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Full Length Research Article

Sugarcane Supplemented with *Zingiber officinale* and *Citrus limon* Enhance Aroma and other Physicochemical Components

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Abstract

The objective of the present work was to evaluate the change in physicochemical characteristics and sensory properties in direct addition of the *Zingiber officinale* and *Citrus limon* on raw and pasteurized juice. The incorporation *of Zingiber officinale* and *Citrus limon* separately on raw cane juices resulted in modulation of total phenolic compound, total flavonoids and total tanins compounds. Pasteurized sugar cane juices supplemented with *Zingiber officinale* and *Citrus limon* showed no significant impact on the physicochemical properties of sugar cane juices. The pH of the raw and pasteurized sugar cane juices were significantly reduced when added with *Citrus limon*. However, the same does not show significant change when added with *Zingiber officinale.* The bacterial count was significantly less in the pasteurized juices added with *Zingiber officinale* and *Citrus limon* compared to the raw cane juices. Sensory evaluation of *Zingiber officinale* and *Citrus limon* enriched raw sugar cane juices were well received compared with the pasteurized juices enriched with the same.

Key words**:** Sugar cane juices, Pasteurization, *Zingiber officinale, Citrus limon,* Physicochemical characterization

The world's most significant commercial crop is sugarcane (*Saccharum officinarum* L.). India is behind Brazil in terms of global sugarcane production, according to the Sugarcane Statistical Report (2008). The main reason sugarcane is grown in India is to make sweeteners like sugar, jaggery, and khandasari. Non-alcoholic sugarcane juice is a viscous, opaque liquid with colour from light to dark green [1]. The pH of sugarcane juice is between 4 and 5 and it is an energetic food with significant amounts of carbohydrates, minerals (potassium, calcium, phosphorus, magnesium, and iron), and vitamins (complex B and C) [2]. The chemical composition of the juice is influenced by the type of sugarcane, its maturity, the climatic and soil conditions, as well as the region of the stalk from which it is extracted.

Numerous phenolic compounds found in sugarcane have been found to have a variety of biological effects, including anti-oxidant, anti-inflammatory, anti-thrombosis, immune-stimulating, and anti-stress properties [3-6]. After only a few hours of extraction, the juice becomes unusable due to rapid fermentation brought on by the high levels of sugars, traces of polyphenols and organic acids, and high polyphenol oxidase activity [7-8]. The main concern is about the quality degradation brought on by microbial spoilage [9]. Despite the enormous potential for the beverage industry, commercialization of sugarcane juice is limited to the immediate consumption of the raw juice shortly after extraction. Few studies reported that pasteurization under suitable conditions could solve these problems [10-11]. But whether pasteurization could keep all the biological components in the raw sugar cane juices without much modification is unknown.

In order to better understand the impact of spices phytochemicals on different physico-chemical properties of cane juice, studies on the enrichment of cane juice with spices may be useful. Consequently, the current investigation is designed to assess the physico-chemical characteristics and sensory evaluation of *Z. officinale* and *citrus limon* enriched cane juice under pasteurize and un-pasteurize conditions

MATERIALS AND METHODS

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 aliquot of sugarcane juice were prepared: Fresh raw juice, raw juice with ginger (1%), raw juice with lemon (1%), raw juice with ginger and lemon (1%), raw pasteurized

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juice; pasteurized juice with ginger (1%), pasteurized juice with lemon (1%) and pasteurized juice with ginger and lemon (1%).

Sugarcane juice processing

The sugarcane stems were sanitized with ethanol, peeled, and the juice extracted by crushing them in a cane grinder. The sugarcane crushing machine was sanitized with ethanol and double distilled water. The cane juice was placed in glass flasks which were cleaned priorly, pasteurization was carried out at 80°C for 20 min in a thermostatic bath and cooled in an ice bath until reaching 37°C. The cane juice was added with ginger and lemon and kept at 4°C in an aseptic environment.

Physico-chemical Characterization

pH

At room temperature, the pH of the cane juices was measured using a pH metre (Systronics India Pvt. Ltd., India) [12].

Color

The color of sugarcane juice was determined at 540 nm using visible spectrophotometer with slight modification [13].

Turbidity

The method described by Guerra and Mujica [12] was slightly modified in order to determine the turbidity of cane juice. There were two portions of cane juice. As a blank, one portion was filtered through Whatman No. 1 filter paper using silica as the filtering aid. In the second part, a visible spectrophotometer was used to calculate the percentage of transmittance at 720 nm.

Filterability

The Guerra and Mujica method were used to test the filterability of sugarcane juices [12]. Under the same circumstances, the rate of filtration of the two sugar solutions (pasteurized and unpasteurized) was compared. The juices were filtered using Whatman No. 1 filter paper for 3 minutes, following which the filtered volume was determined. This process was then repeated for all juices that had been supplemented. The ratio of filtered volumes was used to calculate the percentage of filterability.

Moisture content

The moisture content determined by method given by Khatoon *et al*. [14] using a hot air oven (105 ºC for 6 hours).

ASH content

Ash content estimated by using Muffle furnace (550 ºC for 6 hours) and calculated by weight difference.

Total soluble solids

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

Biochemical components

Determination of total phenol content

Spectrophotometric analysis using Folin-method Ciocalteu's was used to determine the total phenol content of cane juices [15]. 100 mL of the sample (5%), 900 mL of water, 1 mL of the Folin-Ciocalteu reagent (1:2, v/v), and 2 mL of 10% sodium carbonate were added one at a time, thoroughly mixed, and then incubated for an hour at room temperature. A visible spectrophotometer was used to measure the absorbance at 765 nm. As a benchmark, gallic acid was used, and the total phenolic content was expressed as milligram of Gallic acid equivalent (GAE) per gram of sample.

Determination of total flavonoid content

The Aluminium chloride method was used to calculate the total flavonoid content of cane juices [16]. A sample aliquot of 200 µL (5%) was diluted to 2 mL. Incubation took place for 30 minutes at room temperature using 100 µl of 10% aluminium chloride and 100 µl of 1 M potassium acetate. At 415 nm, the absorbance of the reaction mixture was measured. Total flavonoid content was measured as micrograms of quercetin equivalent per gram of sample using quercetin as the reference.

Determination of tannin content

Folin-method Ciocalteu's [17] was used to determine the cane juice's tannin content. Briefly A sample aliquot of 100 µL (5%) was added to 900 µL of water, 1 mL of Folin-Ciocalteu reagent (1:2, v/v), and 2 mL of 10% sodium carbonate. These components were thoroughly mixed, and the mixture was then left to sit at room temperature for 1 h. A visible spectrophotometer was used to measure the absorbance at 765 nm. The amount of total tannin was calculated as milligram of tannic acid equivalent (TAE) per gram of sample using tannic acid as the reference.

Bacterial viable count

Using MRS agar (Himedia, Mumbai, India) and anaerobic incubation (Anaerobac, Probac®) at 37°C for 48 hours, *L. casei* survival on sugarcane juice samples was tested [18]. The plate count method was used to assess the bacterial viable count (CFU/ml). The samples were prepared with various dilutions in 0.2% saline water before being put on MRS agar plates and incubated for 24 hours at 37 °C. To determine the amount of viable bacteria in the medium, the number of colonies obtained from various media was employed.

$$\text{CFU/ml} = (\text{No of colonies} \times \text{Dilution factor}) / \text{Volume of Sample}$$

Sensory attributes

By using an unstructured scaling method or quantitative descriptive analysis (QDA) [19], sensory characteristics such as colour, texture, hardness, sweetness, saltiness, pleasantness, spicy, and overall acceptance of the product were assessed. 20 people, both male and female, in the age range of 20 to 30, made up the panel. They frequently took part in the sensory evaluation of cane juices samples. Oral explanations of the panelists of the sensory descriptors were collected. One of the panelists was given information about *Zinger officinale* and/or *Citrus limon* enriched cane juice, but they were unaware of the same. The scorecard was composed of a 15 cm long horizontal line with anchor points spaced 1.5 cm apart on each end. Each anchor point is identified by a word or phrase.

Statistical analysis

Each experiment was run in triplicate (n = 6), and using Microsoft Excel software, the results were expressed as mean ± standard deviation (SD). ANOVA test was performed between the groups to find out the significant change followed by the post doc test. The sensory scores were subjected to an analysis of variances (ANOVA) to identify preferences for sensory attributes that were statistically significant ($P< 0.05$). Duncan's multiple range testing (DMRT) and least significant difference (LSD) post hoc comparisons. Version 20.0 of IBM SPSS Statistical Software was used to analyses the findings.

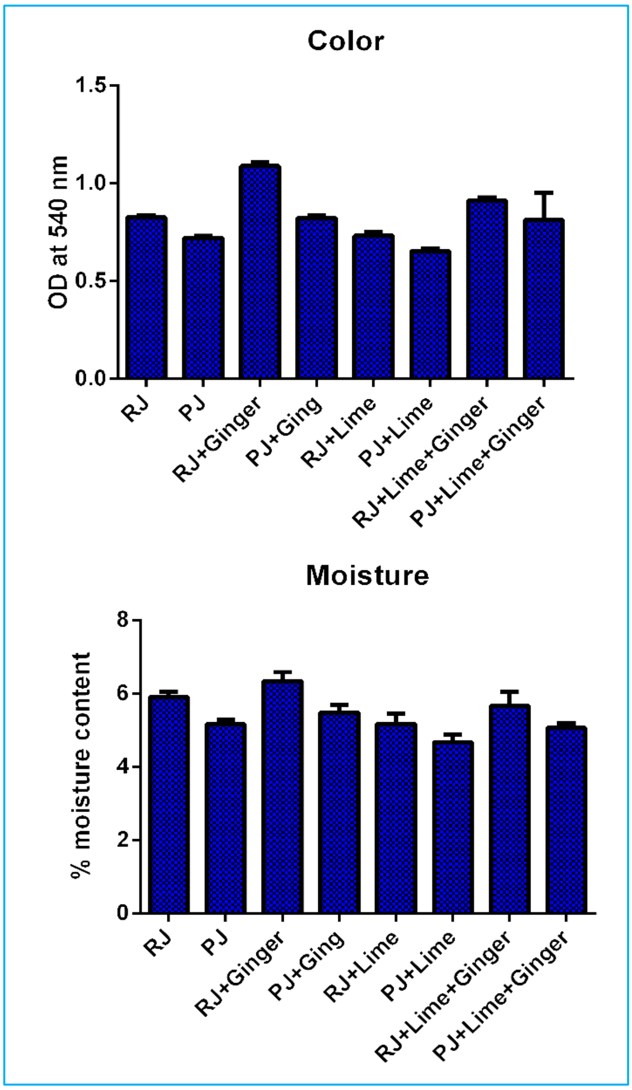

Fig 1 Physicochemical characteristics of the sugarcane juices enriched with *Z. officinale* and *C. limon* –Color and Moisture content. Values are expressed mean ± S.D

RESULTS AND DISCUSSION

The results of physical properties like pH, color, moisture, turbidity, total soluble solids and ash content of *Z. officinale* and *C. limon* enriched cane sugar juice is represented in (Fig 1-2). The color of the sugarcane juice varied much when the raw juice was added to the supplements the maximum absorbance were recorded when the raw juice were analyzed was 0.827 and the same increased to1.09 when the ginger was added. On contrary, the absorbance value decrease when the sugarcane juice is pasteurized. Pasteurized and un-pasteurized juices added with lemon showed decrease in absorbance value.

Moisture content is one of the important parameters determine the quality, stability and shelf-life of foods during storage. *Z. officinale* enrichment showed a marked increase in moisture content in raw juices but a very slight increase in moisture content observed for pasteurized sugarcane juice enriched with *Z. officinale*. Both pasteurized and un-pasteurized added with lemon showed no significant changes in moisture content. However, the pasteurized juice added with lemon showed decrease in moisture content and the same increased when both ginger and lemon was added (Fig 2).

Turbidity of *Z. officinale* and *C. limon* quantified by measuring the percentage of transmittance of *Z. officinale* enriched sugarcane juices at 720 nm. Turbidity of the raw juice was 31.80% and the same increased to 38.58% when *Z. officinale* added to the raw juice. Similarly, the pasteurized cane juice showed a turbidity level of 26.35% and it increased to 33.61% when *Z. officinale* added to the pasteurized cane juice. About 7% increase in turbidity was observed between internal control and *Z. officinale* and *C. limon* enriched juices. Turbidity level showed no change when *C. limon* added to pasteurized and raw juices. On contrary a marginal increase in turbidity was observed when *Z. officinale* and *C. limon* added to the pasteurized and raw juices (Fig 2).

Z. officinale and *C. limon* enriched sugarcane juices showed a marginal increase in filterability upon enrichment with its internal control (pasteurized and raw juices). However, results showed pasteurized juices filterability comparatively higher than the raw juices. Very small increase in filterability observed at *Z. officinale* enriched cane juices compared to the raw juices and slightly progressive increased filterability with Citrus Limon enrichment. In addition, with *Z. officinale*, *C. Limon* enriched pasteurized cane juices showed continuous raise in filterability (Fig 2).

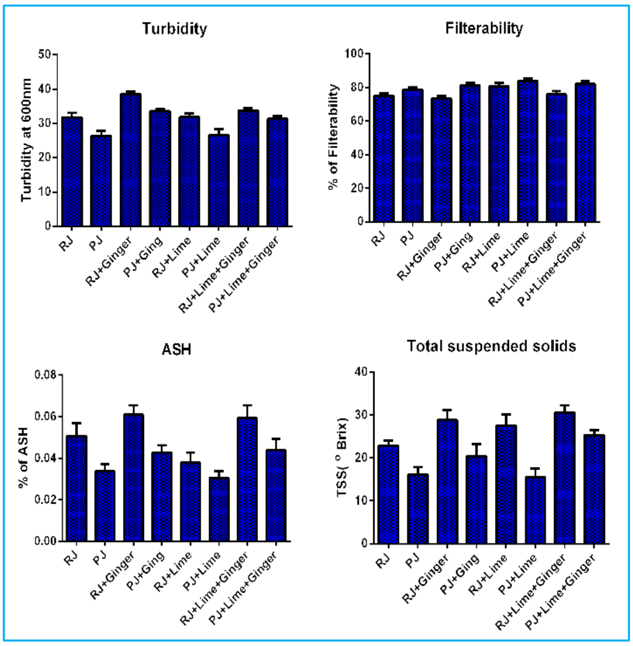

Fig 2 Physicochemical characteristics of the sugarcane juices enriched with *Z. officinale* and *C. limon* –turbidity, filterability, ash and total suspended solid content. Values are expressed mean ± S.D

Ash content in the pasteurized and un-pasteurized sugarcane juices were varied marginally. Pasteurized juices with less ash content were recorded and the same increased when the ginger was added, however such increase was not statistically significant. However pasteurized cane juice added with lemon showed decrease in ash content. The TSS or sugar content, measures the carbohydrates, organic acids, proteins, fats and minerals of the fruit. 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. Significant decrease in TSS value was observed when the cane juice was pasteurized. Raw juices supplanted with *Z. officinale* and *C. limon* shows greater TSS and it shows significant difference with raw juices. Pasteurized juices supplemented with *Z. officinale* and *C. limon* shows higher TSS compared with respective internal control (Fig 2).

Total phenolic content of the sugarcane extract raw juice samples was found to be 45.52 gallic acid equivalents per 100 mg of the extract. The same decreased when the raw sugarcane juices enriched with *Z. officinale* and *C. limon*. Upon pasteurization the phenolic content decreased 17.63 gallic acid equivalents per 100 mg of the extract. Moreover, the Phenolic content decreased when the pasteurized juices added with *Z. officinale* and *C. limon* significantly when compared with the internal control and raw can juices (Fig 3).

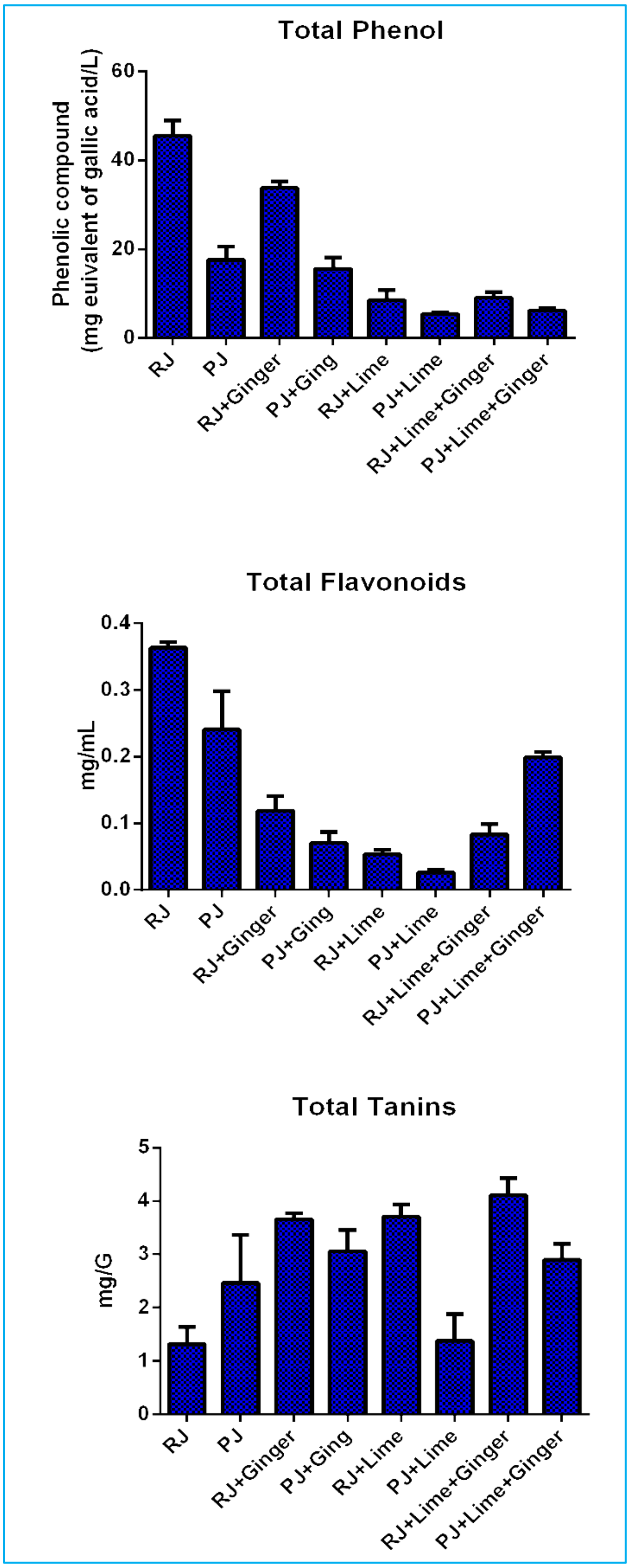

Fig 3 Biochemical components of the sugarcane juices enriched with *Z. officinale* and *C. limon* – total phenol, total flavonoids and total tanin contents. Values are expressed mean ± S.D

The results were expressed in µg of catechin equivalent (CE) per mg of the extracts. Flavonoid content in raw juices was 0.36 and 0.24 µg of catechin in raw and pasteurized cane juices respectively. The results shows that the flavonoid content in the sugar cane juice decrease when it enriched with *Z. officinale* and *C. limon*. Similarly, the cane sugar juices when pasteurized, decreased the flavonoid content, however the same showed marginal increase when the pasteurized juice is enriched with *Z. officinale* and *C. limon* (Fig 2).

The *L. casei* survival during storage is shown in (Fig 4). In the pasteurized sugarcane juice the survivability was significantly lesser than the raw juice. The raw juice added with ginger and lime does not differ much without the supplements. The viability was lesser in the supplement added with pasteurized cane juice (Fig 4).

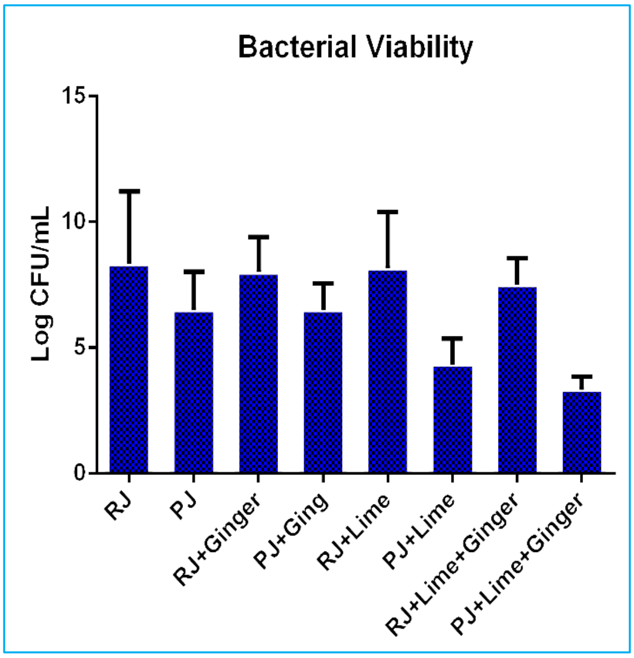

Fig 4 Viability (log CFU/mL) of the *Lactobacillus casei* in different formulations of sugarcane juices. The error bars represent the standard deviation of the mean (n = 6)

The preliminary sensory evaluation was performed to examine the acceptance of *Z. officinale* and *C. limon* juice by consumer against its color, odour and overall acceptability parameters. According to the panelists, the changes in the smell by fermentation were significant. Color of the fermented Sugarcane juice was slightly darkened, but still attractive to the eye. The pasteurized juice enriched with *Z. officinale* and *C. limon* showed a minimal response from the people for the tasts, color and flavours (Fig 5).

Changes in microbial count, pH and sugar content

All of the samples underwent microbiological analysis. The pasteurized samples had fewer bacterial growths overall. Ginger and lemon extracts were combined to control bacterial growth. The bacterial growth is present in raw cane juice, when it is stored for 24 hours. But even when ginger was added to cane juice, the amount of bacterial growth was higher than when lemon was added to raw cane juice. There was no bacterial growth in the pasteurized sample when ginger and lemon were added separately or together. In light of this, the microbiological analysis revealed that the pasteurized juice samples containing ginger and lemon juice were superior to other samples. Similar findings were made by Sangeeta *et al*. [20] and show that anola addition reduced the microbial count in sugarcane juice. Additionally, it stopped the loss of ascorbic acid. The reported changes in the number of microorganisms were within acceptable ranges. The acceptance of the pasteurized and raw cane juices was very low overall. This was due to the off-flavor and off-taste brought on by the bacteria's quick fermentation of the sugars. The pasteurized samples with the addition of ginger and lemon had a higher level of acceptability than the raw cane juice and pasteurized samples without the addition. It is clear that there have been significant changes in the number of microbe cultures because the colour and turbidity parameters of the raw sugarcane juice with ginger and lemon have remained stable. Probiotic cells that have been compromised (lysed or killed) would cause a colour change and an increase in turbidity in fruit-flavored beverages [21-22].

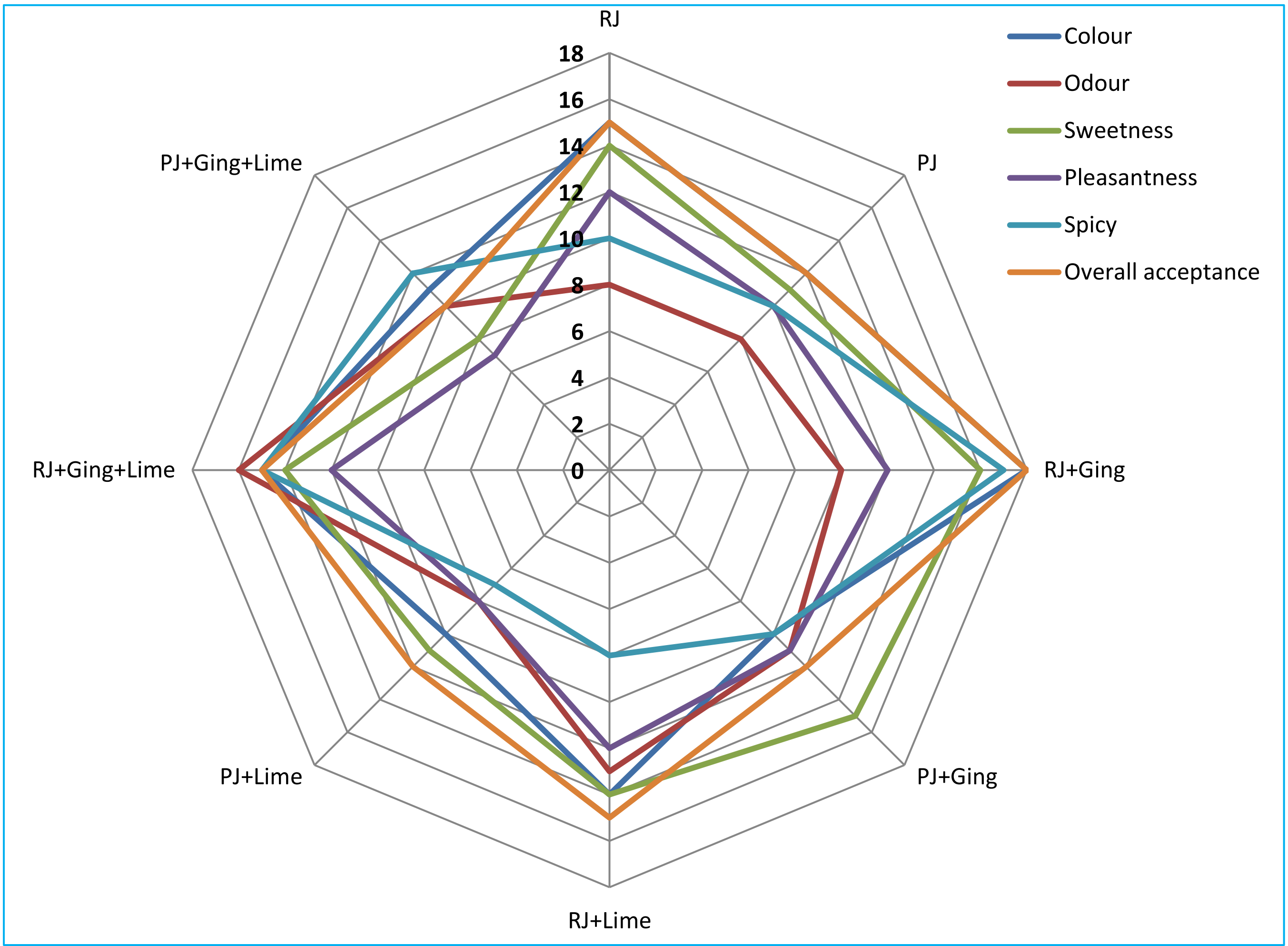

Fig 5 Sensory evaluation of the sugarcane juices enriched with *Z. officinale* and *C. limon*

Due to fermentation, the cane juice's sugar content decreased during storage. The unpasteurized samples' Brix values were higher than those of the pasteurized cane juices. As sugars were fermented by bacteria during storage, the pH dropped. Previous studies involving the addition of anola and lemon to sugarcane juice demonstrated an increase in nutritional value and improved clarification by acting as a coagulant to remove impurities and as a preservative, leading to a high level of acceptability of sugarcane juice overall. Therefore, it is essential to create a preservation method for juice that can easily spoil in a short amount of time and to scale up production to lengthen its shelf life and increase its marketability.

The pH of *Z. officinale* and *C. limon* enriched pasteurized and raw sugarcane juice were in the range of 6.3-3.4 pH that supports the results reported by Guerra and Mujica [12]. The observed pH of sugarcane juice was marginally equal as prescribed by Ecuadorian technical standard (2002). Moreover, *C. limon* lowered the pH of sugarcane significantly in both pasteurized and raw juices.

Since phenolic compounds are more stable under conditions of greater acidity, the higher total phenolic compound content may be related to this acidity [23]. The findings point to good processing and storage conditions because phenolic compounds could oxidize enzymatically and/or chemically if improper processing conditions and lengthy storage times were present [24]. Phenolic compounds function as antioxidants and may help lower the risk of developing chronic non-communicable diseases. They give foods their colour, astringency, aroma, and oxidative stability [25-26]. The raw sugar cane juice is found to have the highest concentration of total flavonoid and phenolic content, according to quantitative analysis of their contents. The total phenolic and flavonoid contents of various plants were found to be higher than average in other studies [27], which made similar findings. A plant extract's medicinal value can be determined in general terms through phytochemical screening. There are a number of secondary metabolites present in sugarcane juices, according to this study's evaluation for qualitative and quantitative estimation of chemical constituents.

The results of the physicochemical parameters show that adding ginger and lemon changes the characteristics of the sugarcane juices marginally, whereas adding the pasteurized sugar cane juices added with ginger and lemon changes the physic-chemical parameters significantly compared with the raw cane juices. In contrast, the stored pasteurized juices added with the supplement even for few hours did not satisfy the raw quality juices. The current work demonstrated that the addition of supplements like ginger and lemon increased the taste, which indirectly increased the number of consumable people. To minimize the physicochemical and sensory changes to the products, the supplement product added to the pasteurized sugar cane juice could be optimized for a longer period of time. The results of this study could help medium-sized and small beverage industries better understand the causes and effects of the additives that are added to raw juice and pasteurized juices before storage, facilitating the commercialization of these products. However, some modifications are necessary to lessen the detrimental effect on the sensory properties.

Natural antioxidants have a significant impact on the food system's safety and acceptability by helping to prevent and stop oxidative damage. By limiting microbial development, they prevent oxidation and maintain the food's stability against oxidation. As added compounds have additional potential for enhancing endogenous antioxidant systems, the conventional practice of adding antioxidants during processing can still play a crucial part. Additionally, plant polyphenols with the ability to donate hydrogen are frequently linked to antioxidant activity because they prevent oxidation caused by free radicals [28]. According to Nayaka *et al*. [29], Duarte Almeida *et al*. [30], the phenolic compounds in sugarcane juice had the potential to be antioxidants and conferred a variety of biological activities.

A food additive made from plants, particularly polyphenolic compounds, has been given health-promoting properties, such as the ability to prevent chronic cardiovascular diseases [31]. *Z. officinale* rhizome has been discovered to have antioxidant action and protection against free radical damage both in vitro and in vivo. It is used as spices and condiments in many food preparations [32-33]. Additionally, rhizome's active components supported a variety of biological processes [34]. Because of the synergistic increase in total phenolic content, flavonoides, and tannins caused by *Z. Officinale* enrichment in the current study, ginger-lemon sugarcane juices are classified as functional foods due to their combination of health benefits.

Consumer preference

A sensory analysis of sugarcane juice with natural extracts was conducted because natural preservatives can change the flavour of food, due to the flavours associated with the substance. According to the results of the overall acceptability test, the scores on a 5-point scale range from 2.3 to 4.1 (Fig 4). Out of all the samples, the raw sugarcane juice with combined ginger and lemon extract received the highest rating.

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