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Investigation of usage of the copper vessel as coconut sap storage and its changes in physicochemical characteristics

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Abstract
This experiment revealed the viability of using copper materials for collecting coconut sap regarding various physicochemical parameter changes in chilled and ambient conditions. In the different storage conditions, the sap's pH, TSS, relative density, and dry matter values all significantly decreased, although the TTA and colour values increased. The glass vessel at ambient condition showed the most significant values of pH, TSS, relative density, dry matter and TTA as 5.0 ± 0.00 to 3.4 ± 0.02, 18.9 ± 0.00 to 10.3 ± 0.14 °brix, 1.1 ± 0.00 to 1.0 ± 0.00, 1.8 ± 0.00 to 1.1 ± 0.01, and 0.0 ± 0.00 to 0.2 ± 0.00 respectively. However, all the parameters were continually maintained when the sap was in the chilled copper vessel. The colour changes in sap held in copper at ambient temperatures was the most noticeable attribute. The L value in the at ambient copper vessel ranged significantly from 12.7 ± 0.04 to 35.8 ± 5.68. Although the sap in glass exposed to ambient conditions had the most degradation, sap exposed to chilling showed less degradation in all parameters. Hence, storage of sap in chilled copper vessel is recommended.

Keywords: Coconut sap, storage, physicochemical change, copper, chilled, ambient

Introduction

The coconut (Cocos *nucifera* L.) of tropical and subtropical crops plays a vital role in economic changes through its different forms of production in India (Hebbar *et al*., 2020; Pandiselvam *et al*., 2021) [10, 17]. Before knowing its health benefits, coconut was used as food in ancient times (Ahuja, 2018) [1]. The coconut nuts can be served as raw materials for producing oil, food, cosmetic and pharmaceutical industries (Ysidor *et al*., 2015) [25]. There are a lot of coconut-derived goods on the market. Some of the most well-known products produced by coconut trees are copra, coconut fiber or coir, coconut peat, coconut charcoal, coconut oil, coconut sap, and palm fronds. The coconut sap or neera is one of the savior products for the coconut farmer, which seems to generate more and quick income than the producing nuts (Ghosh *et al*., 2018; Sudha *et al*., 2019) [8, 21].

Neera is obtained from fully developed, unopened coconut inflorescences, which are golden in color, have a pH of greater than 7, and do not smell bad. The collected fresh sap by coco-sap chiller had been found total sugar (15.18 g/100g), free amino acids (0.245 g/100g), and total phenols (5.10 mg/100g) (Hebbar *et al*., 2015) [9]. It could be an ideal drink if the sap is not fermented (Hebbar *et al*., 2018) [11]. Coconut sap had higher levels of DPPH (23.42%), FRAP (2.09 mM/ml), and ABTS (21.85%) and also contained significant levels of vitamin C (116.19 g/ml) and ash (0.27%), particularly in potassium (960.87 mg/L) and sodium (183.21 mg/L), which also suggests high mineral content. It would be a more useful sugar source than sugarcane and sugar palm juices (Asghar *et al*., 2020) [2]. The sugar prepared from coconut sap can fulfill the global demand for low Glycemic Index (GI) sugar significantly because of its low GI (35±4) (Hebbar *et al*., 2020; Trinidad *et al*., 2010) [10, 22].

The relative and physicochemical changes of coconut sap on different parameters, which were collected using traditional and coco-sap chiller methods, have been reported (Nakamura *et al*., 2004; Pandiselvam *et al*., 2021; Shetty *et al*., 2017; Ysidor *et al*., 2015; Ysidor, 2014) [15, 17, 20, 25, 24]. The available reports were presented on the reaction kinetics of sap during fermentation and physicochemical changes during storage to understand its shelf life under different storage conditions (ambient and refrigerated) and find the appropriate value-added products. Coconut sap powder has been developed and claimed a considerable ergogenic impact and may be widely used as a natural component in energy and sports beverages, which increase the VO2 max (Joseph *et al*., 2021) [12]. This innovative modification of existing products can only be done after examining physicochemical changes in different parameters.

However, the physicochemical changes of pure coconut sap, which is stored in the copper vessel under ambient and refrigerated conditions, are unavailable. Fehling's test, which included copper salt, has been widely recognized for estimating or identifying reducing and non-reducing sugars. The process parameters in such tests were influenced by time, temperature, reagent concentration, and composition (Benedict, 1908) [5]. The glucose reacts with copper to form an activated species, displacing an iodide (Chellquist *et al*., 1997) [6]. There is no uncertainty about the water's ability to fight germs when it is kept in a copper vessel injected with specific bacterial strains (Manikandan *et al*., 2018) [14]. Temperature and pH both had significant effects on inactivation and injury. The quickest inactivation was seen at high temperatures and pH levels that were farthest from neutrality, while the most sub-lethal injury, manifested as sensitivity to traditional aerobic evaluation, was seen at a temperature of 35 °C (Sharan *et al*., 2010) [19]. This study aims to look into the physicochemical changes that occur when fresh coconut inflorescence sap is stored in a copper vessel at ambient and chilled temperatures.

The feasibility of employing copper materials for collecting coconut sap was discovered in this investigation in terms of some physicochemical parameter alterations.

Materials and methods

Sample collection

The fresh coconut sap was directly collected from the coconut tree of the National Institute of Food Technology, Entrepreneurship and Management - Thanjavur, Tamil Nadu, India. The sap was collected by using the coco-sap chiller method. Consecutively, the coconut sap was kept in a copper vessel and then stored at an ambient and chilled temperature. The copper glass (Signora Ware, 100% pure copper) was brought from the Thanjavur Bazar.

Sampling

The sap (200mL each) was poured into two copper vessels and glass container simultaneously, wherein each container (one copper and glass vessel) was kept at ambient temperature (35 ± 4 °C) and the other two at chilled temperature (8 ± 3 °C). The physicochemical variables were examined after 0, 1, 2, 4, 8, 12, 16, 20, and 24 h of storage.

Potential of hydrogen (pH)

The pH of coconut sap under different storage conditions for different time periods was measured using a pH meter (LAQUA PH1100 Horiba scientific). The pH meter probe was clean thoroughly with tissue paper and then deeping into distilled water for neutralization. After that, the pH probe was deeping into the sample which was filled in the tube and took the reading when the displayed numbers gives stable.

Total Soluble Solids

A Digital Pocket Refractometer Pal-1 (ATAGO) was used to measure the number of soluble solids in % Brix. The refractometer has a range of 0 to 53%. This technique was used to determine the sugar content of coconut sap. It was measured by dropping the samples into the refractometer prism with the help of a pipette and then pressing the start button. The displayed value was recorded.

Relative density

The relative density was determined by measuring the mass of 5 mL of sap and dividing by the mass of 5 mL of water.

Dry matter

Dry matter is the substance that remains after the water has been removed, while moisture content indicates how much water is really present in the sample component. The dry matter component of the sample includes the nutrients. The dry matter content was determined by drying 10 g of sap in an oven at 103 °C for 8 hours (Makhlouf-Gafsi *et al*., 2016; Ysidor *et al*., 2015) [13, 25].

Total Titrable acidity

The titration technique outlined by Nielsen (2003) [16] was used to evaluate the titratable acidity of coconut sap samples. 0.01 mol/L NaOH and a 1% (w/v) phenolphthalein solution were used to titrate the palm sap sample. The percent (w/v) of lactic acid was used to represent the titratable acidity (Wiboonsirikul, 2016) [23].

Color value

The sap's color was measured using a colorimeter (Hunter's lab Color Flex EZ's spectrophotometer). Data for the color value were acquired using the CIE "L*" (lightness), "a*" (redness and greenness), and "b*" standards (yellowness and greenness) (Wiboonsirikul, 2016) [23].

Statistical analysis

The sap samples were measured in all trials three times, with the average value being taken. Temperature and amount of time spent storing were the determining variables. Analysis of variance with split plot design (ANOVA) was used to analyze the level of significance at 95% (p<.05). Then, using the Tukey Pairwise Comparisons test, the mean was grouped at Minitab. The correlations between different dependent variables (pH, TSS, Relative density, Dry matter, Total titrable acidity & Color) were evaluated using Origin Pro 2021.

Results

The results of the data's statistical analysis showed that the storage period significantly (p 0.05) affected the physicochemical characteristics of the coconut sap that were provided by the varied storage settings (Table 1).

Table 1: Effect of time-dependent storage conditions on the physicochemical parameters of sap (Mean ± SD)

Time (hours)	Storage condition	pH	TSS	Relative density	Dry matter	TTA
0	Chilled glass	5.0 ± 0.00^{a}	18.9 ± 0.00^{a}	1.1 ± 0.00^{a}	1.8 ± 0.00^{abc}	0.0 ± 0.00^{p}
	Chilled copper	5.0 ± 0.00^{a}	18.9 ± 0.00^{a}	1.1 ± 0.00^{a}	1.8 ± 0.00^{abc}	0.0 ± 0.00^{p}
	Ambient glass	5.0 ± 0.00^{a}	18.9 ± 0.00^{a}	1.1 ± 0.00^{a}	1.8 ± 0.00^{abc}	0.0 ± 0.00^{p}
	Ambient copper	5.0 ± 0.00^{a}	18.9 ± 0.00^{a}	1.1 ± 0.00^{a}	1.8 ± 0.00^{abc}	0.0 ± 0.00^{p}
1	Chilled glass	5.0 ± 0.02^{ab}	18.8 ± 0.00^{a}	1.0 ± 0.00^{mn}	1.7 ± 0.01^{abcd}	0.0 ± 0.00^{op}
	Chilled copper	5.0 ± 0.01^{ab}	15.8 ± 0.14^{ij}	1.0 ± 0.00^{o}	1.6 ± 0.01^{efghij}	0.0 ± 0.00^{p}
	Ambient glass	4.8 ± 0.01^{f}	17.1 ± 0.07^{ef}	1.0 ± 0.00^{n}	1.8 ± 0.01^{abc}	0.0 ± 0.00^{klm}
	Ambient copper	4.8 ± 0.01^{f}	17.3 ± 0.00^{e}	1.0 ± 0.00^{o}	1.7 ± 0.01^{abcd}	0.0 ± 0.00^{klm}
2	Chilled glass	4.9 ± 0.02^{bc}	18.3 ± 0.07^{bc}	1.0 ± 0.00^{cde}	1.7 ± 0.01^{bcdefgh}	0.0 ± 0.00^{nop}
	Chilled copper	5.0 ± 0.00^{ab}	15.2 ± 0.00^{kl}	1.0 ± 0.00^{jk}	1.6 ± 0.01^{fghij}	0.0 ± 0.00^{p}
	Ambient glass	4.4 ± 0.01^{j}	17.0 ± 0.14^{fg}	1.0 ± 0.00^{k}	1.7 ± 0.01^{abcdef}	0.0 ± 0.00^{ijk}
	Ambient copper	4.4 ± 0.01^{j}	17.2 ± 0.00^{ef}	1.1 ± 0.00^{bc}	1.7± 0.00^{abcde}	0.0 ± 0.00^{ij}
4	Chilled glass	4.8 ± 0.01^{de}	18.3 ± 0.14^{b}	1.1 ± 0.00^{bc}	1.7 ± 0.01^{cdefghi}	0.0 ± 0.00^{mno}
	Chilled copper	4.9 ± 0.01^{cd}	15.4 ± 0.00^{kl}	1.0 ± 0.00^{fghi}	1.6 ± 0.01^{ghij}	0.0 ± 0.00^{p}
	Ambient glass	4.1 ± 0.00^{k}	16.6 ± 0.00^{gh}	1.1 ± 0.00^{c}	1.7 ± 0.01^{bcdefg}	0.0 ± 0.00^{h}
	Ambient copper	4.1 ± 0.01^{l}	17.1 ± 0.07^{ef}	1.1 ± 0.00^{b}	1.7 ± 0.01^{abcdefg}	0.0 ± 0.00^{g}
8	Chilled glass	4.8 ± 0.00^{ef}	17.8 ± 0.00^{d}	1.1 ± 0.00^{c}	1.7 ± 0.01^{bcdefghi}	0.0 ± 0.00^{klm}
	Chilled copper	4.8 ± 0.01^{ef}	15.5 ± 0.00^{jk}	1.0 ± 0.00^{ef}	1.6 ± 0.00^{hijk}	0.0 ± 0.00^{lmn}
	Ambient glass	3.8 ± 0.01^{n}	15.8 ± 0.00^{ij}	1.0 ± 0.00^{fghi}	1.6 ± 0.03^{ijkl}	0.1 ± 0.00^{e}
	Ambient copper	4.0 ± 0.01^{m}	16.5 ± 0.21^{h}	1.0 ± 0.00^{efg}	1.7 ± 0.01^{bcdefgh}	0.0 ± 0.00^{f}
12	Chilled glass	4.7 ± 0.01^{g}	18.0 ± 0.07^{cd}	1.0 ± 0.00^{efgh}	1.7 ± 0.01^{cdefghi}	0.0 ± 0.00^{jklm}
	Chilled copper	4.7 ± 0.00^{g}	15.2 ± 0.07^{lm}	1.0 ± 0.00^{fghi}	1.4 ± 0.01^{l}	0.0 ± 0.00^{op}
	Ambient glass	3.6 ± 0.01^{o}	14.0 ± 0.07^{o}	1.0 ± 0.00^{m}	1.5 ± 0.01^{jkl}	0.1 ± 0.00^{d}
	Ambient copper	4.1 ± 0.01^{l}	16.0 ± 0.07^{i}	1.0 ± 0.00^{jk}	1.5 ± 0.03^{kl}	0.1 ± 0.00^{f}
16	Chilled glass	4.7 ± 0.01^{gh}	17.2 ± 0.07^{ef}	1.0 ± 0.00^{efgh}	1.6 ± 0.01^{cdefghi}	0.0 ± 0.00^{jkl}
	Chilled copper	4.7 ± 0.01^{g}	14.7 ± 0.07^{n}	1.0 ± 0.00^{efghi}	1.4 ± 0.01^{l}	0.0 ± 0.00^{lmno}
	Ambient glass	3.5 ± 0.01^{o}	13.0 ± 0.00^{p}	1.0 ± 0.00^{l}	1.2 ± 0.05^{m}	0.1 ± 0.00^{c}
	Ambient copper	4.1 ± 0.02^{l}	16.6 ± 0.00^{gh}	1.0 ± 0.00^{def}	1.6 ± 0.02^{defghij}	0.0 ± 0.00^{fg}
20	Chilled glass	4.7 ± 0.00^{hi}	17.2 ± 0.00^{ef}	1.0 ± 0.00^{hij}	1.6 ± 0.01^{efghij}	0.0 ± 0.00^{ijk}
	Chilled copper	4.6 ± 0.00^{i}	15.1 ± 0.14^{lm}	1.1 ± 0.00^{cd}	1.7 ± 0.02^{cdefghi}	0.0 ± 0.00^{lmno}
	Ambient glass	3.4 ± 0.02^{p}	11.3 ± 0.07^{q}	1.0 ± 0.00^{l}	1.2 ± 0.14^{m}	0.2 ± 0.00^{b}
	Ambient copper	4.1 ± 0.02^{k}	16.5 ± 0.00^{h}	1.0 ± 0.00^{jk}	1.8 ± 0.02^{ab}	0.0 ± 0.00^{fg}
24	Chilled glass	4.6 ± 0.01^{i}	17.4 ± 0.07^{e}	1.0 ± 0.00^{ghij}	1.6 ± 0.01^{fghij}	0.0 ± 0.00^{i}
	Chilled copper	4.6 ± 0.00^{i}	14.8 ± 0.07^{mn}	1.1 ± 0.00^{bc}	1.7 ± 0.01^{abcdef}	0.0 ± 0.00^{lmn}
	Ambient glass	3.4 ± 0.02^{p}	10.3 ± 0.14^{r}	1.0 ± 0.00^{m}	1.1 ± 0.01^{m}	0.2 ± 0.00^{a}
	Ambient copper	4.4 ± 0.02^{j}	16.5 ± 0.07^{h}	1.0 ± 0.00^{ij}	1.8 ± 0.04^{a}	0.0 ± 0.00^{f}

Effect of different storage conditions on Potential of hydrogen (pH)

The pH of the sample before being kept in the copper vessel under different conditions was the same. However, the pH of the sap sample decreased significantly (p<.05) from 5.0 ± 0.00 to 3.4 ± 0.02 during the 24 h of storage in ambient glass (Table 1). In the ambient copper, the pH of the sap decreased significantly (p<.05) for the first 8 h of storage from 5.0 ± 0.00 to 4.0 ± 0.01, which was maintained up to 16 h and increased significantly in 20 & 24 h of storage. In the chilled glass, storage of sap from 4 h had found to be the most significant difference from 5.0 ± 0.00 to 4.6 ± 0.01. The pH of the sap decreased significantly in the chilled copper storage conditions from 5.0 ± 0.00 to 4.6 ± 0.00.

Effect of different storage conditions on Total Soluble Solids

During the 24 hours of storage in the ambient glass vessel, the total soluble solids contents (TSS) drastically dropped significantly from 18.9 ± 0.00 to 10.3 ± 0.14 °Brix. The TSS found the most significant difference in ambient copper storage at 12 h from 18.9 ± 0.00 to 16.0 ± 0.07 °Brix. By statistical values, the TSS decreased significantly in a 4 h gap of storage (Table 1). In the chilled copper, the TSS of the sap was found the most significant at 1 h, which was from 18.9 ± 0.00 to 15.8 ± 0.14 °Brix.

Effect of different storage conditions on Relative density

The relative density of the coconut saps stored in an ambient glass vessel lowered significantly (p<.05) during 24 h of storage from 1.1 ± 0.00 to 1.0 ± 0.00. During the first 8 h of storage, relative density showed the most significant difference, but in between 12 & 24 h did not show any significant difference. Similar results were observed between 16 & 20 h. In the ambient copper, the relative density was found to be most significant in the 2 & 4 h of storage (1.1 ± 0.00 to 1.1 ± 0.00). It was found that the relative density was significantly decreased in the 1 h steps, which was from 1.1 ± 0.00 to 1.0 ± 0.00. There was no significance from the 8 h to 24 h of storage. In the chilled glass, the relative density was most significant in the 1 h of storage (1.1 ± 0.00 to 1.1 ± 0.00). In the chilled copper, the most significant was found during the storage of sap at 1 & 2 h.

Effect of different storage conditions on Dry matter

When considering the two different storage conditions at different times, the dry matter decreased significantly (p<0.05) from 1.8 ± 0.00 to 1.1 ± 0.01 stored in ambient glass conditions. At ambient copper conditions, the dry matter of the sap was a significant difference (p<0.05) (1.8 ± 0.00 to 1.5 ± 0.03) at 12 h of storage, and the dry matter was slightly increased up to 1.8 ± 0.04 from the 16 h of storage which was not significantly different. No significant difference (p<0.05) if chilled glass conditions were only considered. In the chilled copper conditions, it was decreased significantly ($p<0.05$) (1.8 ± 0.00 to 1.4 ± 0.01) at 12 & 16 h and then increased up to 1.7 ± 0.01 in 24 h of storage but not significantly different.

Effect of different storage conditions on Total Titrable acidity

The total titrable acidity (TTA) increased significantly ($p<0.05$) during the 24 h that it was stored in the ambient glass conditions from 0.0 ± 0.00 to 0.2 ± 0.00. It was found to be most significant from the 8 h of storage. In the ambient copper, the TTA values were significantly different up to 8 h, but it was found to be maintained the values from 12 h to 24 h, which did not show any significance. Both chilled glass and chilled copper showed similar, insignificant results.

Effect of different storage conditions on Colour value

The impact of the time of storage on how the colour of the coconut sap changes under various storage conditions (Table 2). The key finding of this investigation is to establish the lightness of coconut sap using the (L*) value rather than the (a*) and (b*) value, which will assist in establishing the sample's redness and yellowness. While progressively changing for the ambient glass conditions, the value of the sap sample increased significantly from (L*) 12.7 ± 0.04 to 27.8 ± 0.65. Coconut sap was often transparent and oyster white. The fermentation process response increased the intensity of coconut sap's lightness and increased the (L*) value. In the ambient copper, the most significant difference was found in 4 h & 24 h of storage from 12.7 ± 0.04 to 35.8 ± 5.68 and 12.7 ± 0.04 to 32.3 ± 0.42 respectively. For the chilled glass, it increased up to 8 h from 12.7 ± 0.04 to 13.0 ± 0.12 and began to decrease at 24 h from 12.7 ± 0.04 to 8.7 ± 0.33 of storage but no significant difference. In the chilled copper, 3 h of storage had the most significant difference from 12.7 ± 0.04 to 3.3 ± 0.01 and started increasing from the 4 h but was not significant (Table 2).

Table 2: Effect of time-dependent storage conditions on the changes in the color of sap (Mean ± SD)

Time (hours)	Storage condition	Color value		
		L*	a*	b*
0	Chilled glass	12.7 ± 0.04^{hijk}	-0.7 ± 0.03^{cde}	-0.2 ± 0.04^{a}
	Chilled copper	12.7 ± 0.04^{hijk}	-0.7 ± 0.03^{cde}	-0.2 ± 0.04^{a}
	Ambient glass	12.7 ± 0.04^{hijk}	-0.7 ± 0.03^{cde}	-0.2 ± 0.04^{a}
	Ambient copper	12.7 ± 0.04^{hijk}	-0.7 ± 0.03^{cde}	-0.2 ± 0.04^{a}
1	Chilled glass	6.0 ± 0.08^{nopqr}	-0.4 ± 0.10^{abc}	-2.5 ± 0.16^{d}
	Chilled copper	5.2 ± 0.07^{pqr}	-0.5 ± 0.06^{abc}	-2.1 ± 0.01^{cd}
	Ambient glass	5.4 ± 0.24^{pqr}	-0.4 ± 0.08^{abc}	-2.2 ± 0.07^{cd}
	Ambient copper	5.5 ± 0.11^{hijkl}	-0.4 ± 0.01^{abc}	-2.1 ± 0.17^{cd}
2	Chilled glass	8.6 ± 0.18^{lmnopq}	-0.6 ± 0.04^{abcde}	-3.2 ± 0.02^{ef}
	Chilled copper	3.3 ± 0.01^{r}	-0.2 ± 0.01^{a}	-1.3 ± 0.00^{b}
	Ambient glass	7.1 ± 0.01^{nopqr}	-0.4 ± 0.03^{abc}	-2.7 ± 0.06^{de}
	Ambient copper	5.0 ± 0.17^{qr}	-0.4 ± 0.02^{abc}	-1.7 ± 0.13^{bc}
4	Chilled glass	12.7 ± 0.13^{hijkl}	-0.7 ± 0.04^{bcde}	-3.8 ± 0.06^{fghi}
	Chilled copper	9.6 ± 0.07^{jklmno}	-0.7 ± 0.01^{cde}	-3.5 ± 0.08^{fgh}
	Ambient glass	16.6 ± 0.52^{fgh}	-0.6 ± 0.03^{bcde}	-4.0 ± 0.06^{ghij}
	Ambient copper	35.8 ± 5.68^{a}	-2.3 ± 0.44^{g}	-3.8 ± 0.83^{fghi}
8	Chilled glass	13.0 ± 0.12^{hij}	-0.7 ± 0.01^{bcde}	-4.1 ± 0.06^{hijk}
	Chilled copper	10.0 ± 0.71^{jklmn}	-0.7 ± 0.11^{bcde}	-3.6 ± 0.00^{fgh}
	Ambient glass	19.7 ± 0.37^{def}	-0.7 ± 0.01^{bcde}	-4.4 ± 0.06^{ijkl}
	Ambient copper	21.5 ± 0.55^{de}	-1.6 ± 0.06^{f}	-4.0 ± 0.06^{ghij}
12	Chilled glass	8.1 ± 0.30^{mnopq}	-0.3 ± 0.14^{ab}	-3.4 ± 0.09^{fg}
	Chilled copper	5.8 ± 0.02^{opqr}	-0.3 ± 0.10^{ab}	-2.2 ± 0.07^{cd}
	Ambient glass	15.5 ± 0.56^{ghi}	-0.5 ± 0.00^{abcd}	-3.9 ± 0.08^{ghij}
	Ambient copper	23.2 ± 0.57^{cd}	-2.1 ± 0.01^{g}	-5.0 ± 0.11^{l}
16	Chilled glass	8.8 ± 0.06^{jklmnopq}	-0.4 ± 0.01^{abc}	-3.6 ± 0.03^{fgh}
	Chilled copper	6.3 ± 0.08^{nopqr}	-0.4 ± 0.11^{abc}	-2.5 ± 0.01^{d}
	Ambient glass	18.0 ± 0.10^{efg}	-0.6 ± 0.00^{bcde}	-4.1 ± 0.01^{hijk}
	Ambient copper	26.6 ± 0.56^{bc}	-3.0 ± 0.07^{h}	-5.8 ± 0.10^{mn}
20	Chilled glass	9.2 ± 0.18^{jklmnop}	-0.6 ± 0.06^{bcde}	-3.5 ± 0.05^{fgh}
	Chilled copper	8.6 ± 0.63^{klmnopq}	-0.6 ± 0.07^{abcde}	-3.4 ± 0.20^{fg}
	Ambient glass	21.3 ± 0.01^{de}	-0.7 ± 0.02^{bcde}	-4.5 ± 0.04^{jkl}
	Ambient copper	27.3 ± 0.08^{bc}	-3.1 ± 0.02^{h}	-6.5 ± 0.08^{n}
24	Chilled glass	8.7 ± 0.33^{klmnopq}	-0.5 ± 0.04^{abc}	-3.3 ± 0.08^{fg}
	Chilled copper	11.6 ± 0.07^{ijklm}	-0.9 ± 0.01^{de}	-4.7 ± 0.02^{kl}
	Ambient glass	27.8 ± 0.65^{b}	-0.9 ± 0.00^{e}	-5.7 ± 0.09^{m}
	Ambient copper	32.3 ± 0.42^{a}	-5.1 ± 0.18^{i}	-7.2 ± 0.04^{o}

Correlations between conditions

Using PCA, the ordination of sap samples from various storage conditions was examined. The principal components explained, respectively, 53.43% (PC1) and 25.75% (PC2) of the total variance (Fig. 1). It was found that dry matter, relative density, and TSS were closely correlated during 24 h of storage of the sap in different conditions. These attributes were acceptable as it lies above the level of variance. The pH value was found to be present along the line of PC1, indicating that this attribute measures the stability of sap during the 24 h of storage period. Titration obtained large negative loading on PC2. When color values were considered, L* values were negatively correlated to a* and b* values.

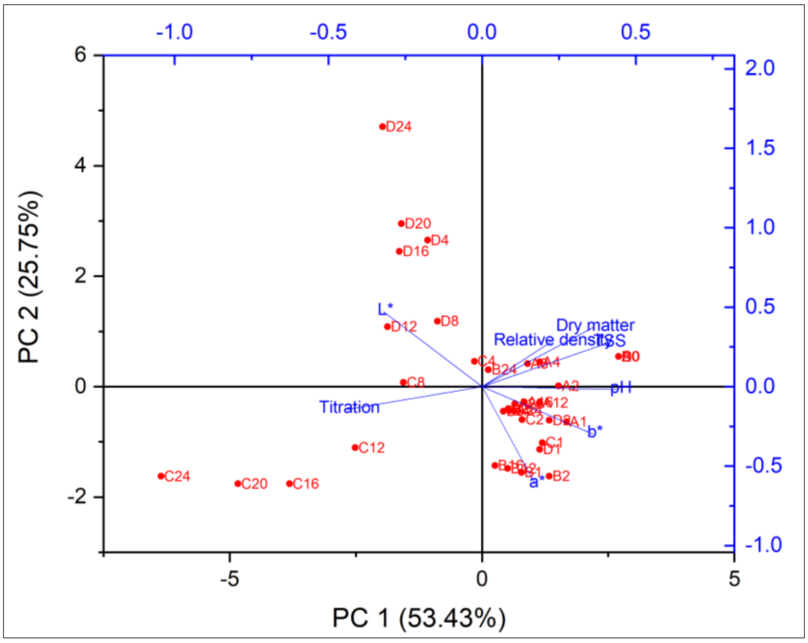

Fig 1: Showing loading and score plot formed by principal components 1 and 2 of physicochemical changes of sap in different storage conditions

Discussions

The sap's pH decreased due to the formation of acetic acid by microbial fermentation (Beegum *et al*., 2018) [4]. The pH of the sap stored in the copper vessel under both conditions was maintained due to inhibiting the microbial fermentation, which inactivates the microbes by copper (Manikandan *et al*., 2018) [14]. The decrease in TSS of the sap was induced by fermentation which is consumed by the microbes (Atputharajah *et al*., 1986) [3]. It was also found that the reduction of TSS was less in the copper vessel under both conditions. The consumption of the sugars by the microbes resulted in the reduction of the relative density of the sap. Therefore, sap stored in the chilled copper conditions shows the highest relative density. From the statistical values, the dry matter decreased to 12 h and increased from 12 h, so copper starts reacting with the sap from the 12th h, whether in chilled or ambient conditions. However, the reactivity of sap to the copper vessel was more in the ambient conditions. The increase of dry matter was due to the formation of some copper oxide residue (Fehling's test). Due to the microbial fermentation that produces acids in ambient glass conditions, the TTA is raised. However, the TTA in both chilled conditions was insignificant since the bacteria in the copper vessel were inactivated, and fermentation slowed down in chilled temperatures.

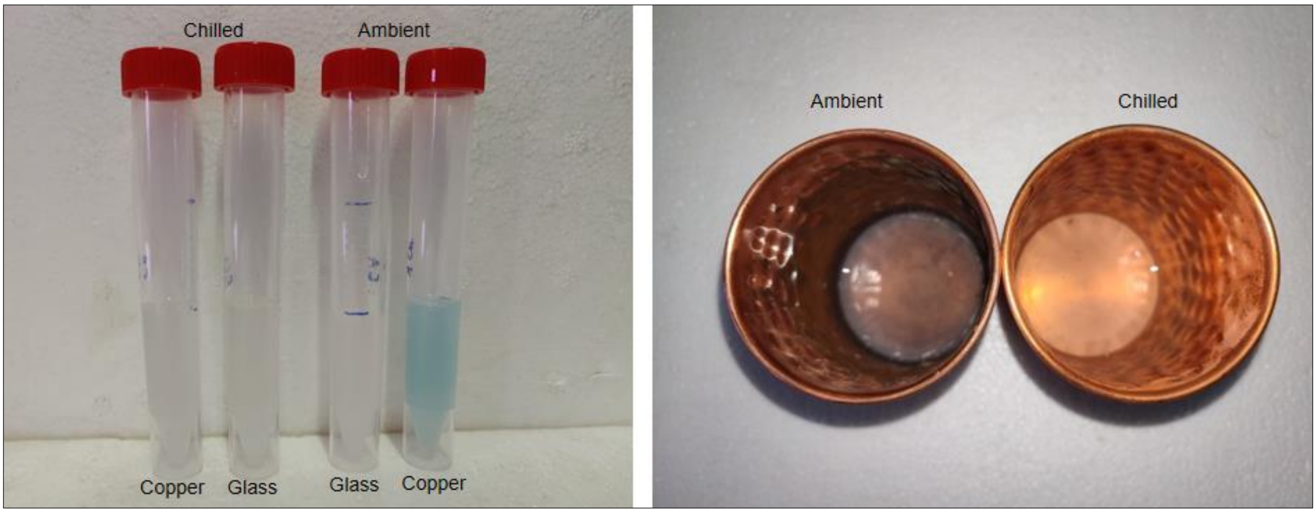

Fig. 2. Color changes in the different storage conditions

In the ambient copper conditions, the color value was significantly increased in (L*), (a*), and (b*), which indicated the increased intensity of light for the sap (Pattee *et al*., 1991) [18]. In addition, under ambient conditions, the color value of the sap drastically changes due to an interaction with the copper ion. When the sap is kept in copper, the bluish color is formed due to a lack or low presents of reducing sugar (Daniels *et al*., 1960) [7]. The sap's color became bluish at 20 h of storage. There was so much difference which can differentiate by our naked in the copper vessel itself after 24 h of storage (Fig. 2).

Conclusions

The pH, TSS, relative density, and dry matter values of the sap were dramatically lowered in the varied storage conditions, whereas the TTA and color values elevated. Temperature and time employed in storage were the parameters considered Nevertheless, all parameters were maintained consistently while the sap was kept in chilled copper. The color changes in sap held in copper at ambient temperatures are the most noticeable. However, other characteristics were kept lower than in sap stored in copper under chilled conditions. Although the sap in glass under ambient conditions was the most damaged, the sap in glass under refrigerated settings likewise showed lower deterioration in all parameters. Storing sap in copper under cold conditions for 24 hours would not cause any issues, but ambient storage would require additional research into the formations caused by interactions with the copper. Due to its strong thermal conductivity, the copper vessel may be used to collect sap at chilled temperatures and will be more effective.

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Conflict of Interest Statement
The authors have no conflict of interest.

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