (C) Calcium hydroxide, (D) Control.
(Counts are mean values from three replications ± standard deviations).

Isolated Bacteria/Yeasts	0 hrs				24 hr				48 hr			
	A	B	C	D	A	B	C	D	A	B	C	D
Acetobacter aceti	-	-	-	-	-	+	-	-	-	+	-	+
Enterobacter sp	-	-	-	+	-	+	-	+	-	+	-	+
Micrococcus sp	-	+	-	+	-	+	-	+	-	+	-	+
Leuconostoc sp	-	+	-	+	-	-	+	+	-	-	+	+
Lactobacillus sp	-	+	-	+	-	+	+	+	-	+	-	+
Bacillus sp	-	+	-	+	-	-	-	+	-	-	-	+
Staphylococcus sp	-	-	-	+	-	-	-	+	+	+	-	+
Saccharomyces cerevesiae	+	-	-	+	+	+	-	+	+	+	-	+
Saccharomyces bailii	+	-	-	+	+	+	-	+	+	-	-	+
Saccharomyces chevavieri	+	-	-	+	+	-	-	+	+	-	-	+
Candida tropicalis	-	-	-	+	+	-	-	+	+	-	-	+

A-Samples treated with sodium metabisulfite; B- sodium benzoate; C- calcium hyroxide; D – control ; (+): Presence; (-): Absence.

Table 3: Microorganisms isolated from the toddy collected with food grade preservatives.

production observed in the case of metabisulfite. Toddy collected in pots containing calcium hydroxide has very low numbers of bacteria as well as yeast cells ranging from 0.495 to 0.521 and 0.203 to 0.256 log cfu/ml respectively. Hence in this treatment, both acidity and alcohol content was less and hence brix value remained was comparatively higher than the other two treatments. Control toddy sample shows higher number of bacteria (8.521 log cfu/ml) as well as yeast cells (8.652 log cfu/ml) which makes toddy more sour and acidic.

Microbial profile

Toddy collected with various preservatives shows the variance in physicochemical properties, ethanol percentage and total plate count. This agree with the difference in the mode of action of preservatives on the microorganisms, mainly on the bacteria and yeast cells. But a significant difference in the physicochemical properties was observed due to the alterations in the microbial profile and hence the bacteria and yeast cells make-up of each sample was identified based on morphological charecters and sugar fermentation studies.

The distribution of microbes present in the toddy samples in relation with the preservatives used and their effect during storage hours is presented in Table 3. A total of 11 isolates from 3 stages during storage of chemically treated toddy samples were identified. This includes 7 bacteria belonging to *Bacillus*, *Enterobacter*, *Micrococcus*, *Leuconostac*, *Lactobacillus*, *Bacillus* and *Staphylococcus* spp. and 3 yeast species of *Saccharomyces* and *Candida tropicalis*. Most of the bacterial species observed were from the control and sodium benzoate treated samples and none of the bacterial species except *Staphylococcus* was observed in the sodium metabisulfite treated sample. This causes the sugary fermentation and the production of alcohol in the sample. But the groups were slightly altered during the storage. The trend was reversed in the sodium benzoate treated sample as there were no yeast cells other than C*andida spp.* but all the other bacteria except *Staphylococcus* were observed. This causes redeuced rate of fermentation and the toddy became more acidic and sour.Bacteria such as *Leuconostoc* and *Lactobacillus spp*, yeast belonging to *Candida sp* and *Saccharomyces cerevesiae* were observed from the calcium hydroxide treated sample stored for 24 hours. In contrast all the bacteria and yeast cells stored coconut toddy.

Conclusion

The results indicate that a diverse group of micorbial flora including pathogenic bacteria such as *Enterobacter*, *Bacillus* and *Staphylococcus* and *Candida tropicalis* were emerging during fermentation of the palm toddy on storage. Further the palm sap collected with the pots containing preservatives revealed that it could be possible to control the development of such pathogenic bacteria during storage at ambient temperature. This initial research on controlling the fermentation rate with food grade preservatives will serve as a guide in the production of safe natural beverage and to select the process for future application, the aim of ongoing research in our laboratory. For complete charecterization of the technological properties, further studies were required on the preservatives properties with organoleptic evaluation.

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