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Hygienic collection and preservation of neera from palmyrah palm

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ABSTRACT

Palmyrah palm (Borassus flabellifer L) produces inflorescence which secrets a sap upon tapping is called neera. In India, palmyrah palm is grown in many states like Andhra Pradesh, Gujarat, Tamil Nadu, Odisha, West Bengal, Bihar, Karnataka, and Maharashtra on large scale. Many challenges are faced by the tappers while tapping neera. The present method of tapping is not scientific. It is highly unhygienic and cross contamination with insects, bacteria and yeast occurs during tapping of neera and fermentation starts quickly after tapping. The fermented product is called toddy which is highly injurious to health. Keeping the problems and challenges of neera tapping and preservation without severe heating, the research investigation entitled "Hygienic collection and preservation of neera from palmyrah palm" has been taken. Application of anti fermenting solution (AFS) of 1500 ppm SO_2 and 0.2% citric acid on the sliced portion followed by collection of neera directly into a polyethylene lined double walled tin container containing 300 g crushed ice and 0.5% lime powder was found effective. The neera was then preserved by thermosonication (35 kHz for 15 and 20 minutes + Pasteurization at 65 for 10 minutes). The preserved neera was successfully stored at 2-3 for 60 days with stable sensorial quality and without any fermentation.

Keywords: *Palmyrah palm, neera, fermentation, hygienic collection, pasteurization, thermosonication and preservation*

Introduction

Palmyrah palm (*Borassus flabellifer* L.) belongs to the family Palmae and grown in many countries of the world. The major growing countries are Srilanka, Pakistan, Bangladesh, Myanmar, Thailand, Malaysia, Indonesia, Nigeria, Congo, Sudan, Tanzania, India, China, Jawa, Sulawesi, Cambodia, Laos and Vietnam [1, 2]. In India, palmyrah palm is grown in many states like Andhra Pradesh, Gujarat, Tamil Nadu, Odisha, West Bengal, Bihar, Karnataka, and Maharashtra on large scale. Among Indian states, Tamil Nadu tops the rank as number one producing state with 60% of total production [3]. According to an estimate, India is a home of approximately 102 million palms and 50% of the total population is found in Tamil Nadu [4]. About 12 to 15 years old palmyrah palm produces inflorescence that produces a sweet sap upon tapping is called neera [2]. It is clear in appearance having pleasant aroma, sweet flavor and possess immense medicinal properties beneficial for human health like anti-phlegmatic agent [5], anti-inflammatory, antibacterial, analgesic and have anti-oxidant properties [6]. It Prevents certain types of cancers in human body [7]. The sugar present in neera has low glycemic index (GI-35) which make it safe to drink for diabetic persons [8]. Sugar prepared from neera is reported to have beneficial for curing liver disorder [9].

Many challenges are faced by the tappers while tapping neera. The present method of tapping is not scientific. It is highly unhygienic. Many cross contamination with insects, bacteria and yeast occurs during tapping of neera and fermentation starts quickly into alcohol (plate 1). A hygienic collection of neera is possible in a polyethylene lined low cost tin box (plate-2) yields fresh neera (plate-3). A front and side view of the same box is shown (plate-4 and 5). Descriptions of plates are mentioned in a*nnexure* I.

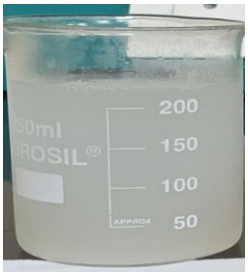

Plate 1 Unhygienic neera Plate 2 Hygienic collection of neera Plate 3 Fresh neera

Plate 4 Neera collection box (front view) Plate 5 Neera collection box (side view)

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Preservation of neera is the biggest challenge faced by palmyrah growers and tappers. It is because of rapid fermentation of neera at ambient temperature. The fermented neera is called toddy. The degree of fermentation of the neera depends on the practice of neera collection and the environmental condition prevailing in the region [10]. Since neera is rich source of sugars, vitamins, proteins and minerals [5], fermentation starts within one or two hours of extraction. A large number of yeast and bacterial strains was found responsible for fermentation of neera [11].The most common microbial flora found in fresh neera are *Bacillus, Lactobacilli, Micrococci, Enterobacter, Leuconostoc, Saccharomyces, Candida, and Pichia* [12]. According to Kurian and Peter, [13], Toddy is the term used to describe the process by which wild yeasts and bacteria ferment sugar sap of neera. The fermented neera (toddy) is a spoiled form of neera. The toddy is, in fact, harmful because it is alcoholic in nature with 5 per cent alcohol and above hence not recommended as a health drink [14]. The business of toddy is prohibited by law in many states of India. It is a total loss for the producers and neera entrepreneurs [15]. Hence collection and preservation of neera by arresting fermentation is challenging and a limiting factor for commercialization of fresh neera without fermentation. Further, any processing or value addition in palmyrah palm is possible only after preservation of neera. Pasteurization is mainly applied for preservation of neera. Mostly pasteurization temperature is kept at boiling temperature. At this temperature, thermal degradation in colour, flavour and nutritional loss is obvious. The most common changes upon heating sap is Millard reaction and Caramelisation [16]. Non-thermal processing like ultrasound coupled with low temperature pasteurization called thermosonication can be applied. It has higher efficiency for killing of microbes and deactivation of enzymes at relatively low temperature [17]. Kapturowska et al. [18] also reported that thermosonication is very effective against yeast. Preserved neera can be used in preparation of various value-added products, which may be a source of employment in the palmyra palm growing areas. Keeping the problems and challenges of neera tapping and preservation without severe heating, the research investigation entitled "Hygienic Collection and Preservation of Neera from Palmyra palm has been taken

Materials and Methods

Collection and preservation of neera

There were two experiments conducted. The experiment I (Hygienic collection of neera) was carried out in the farmer's field situated within a distance of 4-5 km radius surrounding the university, Bihar Agricultural University (BAU), Sabour. The experiment II (Preservation of neera) was conducted in the laboratory, department of Food Science and Postharvest Technology, Bihar Agricultural College (BAC), Sabour, Bhagalpur. For conducting experiment I, neera was tapped/collected from farmer's field in the early morning i.e. before sunrise. It was collected as per treatment details (Table-1).

Table1. Treatment Details of experiment I (Hygienic collection of neera)

Treats.	Treatment details
T_1	No Sanitization, Earthen pot (Labni), Traditional Tapping (Control)
T_2	Sanitization with AFS* Solution (1500 ppm So_2 +0.2% C.A.) +Polyethylene (PE) lined labni
T_3	Sanitization with AFS* Solution (1500 ppm So_2 +0.2% C.A.) +Polyethylene (PE) lined plastic pot
T_4	Sanitization with AFS* Solution (1500 ppm So_2 +0.2% C.A.) +PE lined plastic Pot containing lime (0.5%) + protected by cushioning with thermocol.
T_5	Sanitization with AFS* Solution (1500 ppm So_2 +0.2% C.A.) + PE lined plastic Pot containing (1.0%) + protected by cushioning with thermocol+ 200 g Crushed ice
T_6	Sanitization with AFS* Solution (1500 ppm So_2 +0.2% C.A.) + Plastic Pot lime (0.5%) + protected by cushioning with thermocol+ 300 g Crushed ice

**AFS- Anti-Fermenting Solution*

EXPERIMENT I: Hygienic collection of neera

Under this experiment, neera was collected as per the treatments (table 1). First of all, inflorescence was sliced (plate 6) and sanitized as per treatments by applying an anti fermenting solution on the cut portion as well as on other parts of the inflorescence (plate 7). Now collection container was hanged below the sliced and sanitized inflorescence for collection of neera. The collected neera was brought to the laboratory of the department of Food Science and Postharvest Technology, Bihar Agricultural College, Sabour, Bhagalpur and strained through centrifugation to remove suspended and foreign material (plate 8) and stored at low temperature (0 to 2°C) in order to stabilize the sap and bring it at uniform temperature before initial biochemical analysis.

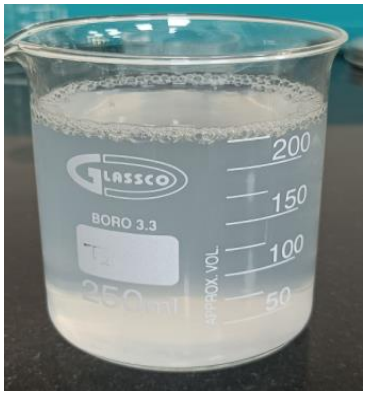

Plate 6. Sliced Inflorescence for Plate 7. Application of anti Plate 8. Filtered neera tapping neera fermenting solution

The biochemical analysis was performed as per standard procedure. For judging the best tapping method, four parameters were used as mentioned in table 2.

Table 2. Parameters for selection of best method of neera collection

Parameters	T_1	T_2	T_3	T_4	T_5	T_6
Alcohol percentage (% v/v)						
pH						
Titratable acidity (%)						
Total Microbial Count (CFU/mL)						

The least fermented neera in terms of minimum % alcohol, minimum change in pH, and titratable acidity (%) and least total microbial count (Cfu/mL)was rated as the best tapping treatment or method.

EXPERIMENT II: Preservation of neera

For conducting experiment II, the neera was collected by the best treatment method identified in experiment I. Treatment T_6 was identified as the best neera collection method where an specially designed box was used. As per treatment details of 2nd experiment, (Table-3), different preservation methods were used for preserving fresh neera. After treatments, neera was packed in 200 mL PET bottle and stored at 2-3°C and quality analysis parameters were recorded initially and after an interval of 15 days for a period of two months. The sensory quality attributes were also evaluated at the same interval.The evaluation of sensory attributes were based on nine-point hedonic scale (*Annexure* I).

Table 3. Experiment -II: Treatment Details (Preservation of Neera)

Treatments	Treatment details
T_1	No Pasteurization (Control)
T_2	Pasteurization at 60°C for 10 minutes
T_3	Pasteurization at 65°C for 10 minutes
T_4	Treatment with ultrasonic (30 kHz) for 10 minutes + Pasteurization at 60°C for 10 minutes
T_5	Treatment with ultrasonic (30 kHz) for 10 minutes + Pasteurization at 65°C for 10 minutes
T_6	Treatment with ultrasonic (35 kHz) for 15 minutes + Pasteurization at 65°C for 10 minutes
T_7	Treatment with ultrasonic (35 kHz) for 20 minutes + Pasteurization at 65°C for 10 minutes

Analysis of fresh neera

Chemical parameters

The physico-chemical parameters were analysed as per standard procedure. Titratable acidity of neera was estimated as per AOAC, [19]. Total soluble solids of neera was estimated by using hand refractometer (Erma hand refractometer with automatic temperature controller, ERB-32, S/N: 600/B19/36) and the results are expressed as degree brix (°B). The pH of the neera was determined with the help of digital pH meter. The pH meter was first standardized with buffers of pH 4.0 and 7.0 before pH measurements of the samples. The percent total and reducing sugars of neera were estimated by Lane and Eynon's method [19] by titrating a known volume of the samples against Fehling's solutions (Fehling A and Fehling B) using methylene blue as indicator. Total phenol content was estimated by using the Folin-Ciocalteau reagent [20].

Microbial analysis

For analyzing microbial load (yeast, mould and bacteria) in neera two different media, (Potato dextrose agar (PDA) and nutrient agar (NA) were used. Potato dextrose agar (PDA) media was used for counting fungal and yeast growth and nutrient agar (NA) was used for bacterial count. The media (PDA and NA) was prepared as described by Opara et al. [21] with required modifications. All laboratory grade chemicals were used for analysis.

Organoleptic evaluation

Organoleptic analysis was carried out by judges comprising of PG students, teaching and non-teaching staffs (six number of judges)as per methods described by Ranganna [22]. The evaluation was for taste and overall acceptability on 9-point Hedonic scale as mentioned in a*nnexure* II.

Statistical analysis

The data of 1stexperiment (experiment I) was analysed by Randomized Block Design (RBD) and 2nd by Controlled Randomized Design (CRD), respectively.

The data were analyzed by analysis of variance (ANOVA) and means were compared by Duncan's Multiple Range Test (DMRT) using SPSS Version 26. Differences between the means at 5 % level were considered significant.

Results

Bio-chemical and microbial estimation of fresh neera

The bio-chemical characteristics of fresh neera as influenced by the tapping method is presented in Table 4. It is clear from the table that the tapping methods affected biochemical parameters. The lowest TSS was observed in control (T_1) (9.50±0.50°B) and highest in T_6 (12.33±0.29°B) followed by T_5 (12.00±1.80°B). The titratable acidity (%) of control (T_1) was found significantly higher from rest of the treatments. The highest titratable acidity (%) was observed in T_1 (1.05±0.18%) and the lowest in T_2 followed by T_4. However, except T_1, all other treatments were found non significant. The pH value of control (T_1) was also found significantly lower from T_5 and T_6. The minimum pH was observed in T_1 (3.94±0.52) and maximum in T_5 (5.02±0.41) followed by T_6 (4.67±0.41). Alcohol percentage was found significantly higher in control sample (5.17±0.58%) as compared to T_6 (3.93±0.35%). Percent reducing sugar was found lowest in control sample and significantly different from T_6 and T_7 treatments but non-significant with other treatments. Similarly, percent total sugar was also found significantly lower in control treatment as compared to rest of the treatments except T_2 which was found at par with T_2. The phenol (mg/100 mL) content was found non significant.

The microbial population in fresh neera is very much important because it is directly related to fermentation and spoilage. The maximum microbial load (bacteria, yeasts and moulds) was recorded in control sample (T_1) and bacterial population was high compared to yeast (figure 1). The best collection treatment was found to be T_6 (Sanitization with AFS* Solution (1500 ppm So_2 +0.2% C.A.) + Plastic Pot lime (0.5%) + protected by cushioning with thermocol+ 300 g Crushed ice) in terms of less microbial load, low acidity, low alcohol percentage and high pH as compared to other treatments.

The acceptability of neera depends upon the organoleptic parameters like taste, odour and over all acceptability (figure 2). It is evident from the figure that there is a significant difference in all three organoleptic parameters (taste, odour and over all acceptability) of fresh neera on 9-point hedonic scale. The highest score (7.33) equivalent to like moderately was observed in T_4 and T_6 and minimum in control sample (3.67±0.58) which was equivalent to dislike moderately. The lowest score was registered in control sample (T_1) and highest in T_6. Other treatments, like T_2 to T_5 were found significantly superior than control but at par to each other. On the other hand, anti fermenting solution that was applied in T_6 (Sanitization with AFS* Solution (1500 ppm So_2 +0.2% C.A.) + Plastic Pot lime (0.5%) + protected by cushioning with thermocol+ 300 g Crushed ice) was found most effective and so the OAA taste was highest in T_6 as compared to others. Hence hygienic collection of the neera preserves the quality for comparatively longer duration [23].

Table 4 . Bio-chemical characteristics of fresh neera as influenced by different tapping methods

Treatments (T)	TSS (°B)	Acidity (%)	pH	Alcohol (%)	Reducing Sugar (%)	Total Sugar (%)	Phenol (mg/100 mL)
T_1	9.50±0.50^{b}	1.05±0.18^{a}	3.94±0.52^{c}	5.17±0.58^{a}	3.68±0.77^{b}	6.70±0.82^{c}	342.43±16.98^{a}
T_2	10.25±0.75^{b}	0.54±0.10^{b}	4.34±0.17^{bc}	4.45±0.78^{ab}	3.76±0.70^{b}	7.42±0.66^{bc}	320.03±62.57^{a}
T_3	10.32±0.12^{b}	0.64±0.23^{b}	4.25±0.08^{bc}	4.35±0.17^{ab}	3.87±0.36^{b}	8.73±0.34^{a}	310.77±175.52^{a}
T_4	10.67±0.58^{ab}	0.60±0.05^{b}	4.44±0.26^{bc}	4.34±0.11^{ab}	4.40±0.15^{ab}	8.75±0.30^{a}	324.64±78.83^{a}
T_5	12.00±1.80^{a}	0.65±0.10^{b}	5.02±0.41^{a}	4.33±0.13^{ab}	4.32±0.50^{ab}	8.63±0.45^{a}	323.93±134.71^{a}
T_6	12.33±0.29^{a}	0.65±0.10^{b}	4.67±0.41^{ab}	3.93±0.35^{b}	4.90±0.12^{a}	8.13±0.31^{a}	307.79±81.99^{a}
T_7	12.29±0.21^{a}	0.64±0.10^{b}	4.66±0.41^{ab}	3.83±0.35^{b}	4.81±0.12^{a}	8.32±0.31^{a}	306.79±81.99^{a}
SEm (±)	**0.509**	**0.071**	**0.160**	**0.250**	**0.284**	**0.322**	**61.431**
CD (P=0.05)	**1.604**	**0.224**	**0.504**	**NS**	**1.021**	**1.015**	**NS**

Table 5.Microbial population of fresh neera.

Treatment	Yeast population	Mould population	Bacterial Population
T_1	2x 10^6	1 x 10^4	3 x 10^6
T_2	2x 10^5	1 x 10^3	3 x 10^4
T_3	2x 10^4	1 x 10^3	3 x 10^3
T_4	2x 10^3	1 x 10^2	3 x 10^4
T_5	2x 10^3	1 x 10^3	3 x 10^4
T_6	2x 10^2	1 x 10^3	3 x 10^4
T_7	2x 10^2	1 x 10^2	3 x 10^3

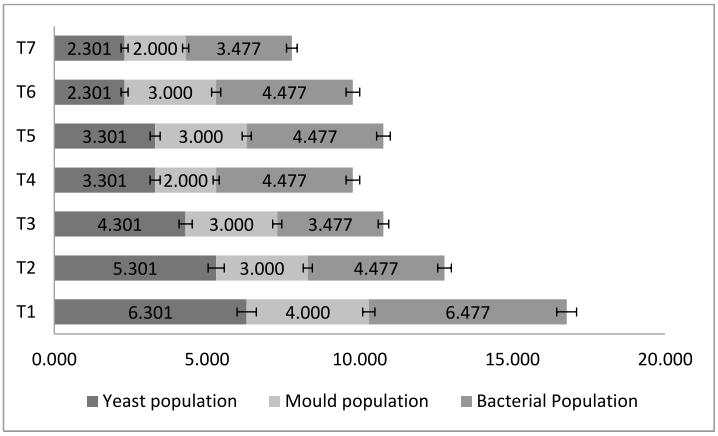

Fig.Microbial population of fresh neera. Population in Log10 transformed value.

Table 6. Organoleptic attributes of fresh neera

Treatments (T)	Taste	Odour	Overall Acceptability
T_1	3.67±0.58^{c}	5.00±1.00^{c}	4.67±0.58^{c}
T_2	6.00±1.00^{b}	6.00±1.00^{bc}	6.00±1.00^{b}
T_3	6.33±0.58^{ab}	6.33±0.58^{ab}	6.33±0.58^{b}
T_4	7.33±0.58^{a}	7.33±0.58^{a}	6.67±0.58^{b}
T_5	5.33±0.58^{b}	5.33±0.58^{bc}	5.67±0.58^{bc}
T_6	7.33±0.58^{a}	6.00±1.00^{bc}	8.00±0.00^{a}
T_7	7.31±0.58^{a}	6.00±1.00^{bc}	8.00±0.00^{a}
SEm (±)	**0.380**	**0.350**	**0.360**
CD (P=0.05)	**1.197**	**1.103**	**1.134**

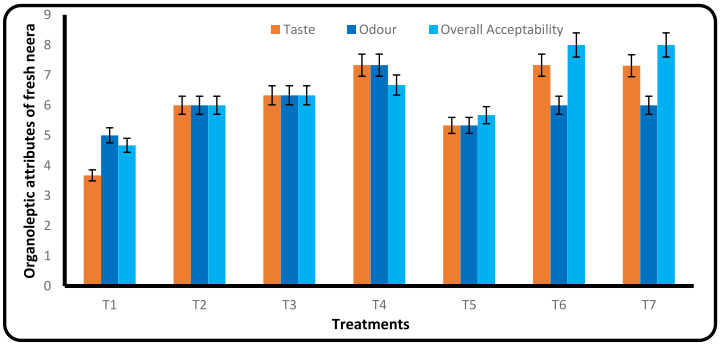

Figure 1. Organoleptic attributes of fresh neera

Effect of preservation methods on biochemical parameters during storage at low temperature(2-3°C)

Under 2nd experiment, the treated neera was packed and stored at low temperature (2-3°C) for quality evaluation at a fixed interval of fifteen days for a total period of 60 days (2-months). During this period, biochemical and sensory observations were recorded. The following parameters were studied during storage.

Titratable Acidity (%) and pH

In general, a successive decreasing pattern in titratable acidity (%) was observed in all treatments from the start of the storage period till the end i.e. up to 60 days (table 7) except few treatments (T_1, T_2, T_3 and T_4) that spoiled before 15, 30 and 45 days, respectively.This decrease in acidity might be the result of acid-mediated hydrolysis of polysaccharides and non-reducing sugars to their simpler components where acid was utilized for converting them to hexose sugars or complexes in the presence of metal ions [24].

The pH value was found decreased in T_1, T_2, T_3 and T_4but an increasing trend was observed in T_5, T_6 and T_7treatments. The decrease in T_1, T_2, T_3 and T_4 might be due to initial lactic acid production by the process of natural fermentation.

Total Soluble Solids (°B), Reducing and Total Sugar (%)

The value of total soluble solids (TSS) is presented in figure 2 and that of sugars (reducing and total sugar) are presented in table 8. It is clear from the figure and table that four treatments,(T_1, T_2, T_3 and T_4) were rejected after noticing spoilage at different storage intervals. T_1 (control) was rejected after 2 days storage only whereas, T_2 after 15 days, T_3 after 30 days and T_4 after 45 days, respectively. Only three treatments namely T_5, T_6, and T_7 successfully stored for 60 days. The TSS and both reducing and total sugars of neera changed significantly during storage of 60 days. The initial value of TSS (°B) of T_5, T_6, and T_7 were found 12.00±1.80, 12.33±0.29 and 12.37±0.30°B, respectively which was found to be increased during 60 days of storage.The initial value of reducing sugar (%) of T_5, T_6 and T_7 were recorded 4.32±0.50%, 4.90±0.12% and 4.87±0.16% which was found to increase during 60 days of storage up to 5.09±0.13%, 5.25±0.05 and 5.17±0.08, respectively.

Table 7.Titratable acidity (%) and pH of treated neera during 60 days storage at low temperature (2-3oC)

	Titratable acidity (%)						pH					
Days / Treatments	0-day	15-days	30-days	45-days	60-days	Mean	0-day	15-days	30-days	45-days	60-days	Mean
T_1	1.05±0.18	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	**0.21±0.44^{d}**	3.94±0.52	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	**0.79±1.64^{f}**
T_2	0.54±0.10	0.82±0.06	0.00±0.00	0.00±0.00	0.00±0.00	**0.27±0.36^{d}**	4.34±0.17	3.88±0.13	0.00±0.00	0.00±0.00	0.00±0.00	**1.64±2.09^{e}**
T_3	0.64±0.23	0.55±0.15	0.93±0.28	0.00±0.00	0.00±0.00	**0.42±0.41^{c}**	4.25±0.08	4.27±0.10	3.76±0.16	0.00±0.00	0.00±0.00	**2.46±2.09^{d}**
T_4	0.60±0.05	0.58±0.06	0.55±0.10	0.75±0.10	0.00±0.00	**0.50±0.27^{b}**	4.44±0.26	4.55±0.19	4.69±0.13	3.71±0.53	0.00±0.00	**3.48±1.85^{c}**
T_5	0.65±0.10	0.58±0.06	0.53±0.04	0.48±0.08	0.47±0.08	**0.54±0.09^{ab}**	5.02±0.41	5.09±0.30	5.25±0.52	5.37±0.36	5.46±0.28	**5.24±0.37^{a}**
T_6	0.65±0.10	0.57±0.16	0.53±0.10	0.50±0.05	0.42±0.03	**0.53±0.12^{ab}**	4.67±0.41	4.78±0.49	5.00±0.12	5.11±0.10	5.22±0.22	**4.96±0.34^{b}**
T_7	0.68±0.06	0.63±0.10	0.60±0.04	0.56±0.02	0.48±0.06	**0.59±0.09^{a}**	4.93±0.54	5.12±0.49	5.13±0.19	5.24±0.31	5.45±0.2	**5.18±0.36^{a}**
Mean	**0.69±0.19^{a}**	**0.53±0.25^{b}**	**0.45±0.34^{c}**	**0.33±0.31^{d}**	**0.20±0.23^{e}**		**4.51±0.48^{a}**	**3.95±1.73^{b}**	**3.41±2.26^{c}**	**2.78±2.53^{d}**	**2.3±2.73^{e}**	
	Days (D)		**Treatment (T)**		**D × T**		**Days (D)**		**Treatment (T)**		**D × T**	
SEm (±)	**0.021**		**0.025**		**0.055**		**0.060**		**0.071**		**0.160**	
CD (P=0.05)	**0.059**		**0.071**		**0.155**		**0.169**		**0.200**		**0.451**	

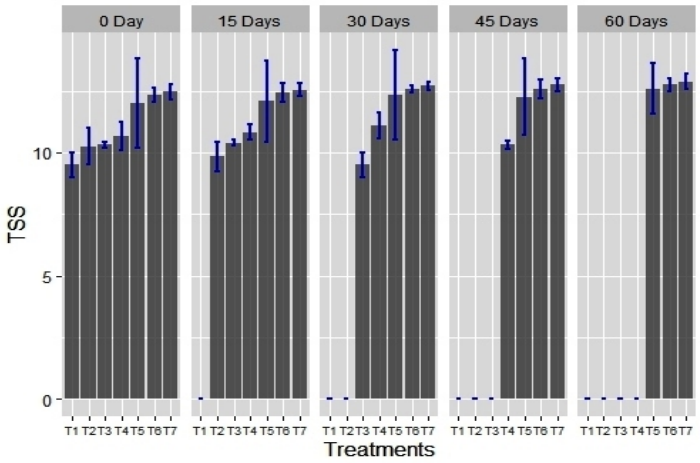

Figure 2. Total Soluble Solids (TSS) (°B)of treated neera during 60 days storage at low temperature (2-3oC)

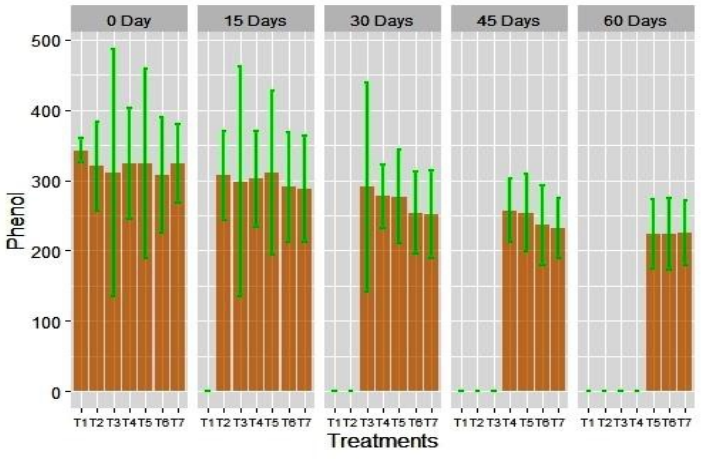

Figure 3.Total Phenol (mg/100 mL)of treated neera during 60 days storage at low temperature(2-3oC)

Total Phenol (mg/100 mL) and Organoleptic properties of neera

The value of total phenol was found in decreasing order till the end of storage (Figure 3). The decrease was observed in all treatments. The initial value of Total phenol (mg/100 mL) ofthe best treatments (T_5, T_6 and $T_{7)}$ were recorded as 323.92±134.73, 307.80±81.98 and 323.73±55.96 (mg/100 mL) which has been reduced to 223.82±50.10, 223.91±51.42 and 225.63±45.7 (mg/100 mL), respectively, at the end of 60 days storage.

Table 8. Reducing and Total Sugars (%) of treated neera during 60 days storage at low temperature(2-3oC)

	Reducing Sugars (%)						Total Sugars (%)					
Days / Treatments	0-day	15-days	30-days	45-days	60-days	Mean	0-day	15-days	30-days	45-days	60-days	Mean
T_1	3.68±0.77	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	**0.74±1.55^{f}**	6.70±0.82	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	**1.34±2.79^{f}**
T_2	3.76±0.70	3.94±0.58	0.00±0.00	0.00±0.00	0.00±0.00	**1.54±1.98^{e}**	7.42±0.66	7.52±0.63	0.00±0.00	0.00±0.00	0.00±0.00	**2.99±3.80^{e}**
T_3	3.87±0.36	4.03±0.53	4.33±0.15	0.00±0.00	0.00±0.00	**2.44±2.09^{d}**	8.73±0.34	9.01±0.18	9.13±0.25	0.00±0.00	0.00±0.00	**5.38±4.55^{d}**
T_4	4.40±0.15	4.48±0.14	4.59±0.05	4.75±0.10	0.00±0.00	**3.64±1.89^{c}**	8.75±0.30	8.85±0.29	9.03±0.24	9.03±0.24	0.00±0.00	**7.13±3.70^{c}**
T_5	4.32±0.50	4.67±0.13	4.74±0.07	4.83±0.08	5.09±0.13	**4.73±0.33^{b}**	8.63±0.45	8.77±0.39	8.89±0.33	8.89±0.33	8.97±0.33	**8.83±0.33^{a}**
T_6	4.90±0.12	4.90±0.12	5.01±0.12	5.10±0.17	5.25±0.05	**5.03±0.17^{a}**	8.13±0.31	8.23±0.38	8.45±0.51	8.45±0.51	8.68±0.52	**8.39±0.43^{b}**
T_7	4.87±0.16	4.84±0.06	4.97±0.25	4.93±0.24	5.17±0.08	**4.96±0.19^{a}**	8.06±0.3	8.12±0.33	8.27±0.52	8.23±0.61	8.57±0.5	**8.25±0.43^{b}**
Mean	**4.26±0.62^{a}**	**3.84±1.66^{b}**	**3.38±2.20^{c}**	**2.8±2.49^{d}**	**2.22±2.62^{e}**		**8.06±0.84^{a}**	**7.21±3.07^{b}**	**6.25±4.07^{c}**	**4.94±4.40^{d}**	**3.75±4.44^{e}**	
	Days (D)		**Treatment (T)**		**D × T**		**Days (D)**		**Treatment (T)**		**D × T**	
SEm (±)	**0.057**		**0.068**		**0.152**		**0.081**		**0.095**		**0.213**	
CD (P=0.05)	**0.161**		**0.192**		**0.429**		**0.228**		**0.268**		**0.601**	

Effect of preservation methods on sensory qualities of neeraduring storage at low temperature(2-3°C)

The initial taste and overall acceptability (OAA) score of treatments, T_1 to T_4 was registered as lower values (6 -7 approx) on hedonic scale confirming that the fermentation started in these treatments and spoiled within few days (table 9). On the other hand, higher scores have been registered inT_5 (6.67±1.15), T_6 (7.33±0.58), T_7 (8.00±0.00) for taste, and T_5 (5.677.00±1.00), T_6 (8.00±0.00), T_7 (8.33±0.58) for OAA, respectively. The final taste score of T_5, T_6, and T_7 were found to be 6.00±1.00, 6.67±0.58 and 7.33±0.58 for taste and 6.00±0.00, 6.67±0.58 and 7.00±0.00 for OAA, respectively. This indicates that both taste and OAA score decreased with the passage of time. Fermentation started immediately in the control sample (T_1) followed by T_2, T_3 and T_4 and hence scored lower values on hedonic scale. On the other hand, higher scores have been registered inT_5, $T_6$and T_7 for both taste and overall acceptability (OAA).The final taste score of T_5, T_6, and T_7 was acceptable even after 60 days of storage but loss in both taste and OAA was observed.

Table 9. Taste and Overall acceptability (OAA) of treated neera during 60 days storage at low temperature (2-3oC)

	Taste						Overall acceptability					
Days / Treatment	0-day	15-days	30-days	45-days	60-days	Mean	0-day	15-days	30-days	45-days	60-days	Mean
T_1	6.33±1.15	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	**1.27±2.66^{f}**	6.00±1.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	**1.20±2.51^{f}**
T_2	6.67±0.58	4.33±0.58	0.00±0.00	0.00±0.00	0.00±0.00	**2.20±2.91^{e}**	6.00±1.00	4.67±0.58	0.00±0.00	0.00±0.00	0.00±0.00	**2.13±2.77^{e}**
T_3	7.33±0.58	6.67±0.58	3.67±0.58	0.00±0.00	0.00±0.00	**3.53±3.27^{d}**	6.33±0.58	6.33±0.58	4.33±0.58	0.00±0.00	0.00±0.00	**3.40±3.00^{d}**
T_4	7.33±0.58	7.00±0.00	6.67±0.58	4.33±0.58	0.00±0.00	**5.07±2.87^{c}**	6.67±0.58	6.67±0.58	6.33±0.58	5.00±1.00	0.00±0.00	**4.93±2.69^{c}**
T_5	7.33±0.58	6.67±0.58	6.67±0.58	6.00±0.00	6.00±1.00	**6.53±0.74^{b}**	7.00±1.00	7.00±0.00	6.67±0.58	6.67±0.58	6.00±0.00	**6.67±0.62^{b}**
T_6	7.67±0.58	7.33±0.58	7.33±0.58	7.33±0.58	7.33±0.58	**7.40±0.51^{a}**	8.00±0.00	8.00±0.00	7.67±0.58	7.00±0.00	6.67±0.58	**7.47±0.64^{a}**
T_7	8.00±0.00	7.67±0.58	7.67±0.58	7.67±0.58	7.67±0.58	**7.73±0.46^{a}**	8.33±0.58	7.67±0.58	7.33±0.58	7.33±0.58	7.00±0.00	**7.53±0.64^{a}**
Mean	**7.24±0.77^{a}**	**5.67±2.61^{b}**	**4.57±3.23^{c}**	**3.62±3.38^{d}**	**3.00±3.61^{e}**		**6.90±1.09^{a}**	**5.76±2.64^{b}**	**4.62±3.19^{c}**	**3.71±3.39^{d}**	**2.81±3.34^{e}**	
	Days (D)		**Treatment (T)**		**D × T**		**Days (D)**		**Treatment (T)**		**D × T**	
SEm (±)	**0.111**		**0.131**		**0.293**		**0.110**		**0.130**		**0.292**	
CD (P=0.05)	**0.313**		**0.369**		**0.826**		**0.310**		**0.367**		**0.824**	

Discussion

Effect of hygienic collection on biochemical, microbial and sensory qualities of fresh neera

It is clear from the table 4 and 5 that the hygienic collection of neera controls changes in biochemical parameters and microbial load of fresh neera, respectively.Biochemical parameters like total soluble solids, percent acidity, pH, percent alcohol, phenol, total and reducing sugars content were found different in different treatments. The titratable acidity (%) of control (T_1) was found significantly different from rest of the treatments. The minimum pH was observed in T_1(3.94±0.52) and maximum in T_5 (5.02±0.41) followed by T_6 (4.67±0.41). High acid and low pH observed in control sample might be due to lactic acid fermentation [25].

Lasekan et al. also observed higher percentage of total acidity and low pH in ten palm sap samples collected from Songkhla province of Southern Thailand. The lowest total soluble solids (TSS)was observed in control(T_1) (9.50±0.50°B) and highest in T_6 (12.33±0.29°B) followed by T_5 (12.00±1.80°B).Alcohol percentage and microbial population were found significantly higher in control sample as compared to T_6. This might be due to the unhygienic collection of neera that might have increased microbial contamination and increased fermentation rate in control sample. The increase in fermentation rate converted more sugar content into alcohol, resulted decrease in TSS content and an increase in alcohol content. Kurniawan et al., [26] also reported that the degree of fermentation of the palmyrah neera depends on the practice of neera collection and the environmental conditions.

Hygiene collection of the neera samples preserves it in fresh form for a comparatively longer duration [23].The maximum value of microbial population in control sample (T_1) be due to unhygienic collection of neera. However, bacterial population was found more than yeast and moulds. This might be due to more bacterial contamination than yeast and moulds. According to Somashekaraiah et al. [11], neera has been reported to have about 75 distinct Lactic acid bacteria (LAB) strains.

The value of organoleptic attributes like taste, odour and over all acceptability (OAA) were found significantly different on 9-point hedonic scale (table 6). The lowest score registered in control sample (T_1) and highest in T_6. The reason might be the microbial contamination in control sample where anti fermenting solution (AFS) was not applied. On the other hand, the most effective anti fermenting solution was applied in T_6(Sanitization with AFS* Solution (1500 ppm So_2 +0.2% C.A.) + Plastic Pot lime (0.5%) + protected by cushioning with thermocol+ 300 g Crushed ice). Hence hygiene collection of the neera preserves it in fresh form for a comparatively longer duration [23]. Kurniawan et al., [26] also reported that the degree of fermentation of the palmyraneera depends on the methods of neera collection and the environmental conditions.

Effect of preservation methods on shelf life of neera at low temperature (2-3°C)

The aim of 2nd experiment was to identify the best treatment that can prevent spoilage up to 60 days. The control sample (T_1) spoiled within 3 days. Treatments like T_2, T_3, and T_4 spoiled with 15, 30 and 45 days, respectively. The thermosensation treatments, T_5, T_6 and T_7 only completed 60 days. Therefore, it was concluded that these three treatments (ultrasonic (30 kHz) for 10 minutes + Pasteurization at 65°C for 10 minutes (T_5), ultrasonic (35 kHz) for 15 minutes + Pasteurization at 65°C for 15 minutes (T_6), ultrasonic (35 Hz) for 15 minutes + Pasteurization at 65°C for 20 minutes (T_7) were effective treatments in preventing fermentation and spoilage of neera. It has been reported that thermosonication has higher efficiency for killing of microbes and deactivation of enzymes at relatively low temperatures [27]. Kapturowska et al. [18] also reported that thermosonication is very effective against yeast.

Effect of preservation methods on biochemical parameters during storage at low temperature(2-3°C)

After 2nd experiment, the samples were stored at low temperatures and again biochemical changes and sensory qualities of neera such as titratable acidity (%), pH, percent reducing and total sugars, total soluble solids (°B), total phenol (mg/100 mL), taste and overall acceptability were analyzed during the storage period of 60 days.In general, a successive decreasing pattern in titratable acidity (%) and percent total phenol were observed.

This decrease in acidity might be the result of acid-mediated hydrolysis of polysaccharides and non-reducing sugars to their simpler components where acid was utilized for converting them to hexose sugars or complexes in the presence of metal ions [24]. Similarly, the decrease in polyphenol content might be either due to various polyphenol mediated reactions during storage or polyphenol compounds might have contributed to haze formation by interacting with proteins through polymerization or oxidation leading to the formation of high-molecular-weight polymeric complexes [28]. At the same time, an increasing pattern was observed in pH, total soluble solids, percent reducing and total sugars in all treatments from the starting of storage period till the end i.e. up to 60 days. Some of the treatments like T_1, T_2, T_3 and T_4not followed the general trend and were spoiled before 15, 30 and 45 days, respectively . In these treatments,an increase in percent acidity and decrease in pH were recorded.

The decrease in acidity and a corresponding increase in pH might be due to fermentation of neera before spoilage. Leena et al. [29] also reported an increase in percent titratable acidity and decrease in pH of palmyrah palm neera due to microbial fermentation of the carbohydrates present in the palmyrah neera. Prashanth and Patil [30] also reported a increase in acidity and a decrease in pH of coconut sap during storage.

The increase in TSS might be due to hydrolysis of polysaccharides and pectic substances into simpler soluble saccharides which might have contributed to an increase in TSS during storage [24]. A nearly similar result was also observed by Nath et al. [31] in ginger- kinnow squash and in Kinnow mandarin juice blends [32]

The increase in reducing sugar (%) might be due to hydrolysis of polysaccharides mainly starch and pectic substances into simpler soluble saccharides which contributed an increase in reducing and total sugar (%) during storage [24]. Naknean et al. [33] reported an increase in reducing sugar due to inversion of sucrose into glucose and fructose. Rao et al. [34] also reported an increase in %reducing and total sugar in palmyra neera during four months of storage and then declined. Naknean et al [35] also reported an increase in reduced sugar of palmyra palm sap during storage after treatment with clarifying agents. Yousaf et al. [36] and Singh et al. [37] also reported increase in reducing sugar during storage of banana juice and processed sugar can juice, respectively.

Effect of preservation methods on sensory qualities of neera during storage at low temperature(2-3°C)

Fermentation started immediately in the control sample (T_1) followed by T_2, T_3 and T_4 and hence scored lower values on hedonic scale. On the other hand, higher scores have been registered inT_5, $T_6$and T_7 for both taste and overall acceptability (OAA). The final taste score of T_5, T_6, and T_7 was acceptable even after 60 days of storage but loss in both taste and OAA was observed. The loss of taste and over all acceptability might be due to undesirable biochemical changes [38] or the degradation of ascorbic acid and furfural production [39]. A similar trend is also reported by Rao et al. [34] during six weeks of storage at 2-4°C.The results are in accordance with [40] in coconut water and [41] in palmyra neera.

Summary and Conclusion

For hygienic tapping of neera from palmyrah palm (experiment I), the treatment T_6 (Sanitization with AFS* Solution (1500 ppm So_2 +0.2% C.A.) + Plastic Pot lime (0.5%) + protected by cushioning with thermocol+ 300 g crushed ice) was selected as the best treatment and carry forwarded for conducting experiment II. Among different treatments of experiment II, treatments T_6 and T_7, (ultrasonic (35 kHz) for 15 minutes + Pasteurization at 65°C for 10 minutes and ultrasonic (35 kHz) for 20 minutes + Pasteurization at 65°C for 10 minutes Pasteurization, respectively, were found the best treatments for preservation of neera at low temperature on the basis of no fermentation, longer shelf life, least changes in biochemical and sensorial qualities.Prashanth and Patil (2020) also reported that pasteurization increases the shelf life of coconut sap.

Hence treatment T_6 of experiment I and treatments T_6 and T_7 of experiment II may be commercially exploited for the safe and higenic collection and preservation of neera from palmyrah palm.

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Thre is no conflict of interest among the authors.

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