VARIATIONS IN VITAMIN C CONTENT AND PHYSICO-CHEMICAL COMPONENTS OF SUGARCANE SUPPLEMENTED WITH *ZINGIBER OFFICINALE* AND *CITRUS LIMON*

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Abstract

A study was conducted to determine the quality of sugarcane juice extract stored at different temperatures. Both raw and pasteurised sugarcane juice are supplemented with *Zingiber officinale* and *Citrus limon*. The mixture is then stored for 21 days at two different temperatures and examined to measure variations in vitamin C concentration and physiochemical parameters like pH, viscosity, titrable acidity, and total soluble solids. The juice stored at room temperature (24°C) could only be kept for a week, whereas the juice stored at low temperature (4°C) could be kept in good condition for two weeks. When cane juice is used as raw, spoilage is higher; when it is pasteurised, it is relatively less. Significant fluctuations in vitamin C levels and the number of microorganisms, particularly lactic acid bacteria, were seen when cane juice was stored.

Key words: Sugar cane juices, pasteurization, *Z. officinale* and *C. limon,* Vitamin C content, Phsicochemical characterization, Shelf-life

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1. Introduction

A wide spectrum of foods and substances with a variety of bioactive components are included in nutritional foods and nutritious products, which are thought to provide health advantages and can help reduce disease. When ingested at effective levels as a component of a varied diet on a regular schedule, foods that are fortified, enriched, or enhanced with products that offer health benefits beyond the inclusion of necessary nutrients like vitamins, minerals, biologically active phenolics, etc., is considered to be functional foods (Galanakis, 2021). Due to its high sucrose concentration and low fibre content, sugarcane—also referred to as noble cane is one of the world's most significant industrial crops. Given that sugarcane accounts for 70% of global sugar production, it serves as the industry's primary ingredient. Due to its numerous health benefits, sugarcane juice is frequently used in traditional medicine to treat jaundice (Sing et al., 2015).

Simple sugars cause sugarcane juice to generally deteriorate fast (Krishnakumar and Devidas, 2006). Enzymatic and chemical (acid) imbalances also have an impact on the quality of cane juice (Singh et al., 2006). Currently, harvested canes are frequently kept at room temperature in the shed until they are processed. When the juice is extracted, it needs to be refrigerated and kept at room temperature right away before being shared. There have been reports of certain juice quality alterations resulting from a delay in the extraction of harvested sugarcane (Mishra et al., 2022).

As the most prevalent water-soluble substance that can participate in one-electron processes, vitamin C is a vital micronutrient and a crucial component of practically every living thing's metabolism (Carr and Maggini, 2017). Vitamin C has a variety of purposes in humans, but its primary roles are as an antioxidant and a cofactor for the enzymes mono- and dioxygenases (Chambial et al., 2013). Numerous scientific studies have proved the functions of vitamin C as an antioxidant in humans. By scavenging free radicals, vitamin C shields lipids, proteins, and DNA from. Although it has a specialised purpose in various organs, vitamin C is employed as an antioxidant throughout the body (Traber and Stevens, 2011).

Only a few vertebrate species humans included that cannot synthesise vitamin C are regarded as vitamins (Drouin et al., 2011). In fact, nutrition is the greatest way for people to get vitamin C, and the main source of this vitamin is plant-based foods. While synthetic vitamin C and vitamin produced from plants are identical chemically, the micronutrients and phytochemicals present in fruits and vegetables can influence the vitamin's bioavailability (Carr and Vissers, 2013).

It has been found that spices include bioactive chemicals that give food items antibacterial, preservative, and antioxidant qualities. The food processing and pharmaceutical sectors have shown a significant deal of interest in the bioactive qualities of chemicals found in ginger (*Zingiber Officinale*). Ginger's stinging flavour, pleasant scent, and carminative qualities make it an essential component in food preparation all over the world. Ginger's active ingredient possesses anti-inflammatory, antibacterial, antioxidant, anti-cancer, and cardiovascular protective properties (Mao et al., 2019). Phenolic chemicals found in plants have a variety of antioxidant and lipoxygenase inhibitory qualities that make them useful for treating inflammatory illnesses. Very little research has been done on the bioactive effects of sugarcane juice enhanced with extract from ginger (*Z. officinale*), aside from its nutritional value.

With regard to the impact of temperature, storage duration, and the addition of a bioactive component (*Z. officinale* and *Citrus limon*), this study examined the correlations between storage parameters and vitamin C content in sugarcane juices. The modification of vitamin C content was determined by selecting two storage temperatures (4ºC and 24ºC) and a storage time of 21 days, either with or without bioactive components (*Z. officinale* and *C. limon*).

2. Materials and Methods

Materials

Sugarcane stems were purchased from the local cultivated land. All the chemical used in the present study were analytical grade

Formulation of sugarcane juices

Eight separate portions of sugarcane juice were made: one each of fresh raw juice, raw juice with ginger (1%), raw juice with lemon (1%), raw juice with ginger and lemon (1%), and raw pasteurised juice. Additionally, there were four different types of pasteurised juice: one with ginger (1%), one with lemon (1%), and one with ginger and lemon (1%).

Sugarcane juice processing

The stems of the sugarcane were cleaned with ethanol, peeled, then crushed in a cane grinder to extract the juice. Ethanol and double-distilled water were used to sanitise the sugarcane crushing apparatus. The cane juice was put into previously cleaned glass flasks, pasteurised for 20 minutes at 80°C in a thermostatic bath, and then cooled in an ice bath until it reached 37°C. Ginger and lemon were added to the cane juice, which was then maintained in an aseptic atmosphere at 4°C.

Storage condition

The formulations were separated into two groups and kept for the full 21-day shelf life of pasteurised commercial fruit juices, at 4°C and 24°C (considered room temperature). Because it is comparable to the temperature of both residential and commercial freezers, the storage temperature of 4°C was chosen to evaluate the items' standard commercialization settings.

PH

At room temperature, the pH of the cane juices was measured using a pH metre (Systronics India Pvt. Ltd., India) (Guerra and Mujica 2010).

Total soluble solids

total soluble solids were measured in accordance with AOAC standards (2004) using a digital refractometer model HI96801 from HANNA Instruments, India and the analysis was carried out six times.

Titrable acidity

0.1 M NaOH was used to calculate the total acidity; this was the tipping point when a pH value of 8.3 was achieved.

Measurement of vitamin-C content

The amount of vitamin C present in the sugarcane juice was measured using the 2, 6-dichlorophenolindophenol under spectrofluorometer technique. The total vitamin C concentration was measured at a wavelength of 350–430 nm (China, 2010; Yang et al., 2022). The substances employed were 5% metaphosphoric acid for the determination of the vitamin-C content. Solutions: 0.025% 2, 6-dichlorophenol indophenol reagent; ascorbic acid standard solution were used.

Measurement of microbial content

The procedure of serial dilution and plating was employed to estimate the bacterial content present. The *Lactobacillus casei* survival on the sugarcane juice was performed on MRS agar (Himedia, Mumbai, India) and anaerobic incubation (Anaerobac, Probac®) at 37°C for 72 h (Tharmaraj and Shah, 2003).

Color

The juice from the sugarcane has a delicate lemony yellow tint. Heating at room temperature causes the colour to fade over time, resulting in a light-whitish, homogeneous turbid solution by the end of the week. This colour fading is relatively slower when stored in a refrigerator or preserved with preservatives after heating.

Statistical analysis

The results were analysed using IBM SPSS Statistical Software, Version 20.0. Every experiment was conducted in triplicate (n = 6), and the mean ± standard deviation (SD) was used to express the data using Microsoft Excel software. Graph were prepared by using Graph pad prism version 9. An Anova test was conducted comparing the groups to determine any significant differences, and this was followed with a post-doctoral test. the least significant difference (LSD) post hoc comparisons and Duncan's multiple range testing (DMRT).

3. Results and discussion

Variation in pH sample

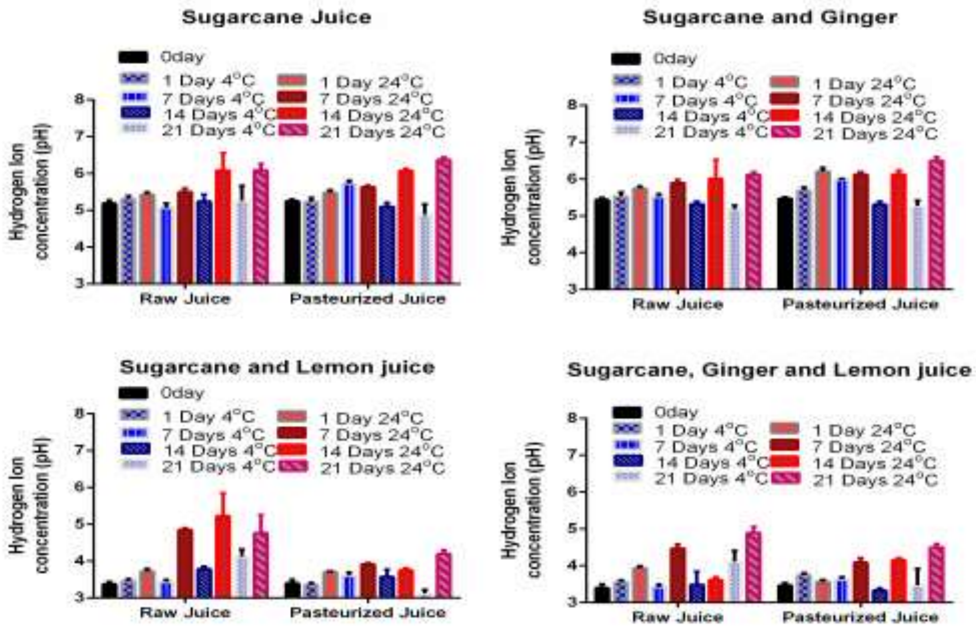


Figure 1. pH variation in sugarcane juice stored at different temperature. Sugarcane juices used as raw juice and pasteurized. pH of the sugarcane juices enriched with Z. Officinale, C. limon and *both Z. officinale and C. limon* respectively were measured. Values are expressed mean ±S.D

The pH of content of the sugarcane raw juice samples was found to be 5.19. The same increased to 6.09 when the sample stored at room temperature for 21 days, on contrary the pH of the raw juices showed no change in pH when it stored in 4ºC after 21 days of storage. When the sugarcane juice is pasteurized and stored at room temperature for 21 days the pH increased to 6.37, which is similar to the pH recorded in non-pasteurized samples. Similar to non-pasteurized sample stored at 4ºC, the pasteurized sample showed decrease in pH value after 21 days of storage. When the raw juice is added with ginger increased the pH in both pasteurized and non pasteurized samples. The pH of the sample increased significantly after 21 days of storage. Interestingly the sample stored at 4ºC retained the pH similar to day one after 21 days of storage. Both pasteurized and non-pasteurized samples added with concentrated lemon extract decreased the pH significantly and such decrease in pH was maintained when the samples were stored at 4ºC, however when the sample stored at room temperature the pH in both pasteurized and non-pasteurized sample showed a considerable increase after 21 days. The raw and pasteurised sugarcane juice enhanced with *Z. officinale* and *C. limon* had pH values between 4.89 and 4.49, which is consistent with the findings published by Guerra and Mujica (2010). As required by the Ecuadorian technical standard (2002), the observed pH of sugarcane juice was somewhat equal. Furthermore, both in pasteurised and raw juices, *C. limon* considerably reduced the pH of sugarcane. Subsequently, the cane stem juice kept at ambient temperature saw a quicker decline than the cane stem juice kept at 4°C. Chauhan et al. (2002) observed similar outcomes regarding the preservation of sugarcane juice.

Variation in titrable acidity

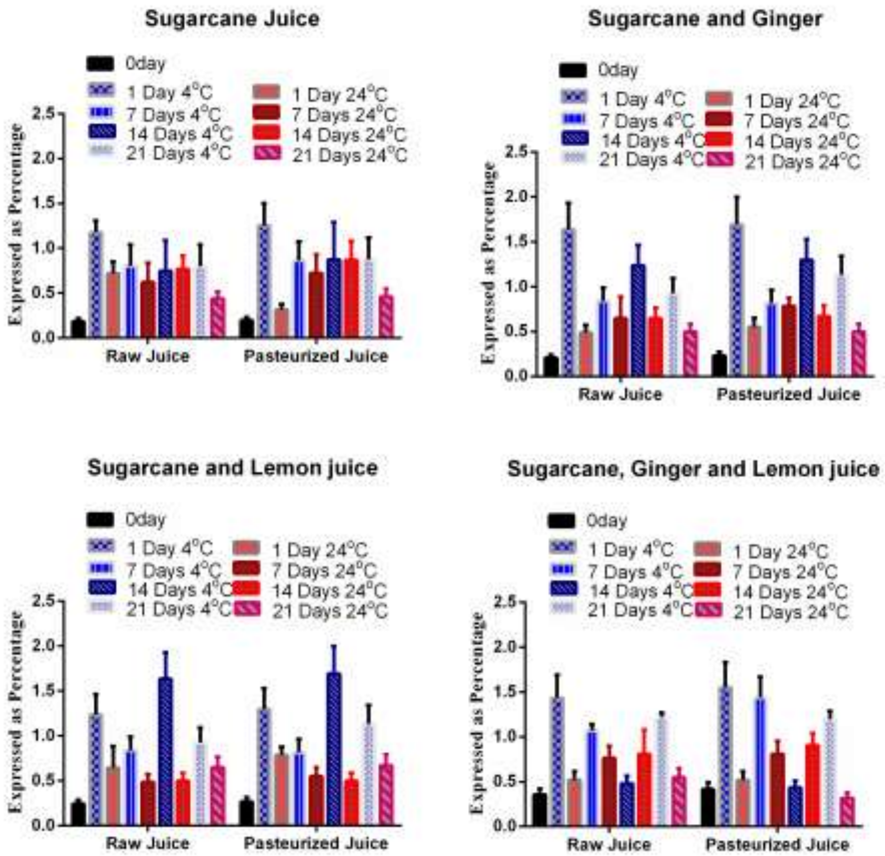


Figure 2. Titrable acidity variation in sugarcane juice stored at different temperature. Sugarcane juices used as raw juice and pasteurized. pH of the sugarcane juices enriched with Z. Officinale, C. limon and *both Z. officinale and C. limon* respectively were measured. Values are expressed mean ±S.D

During storage, the titrable acidity of the raw juice changed considerably in both pasteurized and non-pasteurized, while the raw juice supplemented with ginger and lemon juice increased the titrable acidity initially and decreased as the storage period increased. The supplements might have metabolised some sugars present in the sugarcane juice, resulting in the production of acids and consequent increases in the acidity. The raw juice and the pasteurized juice supplemented with the lemon showed greater rate of acidity.

Figure 2 represent the mean values for sugarcane juice's acidity, indicating significant findings. The sugarcane juice's acidity ranged from 0. 18 to 1.3% depending on the course of treatment and length of storage according to the results. Sample had the lowest acidity value (0.68%) after being kept at room temperature for the first day, while the highest value (0.9%) was observed after the 21st day of storage. Both pasteurised and non-pasteurized samples supplemented with *C. limon* exhibited a similar increase in titrable acidity initially. The value was similar from day 1 to day 14 when the samples stored at 4ºC. Similarly the samples added with ginger and *C. limon* showed no discernible rise in titrable acidity in either pasteurised or non-pasteurized sample when the ample stored at 4ºC on later stage. However when the same sample stored at room temperature for 21 days showed decrease in titrable acidity. A corresponding increase in pH was brought on by a decrease in acidity. However we never found any direct relationship between titrable acidity and pH variation. According to a related finding published by Abbo et al. (2006), the acidity of Sour soup juice rose with a comparable decrease in pH. Production of acetic and lactic acids may be the cause of excessive acidity and low pH. Moreover juice became more acidic throughout period of storage. Sugarcane juice stored for a prolonged period increased the acidity level as reported by Bhupinder et al. (1991).

Variation in viscosity

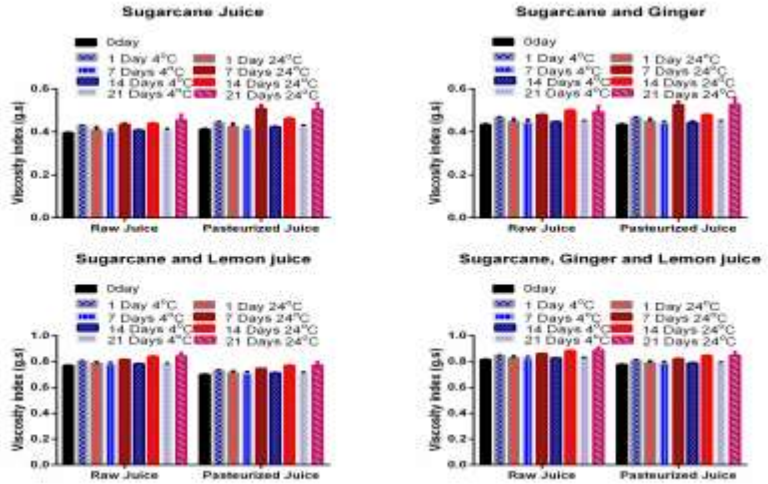


Figure 3. Variation viscosity in sugarcane juice stored at different temperature. Sugarcane juices used as raw juice and pasteurized. pH of the sugarcane juices enriched with *Z. Officinale, C. limon* and *both Z. officinale and C. limon* respectively were measured. Values are expressed mean ±S.D

The viscosity index of the sugarcane juices are presented in figure 3. Regardless of the added component (*Z. officinale* and *C. limon*), all texture attributes remained similar to those observed in the pure product. The formulations showed no differences in the texture parameters during storage. The maintenance of the texture parameters during refrigerated storage (4°C) is important, since it indicates that the addition of the *Z. officinale* and *C. limon*, components does not modify the product and that after 21 days of storage, the sugarcane juices are similar to the newly procured. This observation was supported by the previous study reporting that the viscosity of mandarin juices rose at room temperature and reduced in the refrigerator (Pareek et al., 2011).

Variation in total soluble solids

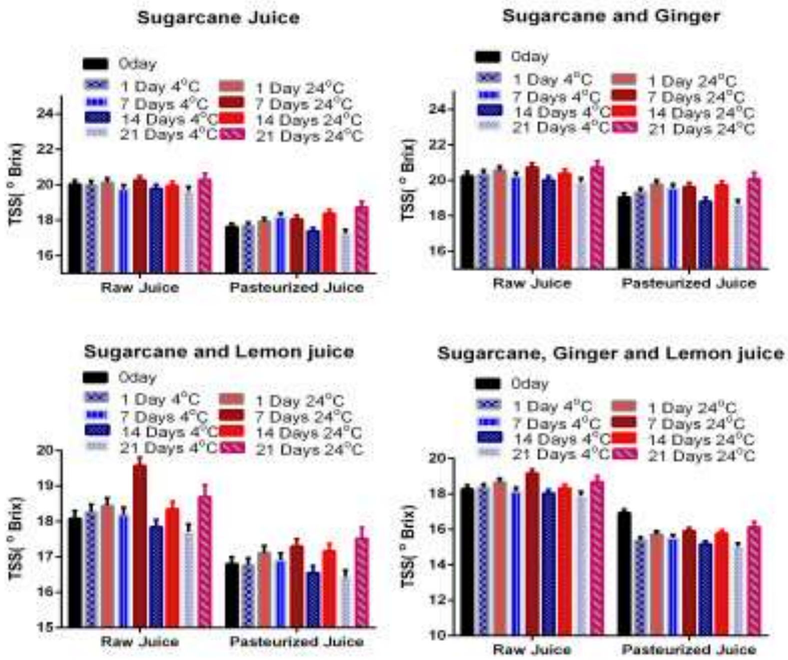


Figure 4. Variation in total soluble solids in sugarcane juice stored at different temperature. Sugarcane juices used as raw juice and pasteurized. pH of the sugarcane juices enriched with Z. Officinale, C. limon and *both Z. officinale and C. limon* respectively were measured. Values are expressed mean ±S.D

TSS is determined by the index of refraction. Brix is widely used to determine the concentration of sugar in a product. Degree Brix usually consider equivalent to sugar in solution. Raw sugarcane juice doesn't showed any significant change in the TSS level when the storage time increased, except on day 7. however significant decrease in TSS value was observed when the cane juice was pasteurized. Raw cane juices supplemented with *Z. officinale* shows greater TSS, on contrary raw juice supplemented with *C. Limon* shows considerable decrease in TSS. Pasteurized juices supplemented with *Z. officinale* and *C. limon* shows lesser TSS compared with respective internal control (Figure 4).

During storage, the sugar level of the cane juice dropped as a result of fermentation. The pasteurised cane juices had lower Brix values than the unpasteurized samples. The pH decreased as carbohydrates were stored because bacteria fermented them. Prior research on the addition of anola and lemon to sugarcane juice showed that these ingredients increased the juice's nutritional content and enhanced its clarity by serving as a preservative and coagulant to remove contaminants, which raised the beverage's overall appeal. Juice may degrade quickly, thus developing a preservation technique and increasing production volume are necessary to extend its shelf life and boost its marketability. A rise in TSS was also seen in the juice sample that was kept at room temperature prior to spoiling. The rise might have been brought on by the juice's microorganisms breaking down the total sugars during storage into simple sugars and acids. Bhupinder et al.'s (1991) findings are consistent with these observations.

Variation in *Vitamin-C*

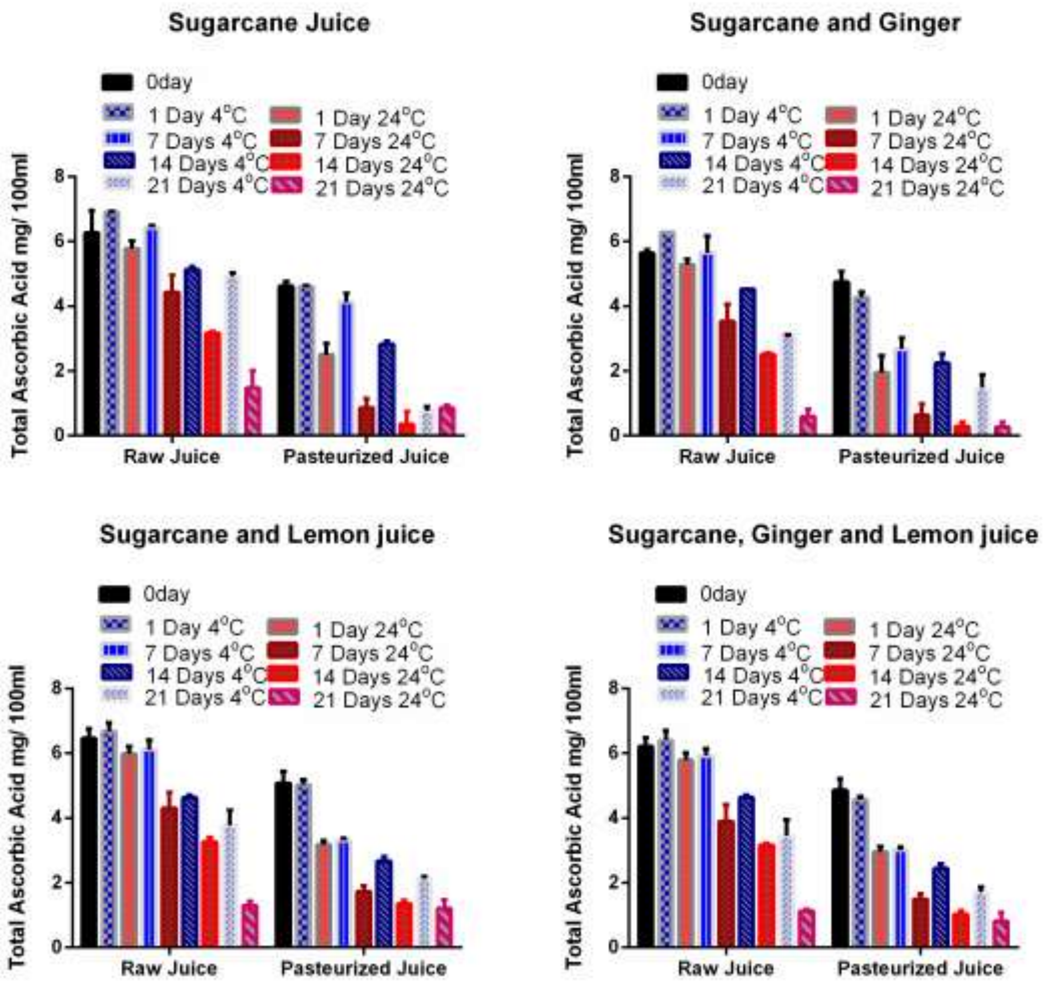
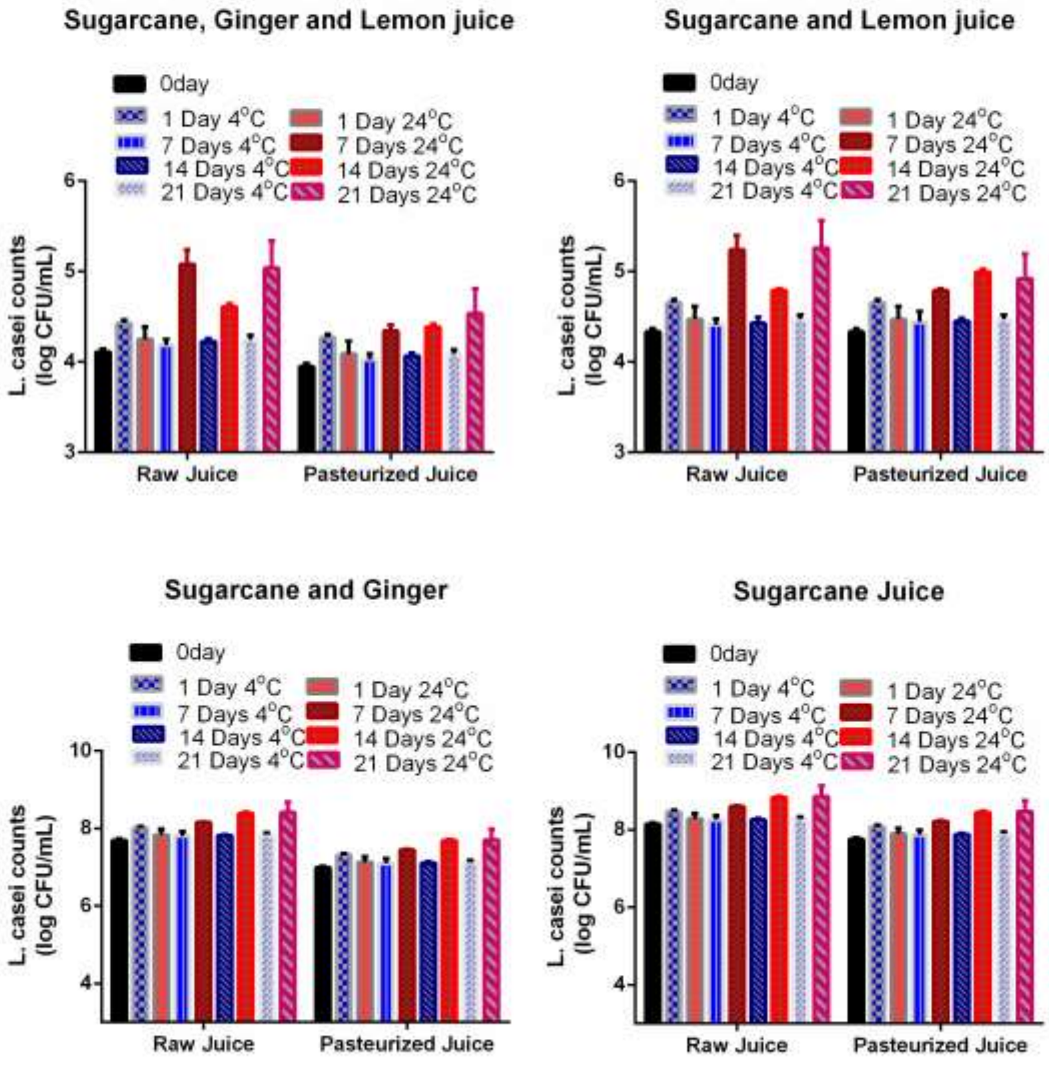


Figure 5. Variation in vitamin C concentration in sugarcane juice stored at different temperature. Sugarcane juices used as raw juice and pasteurized. pH of the sugarcane juices enriched with Z. Officinale, C. limon and *both Z. officinale and C. limon* respectively were measured. Values are expressed mean ±S.D

Strength of Vitamin-C decreases as the storage time increases. In the case of room temperature it was decreasing at a faster rate. In the case of refrigerated sample it was decreasing more slowly than room temperature. In the case of additive like ginger and lemon it was also found to be decreasing but lemon added sugarcane with slower rate as compared with the other group (Figure 5). The vitamin C content of sugarcane juices pasteurized and kept in refrigerator dropped (Figure 5). The decreases in vitamin C detected by the analyses at room temperatures were the same as for the pasteurized juices stored in room temperature, the highest loss of vitamin C values was observed for the raw juice, and the lowest loss was observed for raw juice fortified with *Citrus limon* and stored at 4ºC. Food-related vitamin C depletion is a serious health concern. Nonetheless, it is crucial to remember that visual factors like colour, flavour, consistency, and juiciness are mostly responsible for commercial and consumer appeal. Vitamin C concentration in sugarcane decreased gradually during room temperature storage (Giannakourou and Taoukis, 2021).

Total plate count (TPC)



Mean values regarding the TPC of sugarcane juice are given in Figure 7 which indicated significant results. Results showed that the TPC of sugarcane juice at different treatments and different storage periods ranged from 4.2×10^4 to 8.3×10^4 CFU/mL The minimum value (4.2×10^4) of total plate count has been found in the pasteurized sample on 0th day compared to raw juice without pasteurization whilst the maximum value (8.3×10^5) was exhibited in the jaw juice sample which was stored at room temperature for 21 days. All the samples from (6.96×10^4 CFU/mL), and to (7.92×10^4 CFU/mL) showed near to maximum total plate counts on the 21 day when sugarcane juice samples were kept at refrigeration and room temperature respectively Whereas pasteurized sample added with *C. limon* showed minimum total plate count at room temperature at 0 days. This means that the total plate count is gradually increased during the refrigeration temperature and room temperature. The degree of decrease in the microbial population was also lower at refrigeration as compared to room temperature. The highest total plate counts were found during the storage of controlled samples which are pasteurized juice after that an addition of citric acid. The juice added with *C. limon* had lower bacterial counts as compared to other samples because the juice's acidic state significantly inhibited bacterial development. According to Richa et al. (2011), bacterial contamination can happen at several points during the juice processing process, including on the sugarcane, roller crusher, collecting vessel, ice, staff hands, and filter cloth. It's possible that the organism's development was slowed down by the low storage temperature.

4. Conclusion

Important economic factors in the sugarcane juice industry are the quantity and quality of juice produced. According to the study's findings, sugarcane should be stored at a low temperature in order to preserve its juice output. For two weeks, the canes can be kept at 4°C and still provide high-quality juice. The sugarcane juice's pH, total soluble solids, and other physic-chemical properties began to change on day one when it was held at room temperature, significantly lowering the output of juice. The colours of juice obtained were darker on the second day at room temperature than the juices obtained from canes stored at 4°C and the flavour was also different. The newly extracted, unpasteurized juice could only be stored at 4°C for seven days, according to the results of the experiment's second phase. When *C. limon* is added to juices and they are kept at 4°C, the content of vitamin C is preserved. Furthermore, the addition of *C. limon* by itself and *C. limon* combined with ginger to the juice postponed the quality degradation that occurs during storage, as evidenced by changes in colour and flavour that are followed by a change in viscosity. Bacterial counts also reduced in the sugarcane juice supplemented with *C. limon.*

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