Journal of Food, Nutrition and Diet Science
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Original Research

Comparative study NFC and RFC on nutritional and sensory profile of guava juices

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Abstract: Guava juice, with its distinctive flavour and being rich in vitamin C, lycopene, β- carotene and flavonoids, is known for its health-promoting properties, such as its antioxidant capacity and cancer-preventive effects. As retention of nutrient composition is an important criterion in juice processing, especially loss of nutrients post vacuum concentration and reconstitution, a comparative study was conducted on Not From Concentrate (NFC) and Reconstituted From Concentrate (RFC) juice of Bangalore pink guava. Significant loss of antioxidant activity ($p \leq 0.05$), vitamin C (18.8 %) and β-carotene (13. 0%) ($p \leq 0.01$) was observed in guava RFC as compared to guava NFC, which might be caused by heating of juice under vacuum concentration. The iron bioavailability of guava NFC was significantly ($p \leq 0.01$) higher by 38 % in Caco-2 cell studies. The sensory profile of NFC showed a fresher taste, more freshness, a naturally sweet taste and astringency compared to RFC. Minor deviations were observed in physicochemical parameters. Thus, guava NFC ensures better retention of vitamin C, antioxidant activity, β-carotene, iron bioavailability as well as better in sensorial properties compared to RFC.

Keywords: NFC and RFC Guava juice, Sensory evaluation, Nutrient composition, Antioxidant potential

Introduction

Changes in consumer taste, healthy diet, health and fitness are the main reasons for the consumption of fruit juices across the globe [1] Retail markets supply a large range of packaged fruit juices, which have become indispensable and hence encourage companies to package them for easy transport and consumption.

Fruit-specific methodologies are applied to extract juice, with juice extraction equipment ranging from manual crushing to mechanical extraction. The basic processing technologies of fruit juices undergo modification in different ways as per fruit types. Prior to processing, fruits for juicing are usually inspected for damage, contamination and impurity. After washing, fruits get sorted, peeled and prepared for extraction, either by chopping, pitting, crushing and enzymatic treatment or a combination of these. Extracted juice undergoes filtration, thermal processing, concentration and finally to be bottled and stored [2].

The demand for freshly squeezed direct fruit juices that are sourced from not concentrate (NFC) is increasing [3]. NFC juice is made by processing the fresh fruit to juice and then pasteurizing it. On the contrary, RFC is concentrated fruit juices reconstituted by adding water that has been lost during the process of evaporation [4].

Received: Dec.5, 2023; Revised: Mar.17, 2024; Accepted: Apr.8, 2024; Published: Apr.22, 2024

DOI: https://doi.org/10.55976//fnds.22024123541-50

There are numerous techniques for liquid concentration, such as open-pan boiling, vacuum concentration, freeze concentration, reverse osmosis and electrodialysis, among which vacuum concentration is mostly applied due to its wide availability, and higher popularity [5]. The concentration process may lead to the loss of volatile substances, stripping aromatic volatile compounds and other heat sensitive compounds, which may affect both the sensory and the health attributes [4]. In addition, the nutrient contents are altered regardless of the techniques used, thereby the benefits expected by the consumer are not provided.

Among a variety of fruits, guava predominates the market today as a superfruit and is valued for processing. Guava (Psidium guajava L.), a dicotyledon from the Myrtaceae family, is one of the important commercial fruits grown throughout the tropical regions of the world. Pink guavas are the most aromatic fruits and their colour is due to the naturally occurring plant pigments called carotenoids. Pink guavas have a sweet musky aroma, with moderate acidity and more sweetness [6]. The sensory properties of guava comprising the sweet, tangy taste and tropical notes, have made it a popular fruit for consumption.

Guava juice is well acknowledged for its antioxidant properties and high polyphenol content, which include phytonutrients such as vitamin C, lycopene, β-carotene, flavonoids [7], and catechins, although the presence of these compounds in processed juice is controversial.

As an enhancer of iron bioavailability, ascorbic acid is a rich source of vitamin C naturally present in guava and has a positive effect on absorption of iron [8]. To improve consumer acceptance, the interrelationships between physical, chemical composition and sensory properties need to be investigated. The stability of flavour, which is more important for juice quality, needs to be assessed [9]. The present research focuses on the effects of processing on the nutritional, physicochemical and sensory (colour, aroma, taste, mouthfeel) properties and the overall flavour profile of guava juice reconstituted from concentrate and guava juice not from concentrate.

Only a few works that have been studied focused on fruit juice concentrates [10] or the comparison on nutritional content of fresh juices against commercial juices [11] or on quality evaluation of not from concentrate juice against juice from concentrate, which was studied in orange juice [12]. Limited studies have been conducted to assess the nutritional and physicochemical properties of NFC and RFC juices, therefore further research is needed.

Materials and methods

Chemicals and cell lines

All reagents, standards, solvents and certified reference materials (CRMs) were of analytical grade and purchased from Sigma (Merck). Human epithelial colorectal adenocarcinoma cells (Caco-2), were purchased from the American type culture collection (ATCC). Minimum essential medium (MEM), penicillin and streptomycin and foetal bovine serum (FBS) were obtained from Gibco, Invitrogen, oxygen radical absorbance capacity (ORAC) and antioxidant activity kit were purchased from Zenbio.

Fruit material and juice processing

The pink guava (Psidium guajava L) cultivar Lalith was selected for processing of NFC and RFC juices from guavas (Figure 1). The guavas ready for processing were inspected for spoiled and unripe fruits. Ripened fruits were thoroughly washed with water jets. The stem of the fruits was removed with a scooper and crushed in the fruit miller. The crushed fruit pulp was then passed through a first stage pulper to remove skin and seed particles. Homogeneous pulp was obtained by passing through a second pulping stage to remove fibre particles. The resulting product was divide into two parts. One part of the homogeneous pulp was pasteurised at 104 to 110˚C, applying a holding time of 90 seconds and a filling temperature of 28 to 30˚C. This was then stored aseptically as "Not From Concentrate". The second part of homogeneous pulp was evaporated to remove water in a double-effect evaporator under vacuum 630mm Hg until 19.74 ˚Brix was reached. The concentrate pulp was sterilised at a filling temperature of 30 to 32˚C and stored aseptically as "juice from concentrate". The guava juice concentrate was reconstituted to (RFC) to desired ˚Brix based on Pearson's square formula [13]. Both NFC and RFC were stored at -18˚C before further analysis.

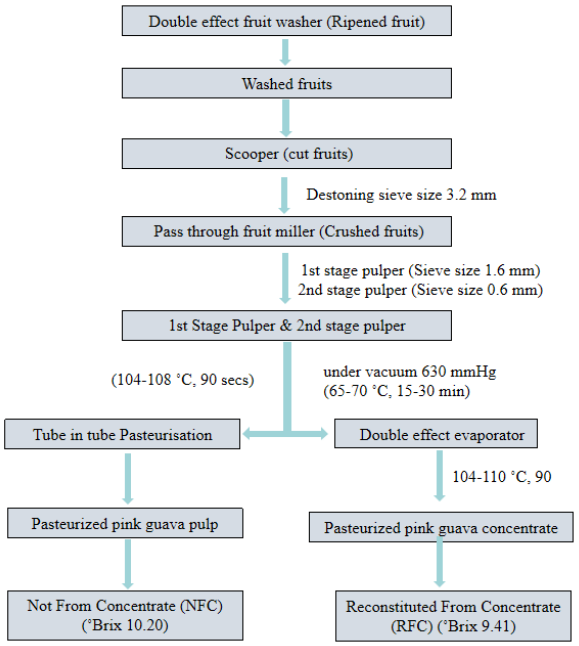

Figure 1. Process chart for guava juice "not from concentrate" (NFC) and "reconstituted from concentrate" (RFC)

Physicochemical paraameters

Physicochemical parameters such as pH, acidity, total soluble solids, total acidity, sugars, proteins and carbohydrates were estimated for both NFC and RFC guava juices. The pH was determined using the 914 Metrohm pH/conductometer (Metrohm, Switzerland) and total soluble solids were measured with an automatic refractometer/densitometer (Metler Toledo, Switzerland). Total sugars and total acidity (as citric acid) were determined titrimetrically as per standard methods [14]. The protein content was determined by Gerhardt protein analyzer (Gerhardt Vapodest 50S, Germany) [15]. The total carbohydrates in the difference were calculated as per the standard method [16].

Vitamin C (ascorbic acid) content

Vitamin C was analysed by modified HPLC method [17]. 2.5 g of NFC and RFC guava juice samples were weighed using a 25 ml volumetric flask and dissolved in 10 ml of water adjusted to pH 2.5 and up to the mark. Both types of samples were filtered through 0.45 µ syringe filters and 10 µl were injected into liquid chromatography (HPLC Agilent-1200, Santa Clara USA) equipped with a UV detector and a C18 Prevail column (250 mm x 4.6 mm) and in particle size (5 µ). 25 mM potassium dihydrogen phosphate was adjusted to a pH of 2.5. Orthophosphoric acid was used as the mobile phase with a flow rate of 1 ml/min and a run time of 30 minutes. The column temperature was kept at 35°C and monitored at a wavelength of 250 nm. An ascorbic acid standard solution (500 mg/L) was prepared and a series of linear standards were run along with the samples.

Analysis of essential elements

Nutritional elements such as sodium, potassium, iron, calcium, magnesium and phosphorus were analyzed by inductively coupled plasma - optical emission spectrometry (720 ICP-OES, Agilent Santa Clara USA) using the AOAC methodology [18]. The samples of NFC and RFC were weighed together with certified reference material into an Xpress Teflon PFA digestion vessel and digested with 5 ml of concentrated suprapur nitric acid (65%) in a MARS-Xpress microwave digester for 15 minutes at 200 °C. The digested samples were cooled to 50 °C. Standards and sample volumes were made up to 25 mL. A stock standard (100 mg/L) was prepared containing the standards sodium, potassium, iron, calcium, magnesium and phosphorus. A series of linear standards ranging from 0.1 to 5 mg/L were aspirated into 720 ICP-OES Agilent and response of detector was calibrated each time.

Total polyphenols

Two grams each of NFC and RFC guava juice were extracted with 80 % methanol for 4 hours. A linear standard curve of gallic acid was plotted at different concentrations (10-100 µg/mL). The ISO standard method [19] was modified 96-well plate method. A 25 µL aliquot of the samples and the standard were treated with 125 µL Folin-Ciocalteau (FC) reagent. After a 5-minute incubation, the plate was neutralized with 100 µL sodium carbonate and incubated in the dark for one hour. The absorbance was then measured at 765 nm using a multiplate plate reader (TECAN GmBH, Switzerland). The total polyphenol content was expressed as mg gallic acid equivalent. The blue color obtained, which has an absorption maximum at a wavelength of 765 nm, was quantified as proportional to the phenolic compounds present.

Lycopene & β-carotene

Lycopene and β-carotene in NFC and RFC were extracted in n-hexane by liquid-liquid extraction. 0.10 g of the samples were weighed in 20 mL of Milli-Q water and sonicated for 10 minutes. This mixture was extracted thrice in 250 mL separating funnel with 10 mL of n-hexane. The combined extracts were made up to 50 mL with n-hexane and the solution was passed through anhydrous sodium sulphate to absorb moisture. The lycopene content was measured spectrophotometrically (Shimadzu UV-1700, Japan) using the molar absorptivity E1 % = 3450 at λmax 470 nm. Beta-carotene from the same mixture was measured using the molar absorptivity E1 % = 2590 at λmax 450 nm.

ORAC value

The guava NFC and RFC juice samples were centrifuged at 8000 rpm for 10 minutes. A range of samples from undiluted to 1:3 dilutions were studied. Prior to the assay, a clear-bottomed black plate, a 96-well plate, was heated at 37 ˚C. The fluorescein solution, Trolox solution and AAPH working solutions were prepared according to the instructions. 150 µL of fluorescein solution was added to the wells. To this 25 µL of the samples with different dilutions and Trolox solution at concentrations ranging from 100 µM to 6.25 µM were added. The plate was incubated at 37˚C for 10 minutes. At the end of the10 minutes, 25 µL of AAPH working solution was added to each well. After this step, a kinetic fluorescence reading was started using a plate reader at an excitation of 485 nm with an emission of 528 to 538 nm. Reading was performed for 30 minutes and the absorbance was recorded every minute. The area under the curve was calculated using the formula and expressed in µM Trolox equivalents.

$$AUC = 0.5 + (F1/F0) + (F2/F0) + \ldots + 0.5*(F30/F0),$$

Where F0 = normalized fluorescence at t = 0 minute.

Iron uptake assay

Guava NFC and RFC were analysed for iron absorption using a slight modification of the prescribed method [20]. These juices were centrifuged at 4000 rpm for 20 minutes, and the supernatant obtained was filtered through a 0.22µ filter. The liquid obtained after filter sterilization was used in assay. Caco-2 cells with a density of 6×104 /mL were seeded in a 12-well plate supplied with a change of medium every alternate day. The medium was supplemented with the following growth factors growth factors: 10 mM/L PIPES, 4 mg/L hydrocortisone, 5 mg/L insulin, 5 µg/L sodium selenite, 34 µg/L T3, 20 µg/L EGF. Cell growth was completed after 10 days (90% confluence) and cells were treated with different concentrations of filter sterilized juices. Both the NFC and RFC samples were treated as described above.

Using ascorbic acid (100 µg/ml) as a positive control, all the wells were treated with 400 µg/ml FeSO4 and incubated at 37 °C, 5 % CO2 for 18-20 hours. The next day, the cells were washed with ice-cold 0.9 % NaCl (saline), followed by washing with 250 µL stop solution containing 140 mM NaCl and 10 mM PIPES – pH 6.7. The cells were then washed with 250 µL removal solution containing 140 mM NaCl, 10 mM PIPES, 5 mM bathophenanthroline disulphonic acid – pH 6.7. Finally, the cells were solubilized using 250 µL of 0.5M NaOH. The harvested cells were stored at -80 °C until further processing. These harvested cells were analysed for total iron using the ferrozine assay and for total protein using the Bradford assay.

Ferrozine assay: 100 µL of harvested cells were mixed with 100 µL of distilled water and 100 µL of 1:1 4.5 % KMnO4 and 1.4M hydrochloric acid and incubated at 60ºC for two hours. After two hours, the incubation mixture was treated with 30 µL of iron detection reagent (6.5 mM 3-(2-pyridyl)-5,6-diphenyl-1, 2, 4-triazine-p, p′-disulfonic acid monosodium salt hydrate [Ferrozine™ iron reagent] 2.5 mM ammonium acetate and 1M ascorbic acid). After 30 minutes of incubation at room temperature, the developed colour was measured spectrophotometrically at 550 nm.

Sensory evaluation

The effects of processing on guava juice in NFC and RFC were investigated by means of flavour profiling, which are considered as a key factor for consumer acceptance. The sensory flavour characteristics for guava juices include pleasant aroma, combined fruity, green and tropical notes and the reason for popularity. The flavour characteristic components commonly associated with guava juice are fruity, fresh taste and an astringent mouthfeel, while others are reminiscent of grapefruit, floral, caramel, seasoning like and metallic [21].

Methodology of sensory evaluation

Evaluation method: For the flavour profile, a descriptive sensory was adapted according to the Sensory Analysis Methodology- Flavour profile methods (ISO 6564:1985/IS 15315:2003).

Panel: A panel of five expert panelists (trained & experienced) was invited to evaluate the descriptive analysis techniques. These panelists included four women and one man, aged 25-45.

Samples and sample serving: On the day of evaluation, thawed (25 ± 2°C) samples were well mixed for homogeneity and about 30 mL was served in glass cup. Drinking water was served as a palate cleanser and the samples were additionally served if required. The samples were coded with three-digit random numbers and served randomly in monadic series.

Sample evaluation procedure: The samples of NFC and RFC guava juice were subjected for the flavor descriptive analysis, in which the sensory characteristic descriptors were identified and quantified to establish the flavour profile of the samples.

An orientation of 3 hours with one hour per day was given to the panelists to make them familiarized with the samples. The panelists received a training on the scales with references to the descriptors wherever it was necessary. The information on the nature of samples provided to the panelists was limited to avoid any bias. For evaluation, 25 mL sample was taken orally and held for 30 seconds and then swallowed. Flavour (aroma, taste and mouthfeel) as a cumulative expression was evaluated when the sample was ingested.

During each session for these two samples evaluation, two replicates were conducted on two different days to identify any variation. The flavour descriptors identified in the sample evaluation were quantified by each panelist individually with assigned intensities to each of the descriptors on a 10 cm line scale (0-10), in which 0 represented very low and 10 represented very high. Increments of 0.5 cm were applied on the scale. Average values of both sessions of the panelist were considered for the final sensory descriptive profile of the samples.

Statistical analysis

Statistical analysis was performed using Microsoft Office software (Vers ion 2016). All the samples were analyzed in triplicates and data were presented as Mean ± Standard Deviation (n=3). Statistical differences were determined using T-test.

Results and discussion

Physicochemical parameters

Table 1. Comparison of chemical composition of NFC and RFC guava juice

SL. No	Parameters	NFC	RFC
1	pH (as Basis)	3.91 ± 0.005[a]	3.74 ± 0.005[b]
2	% Acidity (as citric acid)	0.55 ± 0.01[a]	0.51 ± 0.0075[b]
3	TSS, ˚Brix	10.2 ± 0.026[a]	9.41 ± 0.01[a]
4	Total Protein, g/100g	0.08 ± 0.005[a]	0.15 ± 0.01[b]
5	Total Sugars, g/100g	10.03 ± 0.01[a]	10.24 ± 0.015[b]
6	Carbohydrates, %	12.44 ± 0.02[a]	11.42 ± 0.035[b]
7	Sodium mg/100g	2.35 ± 0.025[a]	2.40 ± 0.030[a]
8	Magnesium mg/100g	5.98 ± 0.03[a]	6.24 ± 0.005[b]
9	Potassium mg/100g	151 ± 1.0[a]	144.76 ± 0.01[b]
10	Calcium mg/100g	7.63 ± 0.00[a]	7.62 ± 0.005[b]
11	Iron mg/100g	0.23 ± 0.02[a]	0.18 ± 0.02[b]
12	Phosphorous mg/100g	8.62 ± 0.026[a]	8.71 ± 0.01[b]

Proximate constituents (Mean ± Standard Deviation, n=3) with significantly differences indicated by different letters ($p \leq 0.05$).

Table 1 showed the physicochemical data and the data on essential elements in quality assessment of NFC and RFC guava juices. The pH values ranged from 3.9 for NFC to 3.7 for RFC, with the low pH range of 2-5 being due to the organic acids content. As the concentrated guava juice was reconstituted to the equivalent ˚Brix of NFC, 9.41 ˚Brix was obtained for RFC. The acidity, total sugar and total protein content of both NFC and RFC guava juices were found to be in the same range.

In addition to functional and nutritional compounds, fruit juices contain many macro and microelements [22]. Like other fruit juices, guava also contains minerals, i.e. alkali cations such as potassium, sodium and alkaline soil elements, especially calcium and magnesium, as well as simple inorganic anions like phosphates and sulphates. Statistical analysis showed a significant difference ($p \leq 0.05$) in the physicochemical composition between NFC and RFC, except for sodium content, which was not significant ($p \leq 0.05$).

Table 2. Phytonutrient comparison of NFC and RFC guava juice

Sl. No	Parameters	NFC	RFC
1	Total Polyphenols, mg/100g	208.32 ±1.59[b]	214.55 ± 0.59[b]
2	Lycopene mg/100g	3.51 ± 0.07[b]	2.92 ± 0.11[b]
3	β- carotene mg/100g	2.9 ± 0.10[b]	2.54 ± 0.05[b]
4	Vitamin C, mg/100g	93.74 ± 0.71[b]	76.13 ± 1.91[b]
5	Trolox equivalent (µM)By ORAC	106.26 ± 3.06[a]	98.19 ± 1.49[a]
6	Iron bioavailability Assay, (µg)/mg protein(CaCo-2 cells)	229.26 ± 48.10[a]	130.23 ± 44.53[a]

Concentration (Mean ± Standard Deviation, n=3) of vitamin C, lycopene, β-carotene, total polyphenols, iron bioavailability and the Trolox equivalent activity of guava juice were evaluated using two different processes. Letter a denotes statistical significance ($p \leq 0.05$) and letter b denotes statistical significance ($p \leq 0.01$, T-test).

Total polyphenols

Typical polyphenol chromatogram of guava juice as studied by Fender [23], separated by high performance liquid chromatography (HPLC) based on spectral study, retention times compared with reference standards gallic acid, catechin, ellagic acid were positively identified. The determination of phenolic compounds by HPLC-PAD on pink guava showed the highest concentration of gallic acid, chlorogenic acid, ellagic acid, catechin and rutin [24]. Based on polyphenol characterization by UPLC, extracts of pink guava and leaves showed the presence of rich composition of phenolic compounds identified as quercetin and catechin oligomers with high antiplatelet potential [25]. As such, there is little published data on guava phenolics with respect to processing [23].

Table 2 illustrated the significant differences ($p \leq 0.01$) captured between the polyphenols content of NFC and RFC guava juices. Processing parameters such as squeezing, pasteurization and concentration had no significant effect on the phenolic composition of orange juices [26]. Although no correlation was observed between HPLC analysis and spectrophotometric quantification of total polyphenols in Thompson Seedless grape juice, Granny Smith apple juice and pear juices, no much differences were observed between bottled and concentrated juices at different stages. The concentration of phenolic compounds quantified by the Folin ciocalteu (FC) assay cannot be overruled by the interference of non-phenolic material in the assay [27].

Lycopene and β-carotene

Red-colored fruits and vegetables such as tomato, papaya, pink guava, watermelon and pink grapefruit contain a red pigment and lycopene in large quantities [28]. The lycopene concentration of the guava used in this experiment (Table 2) is in close agreement with the values (3.6 mg/100g) reported by Ordonez and Ledezma [29]. According to Fender [23], very few studies have been published on the relationship between processed guava and lycopene, while numerous studies have been conducted on thermally processed tomatoes and lycopene. Most carotenoids, including lycopene, are found in natural and unprocessed foods, including guava, mainly in their trans forms. Lycopene undergoes two types of changes during processing, isomerization from all trans to cis forms and oxidation. Depending on the food processing conditions, lycopene undergoes degradation via isomerization under the influence of different factors like heat, light and oxygen [30]. A study by Tola and Ramaswamy [31] indicated that

lycopene degradation in watermelon juice obeys a first-order reaction. At low temperature and heating for shorter period of time, the main reaction is isomerization, with degradation dominating with increasing temperature. In a similar work to tomato products, degradation of lycopene was the main reaction after continuous heating to 150˚C, compared to the isomerization reaction at the beginning [30]. A lycopene study on carrots indicated that lycopene was heat stable at 70˚C for 5 hours; degradation was faster when the temperature increased above 100˚C [32]. Therefore, lycopene degradation was not much higher in the temperature range of guava concentration process, i.e. at 65-70˚C. A significant decrease in values ($p \leq 0.01$) from 3.51 mg/100g lycopene concentration in NFC to 2.92 mg/100g in RFC (-16.80%) might be due to isomerization of the trans form into the cis form, although isomerization was not evaluated during this experiment.

β-carotene, an orange pigment, a fat soluble vitamin with the highest activity of provitamin A, is known as a potent antioxidant and singlet oxygen scavenger and has been found to be able to protect against cancer and ageing [33]. Carotenoids are highly unsaturated and easily susceptible to oxidation and isomerization during food processing. In the present study, a significant decrease ($p \leq 0.01$) in β-carotene concentration in RFC by 13.10% against NFC was observed. As per degradation kinetics studied on watermelon juice, the total carotenoid content decreased after heating for 5 hours at temperature range of 50 to 90˚C [34]. The results of the study [35] on the effect of heat treatment of raw and processed cherry tomato fractions showed a decrease in β-carotene content in all heated products, indicating that heating do have an effect on degradation. For instance [36], it was found that trans-cis isomerization of β-carotene was predominant in carrot juices subjected to blanching and sterilization in varying time and temperature regimes. Vacuum concentration of peach juices at 50°C resulted in a decrease in carotenoid content by 8.78% [37], indicating that the concentration process has a decreasing effect on the concentration of β- carotene. Therefore, a minor reduction in β-carotene was observed in RFC against NFC in the present study, although isomerization reactions due to the concentration process were not investigated during this experiment.

Vitamin C and antioxidant activity by oxygen radical absorbance capacity

Guava has a significant nutritional importance in terms of vitamin C, which is an important enzymatic co-factor. Several factors such as fruit variety, species and harvesting conditions play a role in the variation of vitamin C content [38]. Based on the degree of ripeness, a decrease in vitamin C content was observed in pink guava [39], similar to the study with bergamot juice [40], which was not consistent with the increased vitamin C content as fruit matures [41]. The analysed vitamin C content in NFC and RFC guava juice (Table 2) indicated a significant ($p \leq 0.01$) difference, where 18.8 % loss of vitamin C was observed in RFC guava juice as compared to NFC guava juice. This difference is in close agreement with the percentage loss (15-55 %) of vitamin C content before and after the concentration process at different temperature and vacuum conditions studied in guava and mango juices [42]. The loss of nutrients through the concentration process may result in oxidation of vitamin C, which leads to the formation of carbonyl radicals and produces dark pigments through polymerization. As vitamin C is unstable in the presence of light, oxygen and elevated temperatures, the effect of concentration process leading to changes in vitamin C content needs to be evaluated [42]. As per the studies on orange juice, vitamin C degradation can occur through both aerobic and anaerobic degradation depending on the temperature and availability of oxygen. During processing, the aerobic degradation of vitamin C, which is oxidized to dehydroascorbic acid, predominates [43]. The influence of different factors during processing like temperature, sugar concentration, salt, pH and oxygen affects the concentration of vitamin C, since it is a major antioxidant [44]. According to studies on Sudanese variety, it was observed that white guava lost 32.59 % vitamin C and the pink guava lost 12% vitamin C in concentrate respectively in comparison to pulp [45]. Hence, it can be inferred that varying temperatures and vacuum concentrations play their roles in the degradation of vitamin C.

Antioxidant activity by oxygen radical absorbance capacity

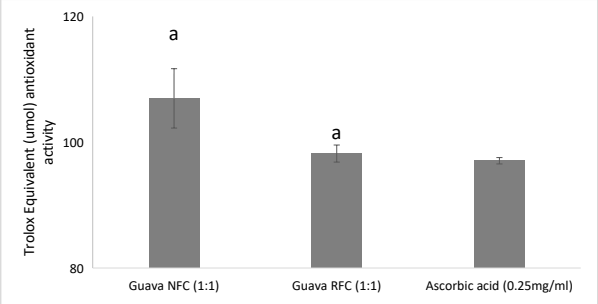

Figure 2. ORAC assay

Antioxidant activity (Mean ± Standard Deviation, n=3) of guava juice was evaluated using two different processes: NFC and RFC, in HepG2 cells. Identical letters denoted that results were statistically significant ($p \leq 0.05$, T-test).

As the most common methods, ORAC was used to determine antioxidant capacity depending on the reaction mechanism, which is typically based on hydrogen atom transfer HAT [46]. This assay has been found useful to study the antioxidant behaviour of tea infusions [46], to conduct a comparative study on common vegetables [47] and also to study the effects of heating on the antioxidant capacity of different oil extracts [48]. This assay measured the properties of guava phytochemicals to scavenge peroxyl radicals generated by 2, 2'-azobis

(2-amidinopropane) dihydro chloride in the presence of fluorescein, the fluorescent probe used as an indicator of oxidation. Antioxidant capacity was calculated by integrating the area under the fluorescence decay curve in the presence of guava phytochemicals and calibrated by using a standard curve of Trolox, which is an analogue of water-soluble vitamin E, with data reported in µM Trolox equivalents. In the present study, the antioxidant activity of Trolox was analysed for samples at different concentration levels, in which the maximum activity was observed at 1: 1 concentration level (Figure 2). NFC guava juice had a significantly ($p \leq 0.01$) higher antioxidant activity as compared to RFC guava juice (Table 2), for which various factors might influence the effectiveness of antioxidants. There were two types of antioxidants, endogenous and exogenous. When endogenous factors cannot provide the complete protection against reactive oxygen species [49], the needs for exogenous antioxidants arise, such as vitamin E, vitamin C, β-carotene, flavonoids and anthocyanins from natural sources. The combination and synergistic effects of bioactive compounds like vitamin C, phenolic compounds, flavonoids and carotenoids contributes to the antioxidant activity in guava fruit. The contributing factors are oxidation conditions, state of oxidisable substrate and partitioning properties of antioxidants between lipid and aqueous phases. As per studies on different guava juices and antioxidant activity of nectars, it was found that ascorbic acid was a major contributor [50]. As per study conducted on fresh guava, guava pulp and paste, 25% decrease in antiradical 1, 1-diphenyl 1-2-picrylhydrazyl (DPPH) activity was reported, where a moderate correlation was verified between DPPH antiradical activity and the content of ascorbic acid [51]. The decrease in antioxidant activity of RFC guava juice might be correlated to decreased content of vitamin C. These studies are in agreement with Corre et al. [52], in which they had reported a high correlation between antioxidant activity and free ascorbic acid along with phenolic compounds, moderate correlation drawn towards antioxidant activity and vitamin C in one of the guava varieties.

Iron bioavailability assay, (µg)/mg protein

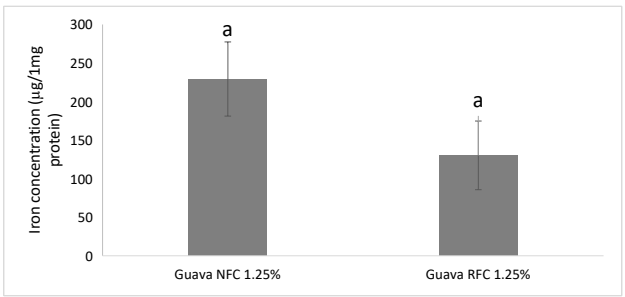

Figure 3. Iron uptake assay

Iron bioavailability (Mean ± Standard Deviation, n=3) of guava juice was obtained using two different processes: NFC and RFC, In HepG2 cells. Identical letters denoted that results were statistically significant ($p \leq 0.05$, T-test).

Caco-2 cells were studied to evaluate the iron bioavailability in the presence of guava juice samples of NFC and RFC. Cells were treated with juice samples together with iron source for 18 to 20 hours. Both these two guava juice samples showed a enhanced uptake of iron in Caco-2 cells (Figure 3) in comparison with ascorbic acid. However, when compared NFC and RFC, NFC guava juice showed an iron uptake significantly ($p \leq 0.05$) more than RFC by 4 % (40 µg/mg protein) at 1.25 % concentration. Two types of dietary iron were present; heme and non-heme iron. Heme iron, which comes from hemoglobin and myoglobin in animal foods, and non-heme iron mainly comes from fruits and vegetables which is less absorbed depending on absorption enhancers and inhibitors [53, 42]. Enhanced iron bioavailability by more than 100 % with the inclusion of guava fruit was clinically studied as part of regular rice-based meal [54]. Vitamin C or ascorbic acid is a potent enhancer for iron bioavailability in a dose-dependent manner. This effect may be due to its ability to reduce ferric to ferrous iron. As cooking, industrial processing degrades ascorbic acid, enhancing the effect on iron absorption may be reduced, which was reflected in this studies on NFC and RFC guava juices [8].

Sensory evaluation

Some of the descriptors that were identified and agreed upon by the panelists for the guava juice were freshness, green peel note, fruitiness, sourness, natural sweet taste, cooked, over-ripened taste, pulpiness, smoothness, astringency and fruity after taste. The T-test statistical analysis on significance showed that all sensorial attributes of aroma and taste profile showed a significant difference, where NFC was better for characteristic of sensorial quality, while RFC showed the processing effects on its sensorial attributes with lower characteristic quality and development of processed components such as cooked and over ripened notes. In the mouthfeel, pulpy and astringent descriptors were found to be significantly different, while the smoothness of NFC and RFC guava juice did not have a significant difference. A graphical representation of data was presented as spider chart in Figure 4, which represented the comparative flavor profile of NFC versus RFC.

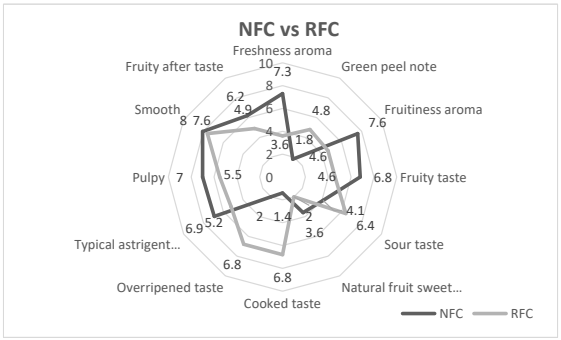

Figure 4. Flavour profile spider chart of NFC vs RFC guava juice

Aroma and taste profile: NFC and RFC showed differences in aroma profile, where the fruity and freshness aroma were high in NFC, while the same aromas were slightly lost in RFC, and at the same time cooked, the aroma of ripened fruit was higher than green peel fruit. The taste profile of NFC juice was found to be better for having more fruity taste, and natural sweet taste, as no heating process was involved and hence no loss of aromatic compounds or breaking down of any components, while RFC samples had higher sour taste, cooked and over ripened fruit taste, which was predominant than the other tastants. The concentration process involved in RFC might be the major cause for both cooked notes and over ripened fruit taste. A similar phenomenon had been studied previously and reported in pomegranate juice [51]. Natural sweet taste was lower while the sour taste was found to be higher in RFC than NFC.

Mouthfeel: NFC showed a slightly higher characteristic astringency than RFC, which is typical of guava fruit. A similar finding was reported in pomegranate juice as well [51]. Pulpiness was also found to be higher, as the fruit pulp was not damaged as much, while it degraded in RFC due to the heat treatment, which was the processing effect. The smoothness remained similar in both NFC and RFC, showing no much impact of processing. This could be due to the fact that the concentration ratio of juice was maintained same in both NFC and RFC, and that there was no processing impact on the same.

Conclusion

Compared to reconstituted juice from concentrate, NFC juices that were freshly extracted without undergoing a concentration process were judged to be of higher quality and also meet consumer requirements in terms of flavour, taste and health profile. Processed guava juices of NFC and RFC were evaluated for their physicochemical, phytochemical and sensory characteristics, and differences were found in some key parameters, attributes and sensorial properties. NFC showed higher antioxidant capacity, vitamin C content and iron uptake as compared to RFC, with slight variation in lycopene and β-carotene content, which could be attributed to the process impact. Higher antioxidant activity in guava NFC juice was influenced by the higher vitamin C content as compared to RFC. The descriptive flavour profile showed that NFC guava juice had a desirable flavour profile with freshness, fruity notes and higher astringency than RFC. The comparitive results showed the process impact on the composition of NFC and RFC juices as well as the importance and superiority of NFC over RFC, differentiating the two products.

Authors' contributions

Brunda G carried out the trials of the study samples NFC and RFC, conducted all the analytical experiments, analysed the data, and wrote the manuscript on Guava juices and analytical testing. Kavyashree Urs conducted the experiments on sensory, analysed the data; wrote the manuscript on sensory aspect. Shilpa S Shetty performed the experiments, analysed the data on cell culture studies and wrote the manuscript on ORAC assay and Iron bioavailability studies. Dr Kirti Sharma planned and designed the study, edited the manuscript.

Conflict of interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

Acknowledgement

Authors thank Central Food Technological Research Institute (CSIR-CFTRI), Mysore for the facility provided for juice processing.

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