https://doi.org/10.17113/ftb.63.04.25.8807 original scientific paper

Implications of Substitution of Natural Sweeteners in Guava Nectar on Qualitative Characteristics and Storage Stability

Running title: Sucrose-Substituted Guava Nectar

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Received: 1 August 2024
Accepted: 13 July 2025

SUMMARY

Research background. Lately, extensive use of refined sugars and artificial sweeteners has led to negative heath implications. Therefore, natural or unrefined sweeteners such as honey, date syrup and jaggery are explored in the present study as a potential alternative ascribed to its nutritional and therapeutic properties.

Experimental approach. The study aimed to optimize level of honey, jaggery and date syrup to substitute sucrose in guava nectar prepared through two processing treatment including hot filling (HF)

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and cold filling (CF). It was further evaluated for its bioactive properties, rheological characteristics, mineral composition (*in-vitro* bioavailable iron) and storage stability. During storage, formation of 5-hydroxymethylfurfural (HMF), effect on antioxidant activity and non-enzymatic browning was focused to indicate changes in overall quality.

Results and conclusions. The level of substitution was optimized at 50, 25 and 30 % in honey, jaggery and date syrup-based guava nectar, respectively, based on organoleptic properties. The optimized formulations depicted a significant improvement in total phenolic content and radical scavenging activity. The guava nectar was found to have pseudo-plastic behavior with a weak gel structure due to the presence of dispersion of pulp particles contributing to its viscoelastic nature below low strain levels (<10 %). The substitution of sweetener resulted in enhanced mineral content; however, the bioavailability of iron (%) considerably decreased. During storage, degradation of ascorbic acid and colour, acceleration of non-enzymatic browning, and development of 5-hydroxymethyl furfural were notably high by the end of the 6th month, but formulations were microbiologically stable.

Novelty and scientific contribution. New products can be formulated using natural sweeteners instead of sucrose which can imply higher nutritional and therapeutic value. However, in the present study, the product can be improved by further research to reduce negative implications on quality characteristics during storage.

Keywords: bioactive compounds; rheological behaviour; *in-vitro* bioavailable iron; 5-hydroxymethyl furfural; non-enzymatic browning

INTRODUCTION

Fruit based beverages are widely consumed and are a significant part of the urban households. Due to the paradigm shift in consumer preference for healthier options against carbonated and artificially flavored soft drinks, a massive surge in fruit juice beverage market has been observed globally. Natural fruit juices are mainly composed of glucose and fructose whereas commercially available ready-to-serve (RTS) or nectars contain appreciable amount of refined sugars in the form of sucrose or high fructose corn syrup (HFCS), which are deliberately added to increase sweetness. Excess consumption of ready-

to-serve beverages poses negative health implications as they are characterized with high glycemic index causing rapid rise in blood glucose and insulin levels along with increased levels of reactive oxygen species, inflammatory mediators and triglycerides in human body, subsequently, increasing the risk of diabetes mellitus and cardiovascular diseases. Moreover, low satiety value of refined sugars leads to over consumption of beverages, leading to obesity (*1,2*).

To overcome the health risks associated with fruit beverage consumption, unrefined sweeteners and artificial sweeteners are recognized as potential alternatives to replace refined sugars. Artificial, non-nutritive sweeteners and low-calorie sweeteners including sugar alcohols can be explored in fruit beverages to reduce the calorie intake and prevent obesity. However, studies have reported artificial sweeteners as one of the contributing factors to various health issues such as coronary heart disease, stroke and mortality (*3*). A recent report by World Health Organization (WHO) has also ruled out that overconsumption of non-sugar sweeteners can substantially increase the risk of type 2 diabetes, cardiovascular diseases and mortality (*4*). Steviol diterpene glycosides (150-450 times sweeter than sugar) are also widely explored as natural sweeteners but may have side effects such as mutagenicity, reduced fertility and allergenic effects (*5*). Moreover, stevia leaf extracts may not provide desired consistency and mouthfeel in a beverage at par with sugar. Overall, it could be emphasized that alternatives for refined and artificial sweeteners must be explored.

Thus, unrefined natural sweeteners *viz*. honey, jaggery and date syrup, could be explored in beverage production as they not only possess significant nutritional compounds such as vitamins and minerals but also have abundant health promoting properties owing to the presence of organic acids, minerals and polyphenolic compounds (*5-7*). Studies conducted in the past have explored the use of honey, jaggery and date syrup to replace/substitute sucrose in beverages, dairy products and baked goods, and have revealed significant alterations in bioactive profile, rheological properties, and colour aspects (non-enzymic browning) of the final products (*8-10*).

Guava is known to be effective in treating diarrhea, hypertension, fighting eczema, pain, caries, toothache and boosting immunity. However, its consumption should be restricted in pregnant and lactating women (*11*). Red fleshed guava, being most suitable for processing, is most consumed fruit in beverage industry. It is also a rich source of citric, ascorbic, malic and succinic acids (*12*), as well as flavones, flavonols, flavonones and polyphenolic compounds *viz.* gallic, chlorogenic, caffeic, *trans*-

cinnamic, vanillic, *p*-coumaric, syringic, ferulic and ellagic acids, and are particularly rich in carotenoids such as all-*trans*-lutein, zeaxanthin, β-cryptoxanthin, α-carotene and β-carotene (*13,14*).

Hence, the present investigation was intended to explore the use of honey, jaggery and date syrup as potential alternatives to sucrose in guava nectar and study the developed product for its impact on qualitative characteristics including bioactive compounds, rheological behavior, mineral composition and *in vitro* bioavailable iron. Furthermore, storage study for six months was conducted to analyze the effect of substitution of various quality parameters such as colour, non-enzymatic browning, and development of 5-hydroxymethylfurfural.

MATERIALS AND METHODS

Procurement of raw materials and chemicals

Red fleshed ripe guava (*Psidium guajava* var. *Punjab Pink*) was procured from Punjab Organic Vegetable and Fruit Producer Co. Ltd., Patiala, Punjab. Honey (Markfed Sohna™, Jalandhar, India), date syrup (Lion™, Tamil Nadu, India), cane jaggery powder (Vedaka™, Nawanshahr, India) and sucrose (good quality refined crystal sugar) were procured from the local market, Ludhiana, India. Chemical reagents (AR grade) were purchased from Sisco Research Laboratories Pvt. Ltd., Mumbai, India.

Preparation of guava nectar

Different formulations of guava nectar in which sucrose was substituted with unrefined natural sweeteners were prepared by following a standard method described in Sidappa *et al.* (*15*), wherein TSS and acidity of the guava nectar was maintained as minimum 15 °B and 0.3 % (maximum 1.5 %), respectively, according to standard specifications laid by FSSAI (*16*). The level of substitution of sucrose was varied at 25, 50, 75 % and 100 % in honey and jaggery-based formulations, whereas it was 20, 30, 40 and 50 % in date syrup-based formulations based on preliminary trials. Control was prepared using 100 % sucrose. The formulations were designed on the basis of TSS and acidity of raw materials *i.e.* guava pulp (TSS=10.2 °Brix and acidity=0.65 %), honey (TSS=82.2 °Brix and acidity=0.15 %), jaggery (TSS=97.8 °Brix and acidity=0.29 %) and date syrup (TSS=72.1 °Brix and acidity=0.47 %).

To prepare guava nectar (control, honey-based, jaggery-based and date syrup-based guava nectar), ingredients were weighed as per formulations. Cold syrup containing water, citric acid and sweeteners

was prepared and filtered through muslin cloth. Guava pulp was added to the syrup and the nectar was homogenized. This was followed by two different processing treatments *i.e.* cold-filling (CF) and hot-filling (HF). Nectar was filled in pre-cleaned glass bottles without pasteurization in cold-filling process, whereas in hot-filling process, pasteurization (at 82-85 ºC for 1–2 min) was carried out followed by filling in glass bottles. The bottles were corked and sterilized in boiling water (100 ºC) for 20 min; labelled and stored under ambient conditions (18–36 ºC) for 6 months.

Analytical methods

Physico-chemical properties

Total soluble solids (°Brix) were estimated using a handheld refractometer with scale ranging from 0-32 °B (Erma, Tokyo, Japan) and were corrected to 20 °C (*17*). pH was measured using pH meter (Mettler Toledo S220, Greifensee, Switzerland) which was calibrated using standard buffer solutions at values 4.01, 7.00 and 9.21. Titratable acidity was estimated as per AOAC (*17*). In brief, 10 mL of nectar sample was diluted to make up volume of 100 mL, and 20 mL of aliquot was drawn, which was titrated against 0.1 N NaOH solution using 1 % phenolphthalein solution as an indicator. Light pink colour was noted as end point (Eq. 1).

$$\text{Titratable acidity (\% citric acid)} = \frac{V_t \cdot m \cdot V_m \cdot M\ \text{NaOH} \cdot 100}{V_s \cdot V_a \cdot 1000} \quad /1/$$

where m is the equivalent mass of citric acid, V_t is the titre value, V_m is the volume made up, V_s is the volume of sample, and V_a is the volume of the aliquot.

Lane and Eynon method was used for the estimation of reducing sugars (*18*). A mass of 4 g of nectar was diluted to 10–15 mL with distilled water and was neutralized with 1 N NaOH using phenolphthalein indicator. A volume of 2 mL of 45 % lead acetate solution was added, and the solution was kept for 10 min, which was then precipitated with 5 mL of 22 % potassium oxalate solution. Final volume was made up to 100 mL. The solution was then filtered using Whatman filter paper. A volume of 5 mL of Fehling solution A and Fehling solution B are taken in a conical flask and boiled with simultaneous addition of 3 drops of 1 % methylene blue indicator. This was titrated against the sugar solution obtained within 1 min. Brick red precipitates were observed as end point (Eq. 2).

$$\text{Reducing sugars (\%)} = \frac{m \cdot \text{DF} \cdot 100}{V_t \cdot m_s \cdot 1000} \quad /2/$$

where m is the mass of invert sugar (mg), DF is the dilution factor, V_t is the titre value, and m_s is the mass of sample.

Non-enzymatic browning was measured as absorbance at 440 nm ($A_{440\ nm}$) (*19*). A volume of 5 mL of nectar sample was diluted to 50 mL and centrifuged at 1000×*g* for 15 min at 4 °C. A volume of 2 mL of supernatant and 3 mL of alcohol were mixed in a test tube thoroughly. Absorbance was measured at 440 nm using aqueous alcohol (60 %) as a blank.

Colour parameters

The *L**, *a**, *b** values of the product were observed using Konica Minolta colour difference meter (CM-5, Osaka, Japan). Chroma and hue were calculated as:

$$\text{Chroma} = \sqrt{a^{*2} + b^{*2}} \quad /3/$$

$$\text{Hue} = \tan^{-1}(b^*/a^*) \quad /4/$$

Organoleptic evaluation

To estimate consumer acceptability, sensory evaluation was carried out using 9 - point hedonic scale rating done by 15–20 personnel for the parameters like colour/appearance, mouthfeel, odor, flavor and overall acceptability (*20*).

Bioactive compounds

Ascorbic acid (mg/100 mL) was estimated using titrimetric method as mentioned in Bal et al. (*18*). A 2,6-dichloroindophenol dye (0.04 %) solution was standardized against mixture of 5 mL of L-ascorbic acid solution (0.1 mg/mL of 0.4 % oxalic acid) and 5 mL of 0.4 % oxalic acid solution. Titre value obtained was used to calculate dye factor (Eq. 5). A volume of 5 mL of sample was diluted to make up volume to 50 mL with 0.4 % oxalic acid solution. The solution was filtered, and 20 mL of its aliquot was titrated against 2,6-dichloroindophenol dye solution. Light pink colour persisting for at least 15 s was considered as the end point (Eq. 6).

$$\text{Dye factor} = \frac{0.5}{V_t} \quad /5/$$

$$\gamma_{\text{ascorbic acid}} = \frac{V_t \cdot \text{DF} \cdot V_m \cdot 100}{V_s \cdot V_a} \quad /6/$$

where $\gamma_{\text{ascorbic acid}}$ is the mass concentration of ascorbic acid (mg/100 mL), DF is the dye factor, V_a is the sample volume, V_m is the volume made up, V_a is the volume of aliqout, and V_t is the titre value.

Total carotenoid sand lycopene contents were determined in acetone-petroleum ether extract by plotting absorbance (*A*) at 452 nm (using β-carotene standard curve) and 503 nm, respectively (Eq. 7 and Eq. 8) as described in Lakhanpal and Vaidya (*8*). A volume of 5 mL of sample was ground in pestle mortar with acetone using sodium sulphate until the residue turned colourless and formed a resinous mass. The filtrate was then transferred to a separating funnel, and 10–15 mL of petroleum ether was added to it. The pigments are transferred to petroleum ether by diluting acetone with water. The petroleum ether extract was filtered, and volume was made up to 25 mL. Absorbance was measured at 452 nm and 503 nm using spectrophotometer (Agilent Technologies, Petaling Jaya, Malaysia) and petroleum ether as blank.

$$\gamma_{TC} = \frac{c \cdot V_m \cdot 100}{V \cdot 1000} \quad /7/$$

$$\gamma_{lyc} = \frac{3.1206 \cdot A_{503} \cdot V_m \cdot 100}{V \cdot 1000} \quad /8/$$

where γ_{TC} is the mass concentration of total carotenoids (mg/100 mL), γ_{lyc} is the mass concentration of lycopene (mg/100 mL), *c* is the concentration from the respective standard curve, V_m is the volume made up, *V* is the volume of sample, $A_{503\ nm}$ is the absorbance at 503 nm.

Total phenolic content was determined using method described in Swain and Hillis (*21*) using gallic acid standard curve. Methanolic extract (100 mL) of the sample was prepared by refluxing 5 mL of the sample with 80 % methanol for 2 h. Methanolic extract (0.2 mL) and 0.8 mL of distilled water were added to a test tube, followed by the addition of 5 mL of Folin-Ciocalteau reagent and 4 mL of saturated sodium carbonate solution. The solution formed was incubated for 45 min and the absorbance of developed colour was observed at 765 nm (Agilent Technologies, Petaling Jaya, Malaysia), and results were expressed as mg GAE/100 mL.

$$\gamma_{TPC} = \frac{c \cdot V_m \cdot 100}{V} \quad /9/$$

where γ_{TPC} is the mass concentration of TPC (mg GAE/100 mL), *c* is the concentration from the standard curve, V_m is the volume made up, and *V* is the volume of the sample.

DPPH radical scavenging activity was estimated as per Shimada *et al.* (*22*). Methanolic extract of the sample was prepared as described for total phenolic content. Methanolic extract (0.5 mL) was mixed

with 0.5 mL of Tris buffer solution and 1 mL of 0.1 mM DPPH (diphenylpicrylhydrazyl) dye. Percent radical scavenging activity was estimated in comparison to the control, which was prepared by adding 0.5 mL of distilled water, 0.5 mL of Tris buffer and 1 mL of 0.1mM DPPH. The solutions were incubated for 30 min and absorbance at 517 nm (Agilent Technologies, Petaling Jaya, Malaysia) was read. Results were expressed as radical scavenging activity (RSA/%) using the following formula:

$$\text{RSA}=\left(\frac{A_o-A_s}{A_o}\right)\cdot 100 \qquad /10/$$

where A_o is the absorbance of blank at 0 min and A_s is the absorbance of the sample after 30 min.

5-hydroxymethyl furfural (mg/100 mL) was estimated as per modified Seliwanoff method (*8*). A volume of 20 mL of sample was diluted with water to 100 mL and centrifuged. The supernatant was filtered through Whatman no. 2 paper. Three successive extractions of 10 mL filtrate were done with 20 mL of ether in a separatory funnel after the addition of 2.5 g of NaCl. A volume of 1 mL of water was added to the obtained extract and evaporated at room temperature in air draft. The volume of residue was made up to 10 mL. A colume of 3 mL of extract was taken in a test tube with the addition of 3 mL of 99.9 % ethyl alcohol and 3 mL of 1 % resorcinol in HCl. The contents were mixed thoroughly and incubated in the dark for 30 min. The absorbance (*A*) was measured at 540 nm (Agilent Technologies, Petaling Jaya, Malaysia), and concentration was calculated via a standard curve of 5-hydroxymethylfurfural.

$$\gamma_{\text{HMF}} = \frac{c\cdot \text{DF}\cdot 100}{V\cdot 1000} \qquad /11/$$

where γ_{HMF} is the mass concentration of 5-HMF (mg/100mL), c is the concentration from the standard curve, DF is the dilution factor, and V is the volume of the sample.

Rheological measurements

The rheological behavior of nectar was analyzed using Physica MCR 101 rheometer (Anton Paar, Graz, Austria) equipped with concentric cylinder probe (DG 267/T 200/AL) having 25 mm inner diameter. Temperature was controlled precisely by the Peltier system.

Flow behavior. Rheological flow behavior was measured at shear rate 0–100 s^{-1} with 30 data points for each curve at 25 ºC. The flow curve for shear stress (τ) versus shear rate was plotted and fitted to Ostwald-de-Waele (Eq. 12) and Herschel-Bulkley (Eq. 13) models using Rheoplus software.

$$\tau=K\gamma^{n} \qquad /12/$$

$\tau=\tau_0+K\gamma^n$ /13/

where τ_0 is the yield stress (Pa), γ is the shear rate (s^{-1}) K is the consistency index (Pa·s) and n is the flow behaviour index.

Oscillatory sweeps. Amplitude sweeps were run to determine the impact of strain (0.01–100 %) on storage modulus (*G'*) and loss modulus (*G"*) at angular frequency 10 rad/s at 25 °C taking 25 data points. The data was recorded using Rheoplus software.

Mineral composition and i*n vitro* bioavailable iron

A volume of 5 mL of nectar was digested using 10 mL of concentrated nitric acid and concentrated perchloric acid in a ratio 3:1. The solution was kept overnight, followed by heating until a clear solution was obtained. Volume is made up to 25 mL and mineral content (Ca, K, Na, P, S, Mg, Mn, Cu, Zn, B, Fe) (reported in mg/L) is analyzed through inductively coupled plasma-optical emission spectrometry (Agilent Technologies, Petaling Jaya, Malaysia).

Bioavailable iron has been estimated through *in vitro* method described in Rao and Prabhavati (*23*). A weighed amount of sample was digested using pepsin-hydrochloric acid (0.5 % pepsin in 0.1 N HCl) and was incubated at 37 °C for 90 min after adjusting pH to 1.35. The contents were centrifuged, and the filtrate was again incubated at 37 °C for 90 min after adjusting pH to 7.5. The ionizable iron content in the extract formed was estimated using atomic absorption spectrometry (Agilent Technologies, Petaling Jaya, Malaysia). *In vitro* bioavailable iron was calculated based on the prediction equation suggested:

$Y=0.4827+0.4707\cdot X$ /14/

where X is the percentage of ionizable iron at pH=7.5 and Y is the percentage of iron absorbed in adult men.

Total plate count

To ensure the microbial load of the product to be in prescribed limits *i.e.* 50 CFU/ mL as per FSSAI (2011) (*16*), total plate count (CFU/mL) was carried out using pour plating method with standard Plate Count Agar media (SRL, Mumbai, India) as per Ranganna (*18*).

Statistical analysis

Data obtained was subjected to statistical analysis using various ANOVA techniques with post-hoc Tukey's test to evaluate significant differences between means using STATISTIX v. 10.0. software (*24*).

RESULTS AND DISCUSSIONS

Effect of substitution level of sweetener and processing treatment on physico-chemical and colour properties

The effect of sweetener substitution and processing treatment on physico-chemical properties and colour is depicted in Table S1. pH values increased in sucrose substituted guava nectar with an increase in concentration of honey, jaggery and date syrup. The trend was comparable to Cerevera-Chiner *et al.* (*10*), who reported a rise in pH value from 3.58 to 3.75 and 3.45 to 3.82 in kiwifruit and strawberry jams, respectively, with an increase in jaggery concentration from 0-75 %. It is suggested that the higher pH of jaggery might be due to the addition of lime during the purification process in jaggery making. Farahnaky *et al.* (*25*) reported the pH of date syrup as 4.24-4.62, and Belay *et al.* (*26*) reported that the pH of honey from different origins can vary from 3.38-4.57. Therefore, it can be inferred that higher pH of sweeteners might have contributed to the increased pH of sucrose substituted guava nectar. The effects of hot-filling and cold-filling were found to be non-significant ($p \leq 0.05$).

An increment in reducing sugar content was observed with an increase in the level of honey and date syrup, whereas the trend reversed in jaggery-based guava nectar. It was found to decrease up to two-fold on increasing the level of jaggery from 25 to 100 %. This could be ascribed to the presence of a very high amount of reducing sugars, *viz*. glucose and fructose in honey and date syrup (*26-28*). However, Cerevera-Chiner *et al.* (*10*) also depicted depletion in glucose and fructose content on increasing the level of substitution of jaggery in strawberry and kiwifruit jams and suggested that sugars in jaggery might be less prone to hydrolysis during processing. Also, the higher pH of jaggery, due to remnants of lime used during processing, might have interfered with the process of inversion. Reducing sugars were found to be significantly higher ($p \leq 0.05$) in HF in comparison to CF. Adulvitayakorn *et al.* (*29*) implied a breakdown of sucrose into glucose and fructose upon intensive heating.

The level of substitution of unrefined natural sweeteners also had a notable impact on colour characteristics of the red-fleshed guava nectar. It has been observed that irrespective of the natural

sweetener, *L** values have significantly ($p \leq 0.05$) decreased, and *a** and *b** values increased with the increase in level of substitution. Correspondingly, a change in chroma indicated enhanced saturation, and hue values indicated a loss of redness. An overall shift from redness to yellowness on the CIE colour wheel can be observed, depicting a paradigm shift from characteristic pink colour to pink orangish tonalities. This could be ascribed to the presence of red, yellow and brown coloured compounds in natural sweeteners. Brown colour of unrefined sugars (jaggery) might be due to the presence of molasses, phytochemical pigments and amino acids. Subsequently, the use of high temperature during the processing of jaggery could also contribute to browning (*30*). However, the brown colour of honey depends on the composition of nectar, the process of acquisition, pigments present, temperature, light and storage time (*31*). Similarly, the colour of date syrup could also vary from yellow to red brown depending upon colour of date flesh and processing temperature used to obtain syrup, as explained by Julai *et al.* (*32*). They noted that the *L** value of date syrup prepared by vacuum evaporation was two-fold higher than open heating.

Although there was no apparent difference perceivable to the naked eye between HF and CF samples for a particular level of sweetener, data in Table S1 elucidated that heating has a pronounced effect on colour values. Statistically significant ($p \leq 0.05$) reduction in *L** values and surge in *a** and *b** values have been observed in hot-filled samples in comparison to cold-filled. This might be due to the formation of Maillard's reaction products during heating that are brown in colour as Tamanna and Mahmood (*33*) suggested that processing temperature may contribute to the formation of furoylmethyl derivatives in processed fruits and juices.

Optimization of substitution level based on organoleptic properties

In comparison to control guava nectar, 50 % level of honey, 25 % level of jaggery and 30 % level of date syrup for substitution of sugar in HF samples was selected for further assessment (Table S2, Table S3 and Table S4) as panelists suggested that HF samples had a comparatively richer mouthfeel than cold-filled samples. An increase in the substitution level of sweeteners at a higher level led to the darkening of the product. In addition, the favor profile was also immensely influenced at higher levels. Honey imparted astringent aftertaste, jaggery contributed caramelized notes and concealed flavor of guava, and date syrup imparted an over-sweet aftertaste along with a thick, gel-like consistency to the

nectar.

Bioactive characterization of sucrose substituted guava nectar

A significant change in bioactive content of sucrose-substituted guava nectar in comparison to the control was observed (Table 1). Substitution of sucrose with natural sweeteners immensely improved the total phenolic content (TPC) and antioxidant activity. It was found to be maximum in date syrup-based guava nectar followed by honey based and jaggery based guava nectar. Ascorbic acid content was also found to be maximum in date syrup-based guava nectar. However, the change in honey-based guava nectar and jaggery-based guava nectar was statistically non-significant ($p \leq 0.05$). Results were supported by studies conducted earlier, as Cerevera-Chiner *et al.* (*10*) also reported improved total phenolic content and DPPH activity in jaggery-substituted strawberry and kiwifruit jams with increased levels of substitutions. Inherent presence of phenolic compounds in honey (gallic acid, chlorogenic acid, caffeic acid, coumaric acid, pinocembrin, chrysin, quercetin, abscisic acid), jaggery (gallic acid, protocatechuic acid, gentistic acid, 4-hydroxyphenylacetic acid, vanillic acid, syringic acid, *p*-coumaric acid and ferulic acid) and date syrup (catechin, caffeic acid, vanillic acid, syringic acid, ferulic acid, *p*-coumaric acid, sinapic acid) (*34-36*) may have contributed to high phenolic content which attributed to improved antioxidant potential to the product.

In contrast to the results reported by Lakhanpal and Vaidya (*8*), the substitution of natural, unrefined sweeteners has led to a significant decrease in carotenoids and lycopene content in the beverage. It could be suggested that the presence of metal ions in honey, jaggery and date syrup (as depicted in Table 1) could have contributed to the higher degradation of carotenoids during heat processing. Penicaud *et al.* (*37*) suggested that, particularly at low pH (which was found to be 3.44–3.97 in prepared product), transition metals can oxidize carotenoids (unsaturated lipids). In addition to this, ascorbic acid content which could act synergistically to prevent oxidation of carotenoids was not significantly higher in this case.

Rheological characterization of sucrose substituted guava nectar

The flow behavior of guava nectar has been studied through the application of two different models: Ostwald-de-Waele model and Herschel-Bulkley model described in Table 2. According to the correlation

coefficient obtained, Herschel-Bulkley model was found to be a better fit to study characteristics of guava nectar. Table 2 elucidates rheological properties such as flow behavior index, consistency index and yield stress.

Flow curves adjusted according to Herschel Bulkley model (Fig. 1a) represented an increase in shear stress with an increase in shear rate. This indicated a decrease in viscosity with an increase in shear rate and values of flow behavior index for control and sucrose substituted nectar was also less than 1 which represented shear-thinning behavior. Therefore, the sucrose substituted guava nectar could be characterized as Herschel-Bulkley fluid ($\tau_0 \neq 0$). Similar results were reported by Peasura and Sinchaipanit (*38*) in which guava nectar substituted with neotame (0.01 %) and stevia (0.05 %) depicted shear-thinning behaviour.

However, wide variation in rheological properties in control and honey, jaggery and date syrup-based guava nectar could be explained as an overall impact of high temperature processing and variability in total solids, insoluble solids and pulp solids due to substitution with sweeteners which can have a significant impact on viscosity as with higher amount of solids concentration, consistency coefficient increases and flow behavior index decreases (*39*). As suggested by Bhandari *et al.* (*40*) viscosity of honey might be affected by the amount of monosaccharides and disaccharides as the molecular chain length of sugars affects the viscosity of honey. The higher viscosity in date syrup-based guava nectar could be due to presence of pectin and fibre content. Furthermore, high temperature during heating provides a higher level of molecular energy, which facilitates molecular movement and causes a decrease in the consistency coefficient. However, high temperature processing can also lead to the alteration in microstructure of the product, enzyme inactivation, causing a lesser degree of pectin degradation, leading to an increase in consistency (*39*). Therefore, a detailed study about sugar composition and its changes upon heating could be carried out to study its effect on rheological parameters.

The graph for storage modulus (*G'*) and loss modulus (*G"*) plotted against strain (%) (Fig. 2) explicated that the value for *G'* was higher for all the samples was initially higher than *G"* but *G'*<*G"* when the strain exceeded. The graph shows that the solid structure was predominant. This could be due to the dispersion of pulp particles containing cell wall material such as cellulose, hemicellulose, lignin and pectic materials in guava nectar, which may include fibre and represent a weak gel structure. The graph

elucidates that elastic properties were dominant over viscous properties, and thus it can be suggested that prepared nectars could be considered viscoelastic liquids under low strain amplitude (<10 %) and viscous liquids at higher strain amplitude. Similar results were reported by Augusto *et al.* (*41*) in peach juice with the addition of fibre (suspended solids) at 12.5 % depicted higher *G'* than *G"* throughout. The study suggested that the addition of fibre caused a change in Newtonian behavior to shear-thinning behavior. Thus, it could be concluded that nectar prepared exhibits a dispersion of insoluble polymeric clusters in a viscous medium composed of soluble polysaccharides, sugars and acids in water. Interaction between hemicellulosic polysaccharides and pectic polysaccharides forming a network could contribute to the elastic component of nectars (*41*).

Mineral composition and in-vitro bioavailable iron content of sucrose substituted guava nectar

Mineral composition (Table 3) represents that substitution of honey, jaggery and date syrup at 50, 25 and 30 % level respectively, have significantly ($p \leq 0.05$) improved contents of calcium, potassium, sodium, phosphorus, sulphur, magnesium, manganese and boron as honey, jaggery and date syrup are rich source of minerals (*6-7,9*). Bread substituted with date fruit pulp meal and soursop drink with honey has also found to have higher mineral content as reported by Obiegbuna *et al.* (*9*) and Olagunju and Sandewa (*42*), respectively. Iron was focused on the present product, as guava is a rich source of organic acids and ascorbic acids, and studies suggest a synergistic effect of these compounds in the absorption of iron (*43*). Iron content has also been found to be significantly ($p \leq 0.05$) higher in jaggery and date syrup-based guava nectar and insignificantly in honey-based guava nectar than control. However, bioavailability of iron (%) was found to be maximum in control (48.68 %) followed by honey-based (45.30 %), jaggery-based (30.86 %) and date syrup-based (27.25 %) guava nectar. This could be attributed to the lower acidity and high pH (as presented in Table S1), as organic acids can have synergistic effects on enhancing iron-absorption as suggested by Teucher *et al.* (*43*). Govindaraj *et al.* (*44*) have also concluded that the addition of citric acid and tartaric acid has increased the bioavailability of iron in iron-fortified biscuits. Therefore, citric acid supplementation can be done to promote iron-absorption in body and food products such as beverages, which are widely consumed and could be used as food vehicles to promote iron absorption.

Effect on qualitative characteristics during storage

During storage at ambient temperature for a period of 6 months, extensive and crucial changes in qualitative characteristics were observed (Table 4).

Reducing sugars initially increased during the storage period up to 4 months, then decreased further. Bal *et al.* (*18*) also reported a surge in reducing sugars in guava nectar during storage due to partial hydrolysis of starch from acid and inversion of non-reducing sugars into reducing sugars. Further, a decrease in reducing sugar content during storage was observed, which could be explained by the participation of reducing sugars in the formation of 5-HMF, which was found to be in the range of 5.89 to 8.44 mg/100 mL in the 6th month of storage. Cavaco *et al*. (*45*) also suggested carbohydrate degradation during thermal processing that could contribute to the formation of 5-HMF. The pH significantly ($p \leq 0.05$) increased over the period of 6 months, except in the case of honey-based guava nectar. This could be due to the conversion of organic acids present in juices into simple sugars and salts due to the action of invertase (*46*).

Ascorbic acid degradation was prominent during storage in guava nectar, particularly in jaggery-based guava nectar. Ascorbic acid retention was found to be the least in jaggery-based guava nectar (23.81 %), followed by honey-based guava nectar (50 %), date syrup-based guava nectar (41.33 %), and control (63.50 %). The observations were in line with results obtained by Hariharan and Mahendran (*47*), which suggested a reduction in ascorbic acid during storage as, is prone to oxidation and its conversion into dehydroascorbic acid in ginger-lime RTS beverage sweetened by palmyra sugar. Therefore, headspace in the glass bottle might have a considerable impact on the stability of ascorbic acid in the beverage. According to Tiwari *et al.* (*48*), ascorbic acid degrades aerobically at first and then anaerobically during storage in thermally processed orange juice. Sheraz *et al*. (*49*) suggested that the stability of ascorbic acid is also influenced by oxygen, temperature, pH of the medium, and the presence of metal ions such as Cu^{2+}, Fe^{2+} and Zn^{2+} that aid in catalysis of degradation reactions. Therefore, it can be inferred that higher pH and presence of metal ions (Table 3) in sucrose substituted nectar might be responsible for reducing the stability of ascorbic acid in guava nectar.

Initially, DPPH radical scavenging activity decreased and increased during 6th month of the storage period. This could be attributed to the oxidation of phenolic compounds to its polymeric forms during storage (*50*). Results obtained were in the agreement with Klimczak *et al.* (*51*), as it also suggested a decrease in antioxidant activity during the storage period of 6 months in orange juices and a sudden

increase in antioxidant activity that is attributed to the formation of Maillard's reaction product. This was evident in the present study as 5-HMF content was significantly higher in the 6th month of storage.

Furthermore, an increase in non-enzymatic browning (NEB) values during storage, along with a decrease in *L** values and subsequent increase in *a** and *b** values, depicts darkening of the product during storage. This could be attributed to chemical reactions such as oxidation of phenolic compounds and other reactions involving reducing sugars and organic acids, which could lead to the formation of brown pigments (*46*). Discolouration of juices due to the formation of brown pigments and the inherent dark colour of sweeteners is responsible for masking of characteristic pink colour of guava nectar and shifting its hue towards yellow.

5-HMF content by the end of the storage period was found to be lower in the control than sugar substituted guava nectar. Talcott *et al.* (*52*) also reported increased browning, higher levels of 5-HMF and decrease in *L** values in passion fruit juice during storage of 28 days. It also suggested that colour degradation is proportional to loss in ascorbic acid, which could be validated in the present study. Shinoda *et al.* (*53*) suggested that browning in orange juice due to the formation of HMF and other browning compounds are stimulated by the presence of ascorbic acid, sugars, citric acid, storage time and absence of head space whereas presence of chelating agents and radical scavenger inhibits formation of compounds contributing to browning of juices. Also, the presence of metal ions such as Fe^{2+} and Cu^{2+} (as natural sweeteners are a rich source of minerals) promotes browning through Maillard's reactions (*54*). Recent studies in the past have depicted apprehensions about the toxic potential, carcinogenicity and genotoxicity of 5-HMF. According to Abraham *et al.* (*55*), 5-HMF levels in the range of 80-100 mg/kg body mass per day could be consumed safely without any adverse effects. Therefore, it can be concluded that sucrose substituted guava nectar can be consumed safely up to the shelf life of 6 months. The value for total plate count increased significantly during the storage period of six months (0-5 CFU/mL) but was lower than the limit specified by FSSAI *i.e.* 50 CFU/mL. Therefore, microbiologically also, sucrose substituted guava nectar can be considered safe for consumption for up to six months.

CONCLUSIONS

Sucrose substituted guava nectar could be successfully prepared using natural sweeteners: honey, jaggery and date syrup at 50, 25 and 30 % substitution levels, respectively, through hot filling method.

With respect to harnessing health benefits of the natural sweeteners to the maximum, red-fleshed guava nectar with 30 % date-syrup has been found to have higher values of ascorbic acid content, total phenolic content and antioxidant activity in comparison to honey/jaggery-based nectar. However, a considerable reduction of carotenoids and lycopene content was observed, which could be attributed to the presence of transition metals in sweeteners. The substitution of natural sweeteners also led to substantial improvement in mineral content of the product, except copper. Although iron content was enhanced upon substitution of natural sweeteners, its bioavailability (%) decreased, which could be associated with higher pH in comparison to the control, as the presence of organic acids have a synergistic effect on improving iron bioavailability. Study of rheological properties depicted non-Newtonian (pseudoplastic) behavior of the nectar due to the presence of dispersion of pulp particles, which further contributed to the weak-gel structure of the nectar, resulting in its viscoelastic properties below 10 % strain. Microbiologically, it could be safely stored at ambient temperature for a period of 6 months. However, its degrading effects on quality parameters such as colour, non-enzymatic browning, ascorbic acid and 5-HMF development, which are highly correlated, must be considered, and methods for improvement could be suggested. Hence, based on the results, it can be concluded that substitution of honey, jaggery and date syrup could be widely explored for enrichment in nutritional and therapeutic properties of beverages.

FUNDING DECLARATION

No funding was received for conducting this study.

CONFLICT OF INTEREST

The authors report there are no competing interests to declare.

AUTHORS' CONTRIBUTIONS

M. Gupta contributed to conceptualization, methodology, analysis and investigation, writing original draft and editing. S. Kapoor contributed to conceptualization, methodology, supervision and editing. M. Bala and B.V.C. Mahajan provided resources and edited the manuscript.

SUPPLEMENTARY MATERIAL

Supplementary material is available at www.ftb.com.hr.

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Table 1. Bioactive compounds of sucrose substituted guava nectar

Bioactive compounds	Control	Honey-based guava nectar	Jaggery-based guava nectar	Date syrup-based guava nectar
$\gamma_{\text{ascorbic acid}}$/(mg/100 mL)	(14.80±0.24)^{b}	(14.94±0.42)^{b}	(14.53±0.42)^{b}	(16.74±0.24)^{a}
γ_{TPC}/(mg GAE/100 mL)	(85.31±0.25)^{d}	(99.09±0.32)^{b}	(94.40±0.27)^{c}	(117.37±0.41)^{a}
γ_{TC}/(mg/100 mL)	(46.41±0.21)^{a}	(41.58±0.12)^{b}	(33.36±0.24)^{d}	(34.12±0.11)^{c}
γ_{lyc}/(mg/100 mL)	(1.05±0.02)^{a}	(0.96±0.03)^{b}	(0.85±0.03)^{c}	(0.94±0.03)^{b}
RSA/%	(56.43±0.25)^{d}	(59.61±0.27)^{b}	(58.54±0.41)^{c}	(64.48±0.25)^{a}

Data is represented as mean value±S.D. (N=3). Values with different superscripts (a,b,c,d) depict statistical differences between samples ($p \leq 0.05$).
RSA=radical scavenging activity

Table 2. Rheological parameters of Ostwald-de-Waele model and Herschel-Bulkley model in sucrose substituted guava nectar

	Ostwald-de-Waele model			Herschel-Bulkley model			
	K	*n*	R^2	Yield stress	*K*	*n*	R^2
Control	13.336	0.221	0.77	0.823	4.946	0.477	0.97
Honey-based guava nectar	8.749	0.372	0.92	10.937	2.188	0.629	0.93
Jaggery-based guava nectar	9.672	0.184	0.85	1.229	5.810	0.299	0.93
Date syrup-based guava nectar	17.548	0.205	0.83	1.253	9.789	0.349	0.94

K=consistency coefficient, *n*=flow behaviour index

Table 3. *In-vitro* bioavailable iron and mineral composition of sucrose substituted guava nectar

Sample	*γ*/(mg/L)											
	Calcium	Potassium	Sodium	Phosphorus	Sulphur	Magnesium	Manganese	Copper	Zinc	Boron	Iron	Bioavailable iron
Control	(86.53±0.30)^{d}	(642.22±0.14)^{d}	(45.23±0.16)^{d}	(33.72±0.18)^{d}	(183.66 0.18)^{d}	(42.67±0.29)^{d}	(0.28±0.014)^{c}	(0.20±0.01)^{a}	(2.11±0.11)^{bc}	(0.66±0.01)^{d}	(3.28±0.06)^{c}	(1.60±0.01)^{a}
Honey-based guava nectar	(99.17±0.20)^{c}	(792.57±0.21)^{c}	(53.63±0.14)^{b}	(40.54±0.10)^{c}	(201.93 0.08)^{c}	(49.09±0.18)^{c}	(0.31±0.007)^{c}	(0.21±0.01)^{a}	(2.91±0.06)^{a}	(1.16±0.02)^{b}	(3.48±0.04)^{bc}	(1.57±0.03)^{a}
Jaggery-based guava nectar	(117.17±0.14)^{b}	(925.15±0.20)^{b}	(148.32±0.14)^{a}	(82.43±0.18)^{a}	(229.51 0.13)^{a}	(80.29±0.23)^{a}	(0.38±0.011)^{b}	(0.25±0.007)^{a}	(2.50±0.09)^{b}	(0.94±0.01)^{c}	(3.95±0.04)^{a}	(1.22±0.01)^{b}
Date syrup-based guava nectar	(123.76±0.30)^{a}	(1128.51±0.27)^{a}	(46.073±0.06)^{c}	(64.30±0.06)^{b}	(221.72 0.36)^{b}	(67.41±0.24)^{b}	(0.49±0.005)^{a}	(0.22±0.01)^{a}	(1.72±0.10)^{c}	(1.37±0.01)^{a}	(3.72±0.09)^{ab}	(1.01±0.02)^{c}

Data is represented as mean value±S.D. (*N*=3). Values having different superscripts (a,b,c,d) depict statistical differences between samples ($p \leq 0.05$)

Table 4. Effect on qualitative characteristics during storage

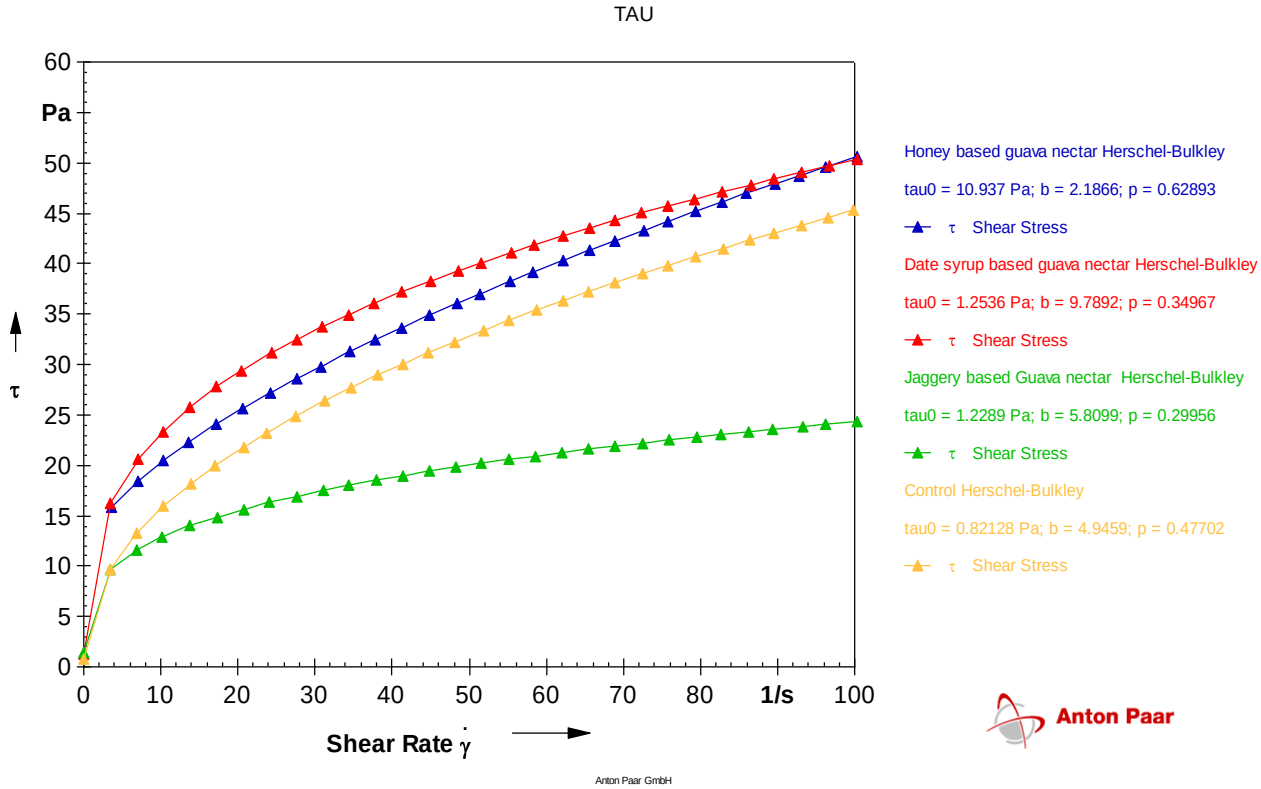
Quality parameter	t(storage)/month			
	0	2	4	6
	Control			
w(reducing sugar)/%	(5.11±0.12)^{c}	(7.81±0.37)^{b}	(10.82±1.55)^{a}	(7.98±0.19)^{b}
pH	(3.41±0.01)^{b}	(3.43±0.01)^{b}	(3.46±0.01)^{a}	(3.46±0.01)^{a}
$\gamma_{\text{ascorbic acid}}$/(mg/100 mL)	(14.80±0.24)^{a}	(10.79±0.42)^{b}	(9.96±0.42)^{bc}	(9.41±0.24)^{c}
RSA/%	(56.43±0.25)^{a}	(50.30±0.15)^{b}	(26.55±0.44)^{d}	(37.37±0.29)^{c}
NEB	(0.052±0.001)^{d}	(0.072±0.001)^{c}	(0.087±0.002)^{b}	(0.092±0.001)^{a}
*L**	(50.35±0.11)^{a}	(48.78±0.20)^{b}	(44.42±0.27)^{c}	(44.73±0.26)^{c}
*a**	(15.40±0.18)^{d}	(16.57±0.22)^{c}	(20.23±0.20)^{b}	(22.54±0.09)^{a}
*b**	(26.73±0.24)^{d}	(28.26±0.14)^{c}	(31.33±0.15)^{b}	(31.88±0.11)^{a}
γ_{HMF}/(mg/100 mL)	BLD	BLD	(1.01±0.03)^{b}	(5.89±0.03)^{a}
	Honey-based guava nectar			
w(reducing sugar)/%	(10.54±0.32)^{a}	(13.40±0.59)^{a}	(13.45±0.44)^{a}	(12.50±0.20)^{a}
pH	(3.42±0.02)^{a}	(3.44±0.01)^{a}	(3.44±0.01)^{a}	(3.45±0.02)^{a}
$\gamma_{\text{Ascorbic acid}}$/(mg/100 mL)	(14.94±0.42)^{a}	(9.55±0.42)^{b}	(8.55±0.24)^{c}	(7.47±0.42)^{c}

RSA/%	(62.46±0.23)^{a}	(43.54±0.38)^{b}	(24.46±0.50)^{d}	(36.49±0.30)^{c}
NEB	(0.056±0.002)^{d}	(0.069±0.001)^{c}	(0.078±0.001)^{b}	(0.095±0.001)^{a}
*L**	(47.28±0.29)^{a}	(45.26±0.11)^{b}	(42.61±0.19)^{c}	(41.47±0.27)^{d}
*a**	(16.31±0.10)^{d}	(17.21±0.08)^{c}	(19.79±0.11)^{b}	(20.06±0.13)^{a}
*b**	(34.28±0.13)^{d}	(35.50±0.16)^{c}	(39.82±0.07)^{b}	(41.69±0.11)^{a}
γ_{HMF}/(mg/100 mL)	BLD	BLD	(0.93±0.03)^{b}	(6.64±0.03)^{a}
		Jaggery-based guava nectar		
w(reducing sugar)/%	(4.53±0.28)^{c}	(5.13±0.13)^{c}	(7.38±0.32)^{a}	(6.61±0.33)^{b}
pH	(3.81±0.01)^{b}	(3.82±0.01)^{b}	(3.84±0.01)^{a}	(3.85±0.01)^{a}
$\gamma_{ascorbic\ acid}$/(mg/100 mL)	(14.53±0.42)^{a}	(7.19±0.24)^{b}	(6.50±0.24)^{b}	(3.46±0.24)^{c}
RSA/%	(58.54±0.41)^{a}	(39.53±0.21)^{b}	(24.63±0.38)^{d}	(32.57±0.19)^{c}
NEB	(0.069±0.001)^{d}	(0.077±0.001)^{c}	(0.089±0.003)^{b}	(0.099±0.001)^{a}
*L**	(46.73±0.17)^{a}	(45.31±0.12)^{b}	(43.39±0.23)^{c}	(37.33±0.22)^{d}
*a**	(17.59±0.23)^{c}	(18.08±0.12)^{b}	(18.48±0.17)^{b}	(21.14±0.07)^{a}
*b**	(37.52±0.26)^{d}	(40.37±0.23)^{c}	(41.33±0.11)^{b}	(44.67±0.09)^{a}
γ_{HMF}/(mg/100 mL)	BLD	BLD	(0.81±0.01)^{b}	(7.74±0.06)^{a}
		Date syrup-based guava nectar		
w(reducing sugar)/%	(7.89±0.11)^{c}	(9.67±0.21)^{b}	(12.37±0.42)^{a}	(8.38±0.17)^{c}
pH	(3.64±0.01)^{b}	(3.67±0.01)^{ab}	(3.68±0.02)^{a}	(3.68±0.01)^{a}
$\gamma_{ascorbic\ acid}$/(mg/100 mL)	(16.74±0.24)^{a}	(8.58±0.24)^{b}	(7.74±0.24)^{c}	(6.92±0.24)^{d}

RSA/%	$(64.48\pm0.25)^{a}$	$(38.70\pm0.25)^{b}$	$(27.31\pm0.28)^{d}$	$(32.21\pm0.23)^{c}$
NEB	$(0.105\pm0.001)^{d}$	$(0.118\pm0.001)^{c}$	$(0.172\pm0.002)^{b}$	$(0.187\pm0.002)^{a}$
*L**	$(38.58\pm0.11)^{a}$	$(37.31\pm0.27)^{b}$	$(35.69\pm0.20)^{c}$	$(34.44\pm0.30)^{d}$
*a**	$(21.36\pm0.22)^{d}$	$(22.39\pm0.18)^{c}$	$(23.25\pm0.16)^{b}$	$(24.28\pm0.25)^{a}$
*b**	$(42.50\pm0.12)^{b}$	$(47.33\pm0.25)^{a}$	$(47.34\pm0.15)^{a}$	$(47.48\pm0.18)^{a}$
$\gamma_{HMF/}$(mg/100 mL)	BLD	BLD	$(1.25\pm0.04)^{b}$	$(8.44\pm0.04)^{a}$

Data is represented as mean value±S.D. (*N*=3). Values having different superscripts a,b,c,d depict statistical differences between samples ($p\leq0.05$).
NEB=non-enzymatic browning, HMF=5-hydroxymethylfurfural, RSA=radical scavenging activity, BLD=below limit of detection

a)



b)

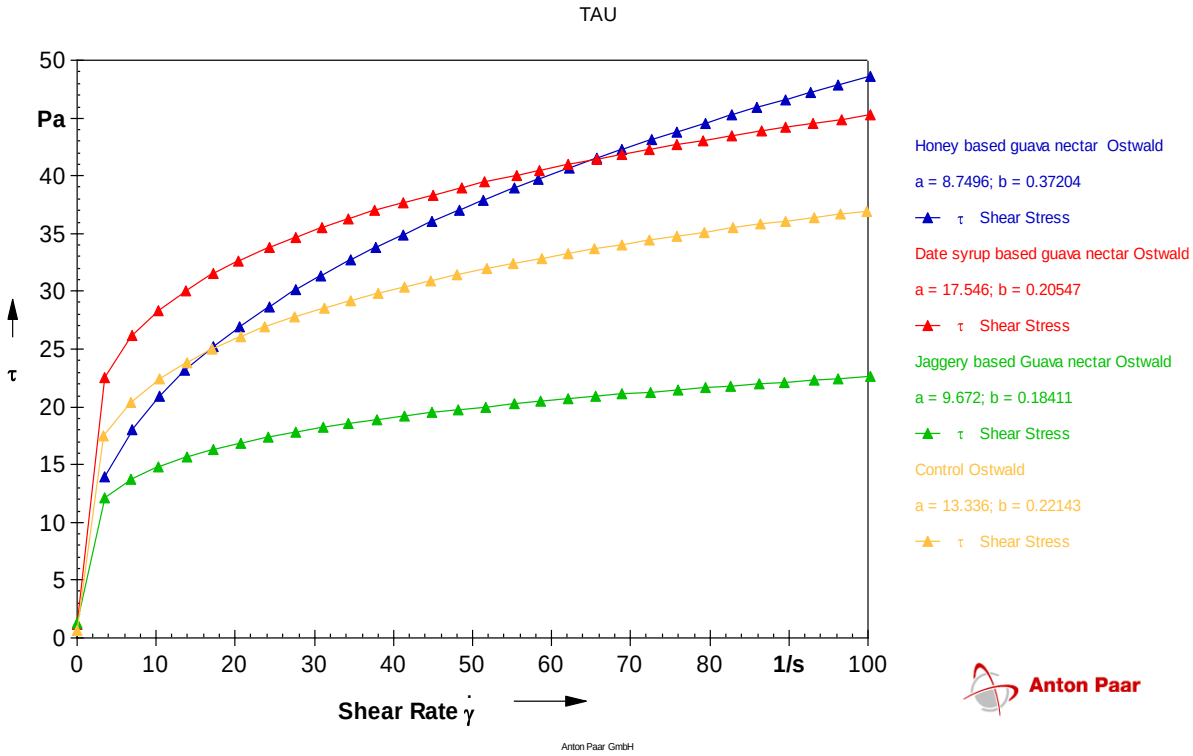


Fig. 1. Steady state rheology for sucrose substituted guava nectar adjusted according to: a) Herschel-Bulkley and b) Ostwald model

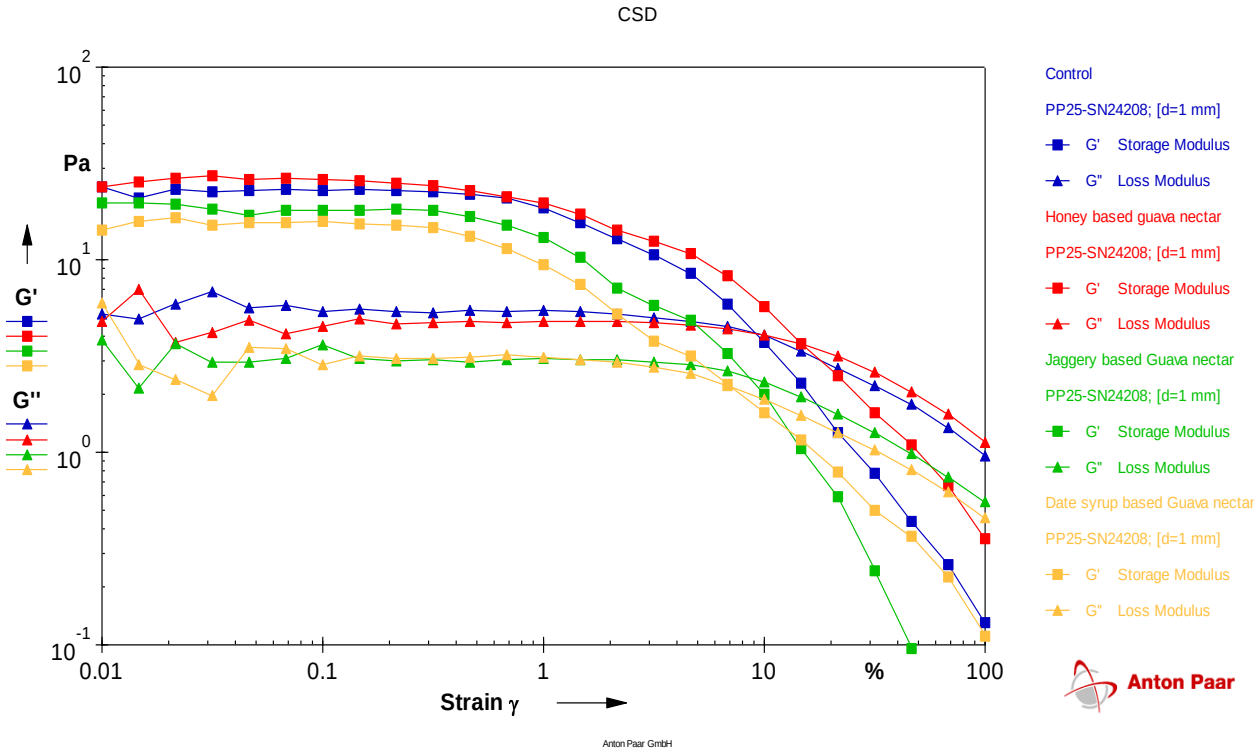


Fig. 2. Amplitude sweeps for sucrose substituted guava nectar

SUPPLEMENTARY MATERIAL

Table S1. Physico-chemical and colour properties of sucrose substituted guava nectar with honey, jaggery and date syrup at different levels

Level of sugar substitution/%	Processing treatment	Parameter				
		pH	*w*(reducing sugar)/%	*L**	*a**	*b**
Control	HF	(3.39±0.01)^{a}	(5.39±0.26)^{a}	(50.34±0.11)^{a}	(15.39±0.18)^{a}	(26.73±0.24)^{a}
	CF	(3.38±0.01)^{a}	(4.51±0.17)^{b}	(52.32±0.11)^{b}	(13.66±0.37)^{b}	(26.16±0.06)^{b}
25 % Honey	HF	(3.42±0.01)^{cd}	(7.44±0.21)^{e}	(51.17±0.04)^{b}	(15.33±0.10)^{e}	(29.36±0.08)^{f}
	CF	(3.41±0.01)^{d}	(6.52±0.31)^{f}	(51.82±0.10)^{a}	(13.43±0.16)^{g}	(29.12±0.01)^{f}
50 % Honey	HF	(3.44±0.02)^{bc}	(8.51±0.23)^{d}	(47.28±0.28)^{d}	(16.31±0.09)^{d}	(34.28±0.13)^{e}
	CF	(3.43±0.01)^{cd}	(7.26±0.18)^{e}	(48.40±0.20)^{c}	(14.52±0.20)^{f}	(34.37±0.30)^{e}
75 % Honey	HF	(3.48±0.01)^{a}	(11.94±0.18)^{b}	(45.93±0.05)^{e}	(17.81±0.06)^{c}	(38.14±0.10)^{b}
	CF	(3.46±0.01)^{ab}	(9.87±0.38)^{c}	(46.32±0.10)^{e}	(16.15±0.10)^{d}	(36.66±0.10)^{d}
100 % Honey	HF	(3.49±0.01)^{a}	(13.50±0.15)^{a}	(43.47±0.08)^{g}	(19.45±0.18)^{a}	(38.60±0.18)^{a}
	CF	(3.46±0.02)^{ab}	(11.53±0.30)^{b}	(44.71±0.05)^{f}	(18.25±0.20)^{b}	(37.52±0.11)^{c}
25 % Jaggery	HF	(3.72±0.01)^{e}	(9.53±0.25)^{a}	(46.73±0.17)^{b}	(17.59±0.23)^{d}	(37.52±0.26)^{g}
	CF	(3.71±0.01)^{e}	(9.28±0.34)^{ab}	(48.25±0.17)^{a}	(15.12±0.11)^{g}	(40.82±0.15)^{h}
50 % Jaggery	HF	(3.97±0.01)^{d}	(8.74±0.18)^{bc}	(46.28±0.04)^{c}	(18.54±0.09)^{c}	(43.85±0.10)^{e}

	CF	(3.96±0.02)^{d}	(8.56±0.12)^{c}	(47.85±0.14)^{a}	(15.88±0.10)^{f}	(41.34±0.13)^{f}
75 % Jaggery	HF	(4.22±0.01)^{b}	(5.60±0.25)^{d}	(44.39±0.15)^{d}	(19.49±0.16)^{b}	(49.16±0.14)^{c}
	CF	(4.17±0.01)^{c}	(4.79±0.15)^{e}	(46.86±0.14)^{b}	(16.36±0.12)^{e}	(47.36±0.12)^{d}
100 % Jaggery	HF	(4.40±0.01)^{a}	(4.30±0.06)^{ef}	(38.57±0.21)^{f}	(20.23±0.08)^{a}	(52.38±0.21)^{a}
	CF	(4.39±0.01)^{a}	(3.85±0.10)^{f}	(40.14±0.10)^{e}	(17.55±0.11)^{d}	(50.53±0.26)^{b}
20 % Date syrup	HF	(3.61±0.01)^{de}	(6.14±0.08)^{d}	(42.45±0.09)^{b}	(20.87±0.11)^{de}	(41.47±0.23)^{g}
	CF	(3.59±0.01)^{e}	(5.01±0.19)^{e}	(46.62±0.10)^{a}	(19.50±0.16)^{f}	(41.31±0.07)^{g}
30 % Date syrup	HF	(3.65±0.01)^{c}	(7.73±0.17)^{b}	(38.57±0.11)^{f}	(21.35±0.21)^{cd}	(42.50±0.11)^{f}
	CF	(3.63±0.01)^{cd}	(7.21±0.21)^{bc}	(41.34±0.09)^{c}	(20.63±0.16)^{e}	(44.45±0.22)^{e}
40 % Date syrup	HF	(3.73±0.01)^{b}	(8.61±0.16)^{a}	(37.45±0.10)^{g}	(23.26±0.33)^{b}	(49.66±0.10)^{c}
	CF	(3.73±0.01)^{b}	(6.84±0.11)^{c}	(40.89±0.09)^{d}	(21.47±0.12)^{c}	(48.74±0.17)^{d}
50 % Date syrup	HF	(4.28±0.01)^{a}	(8.74±0.23)^{a}	(35.85±0.10)^{h}	(24.51±0.27)^{a}	(52.57±0.11)^{a}
	CF	(4.25±0.01)^{a}	(7.54±0.26)^{b}	(39.46±0.10)^{e}	(21.83±0.18)^{c}	(51.42±0.18)^{b}

Data is represented as mean value±S.D. (N=3). Values with different superscripts (a,b,c,….) depict statistical differences between levels of substitution and processing treatment ($p \leq 0.05$) column wise for a particular substituted.sweetener. HF=hot filled, CF=cold filled

Table S2. Optimization of level of honey in guava nectar based on organoleptic evaluation

Level of sugar substitution/%	Processing treatment	Parameter				
		Colour	Odour	Mouthfeel	Flavour	Overall acceptability
Control	HF	(7.70±0.67)^{a}	(7.50±0.71)^{ab}	(7.60±0.65)^{ab}	(7.70±0.27)^{abc}	(7.63±0.51)^{ab}
	CF	(7.80±0.57)^{a}	(7.40±0.65)^{abc}	(7.70±0.67)^{ab}	(7.90±0.42)^{ab}	(7.70±0.41)^{ab}
25 % Honey	HF	(7.70±0.57)^{a}	(7.80±0.57)^{ab}	(7.70±0.84)^{ab}	(7.50±0.71)^{abc}	(7.68±0.63)^{ab}
	CF	(7.80±0.57)^{a}	(7.90±0.42)^{ab}	(7.80±0.45)^{a}	7.70±0.45)^{abc}	(7.80±0.38)^{ab}
50 % Honey	HF	(7.60±0.65)^{a}	(8.30±0.27)^{a}	(8.20±0.45)^{a}	(8.20±0.45)^{a}	(8.08±0.24)^{a}
	CF	(7.70±0.57)^{a}	(8.00±0.35)^{ab}	(8.10±0.42)^{a}	(8.10±0.42)^{a}	(8.03±0.24)^{a}
75 % Honey	HF	(7.10±0.55)^{ab}	(6.90±0.74)^{bcd}	(6.90±0.65)^{abc}	(6.90 ±0.65)^{bcd}	(6.98±0.63)^{abc}
	CF	(7.10±0.55)^{ab}	(6.90±0.74)^{bcd}	(6.70±0.76)^{abc}	(6.70±0.84)^{cd}	(6.90±0.72)^{bc}
100 % Honey	HF	(6.00±0.61)^{b}	(6.00±0.71)^{d}	(6.30±0.84)^{bc}	(6.20±0.84)^{d}	(6.13±0.70)^{c}
	CF	(6.10±0.65)^{b}	(6.10±0.74)^{cd}	(6.00±0.61)^{c}	(6.10±0.42)^{d}	(6.08±0.57)^{c}

Data is represented as mean value±S.D. (*N*=3). Values with different superscripts (a,b,c,….) depict statistical differences between samples ($p \leq 0.05$) column wise. HF=hot filled, CF=cold filled

Table S3. Optimization of different level of jaggery in guava nectar based on organoleptic evaluation

Level of sugar substitution/%	Processing treatment	Parameter				
		Colour	Odour	Mouthfeel	Flavour	Overall acceptability
Control	HF	(8.00±0.35)^{ab}	(7.90±0.42)^{abc}	(8.10±0.42)^{ab}	(8.10±0.42)^{ab}	(8.03±0.36)^{abc}
	CF	(8.00±0.35)^{ab}	(7.90±0.42)^{abc}	(7.60±0.55)^{abc}	(7.90±0.22)^{abc}	(7.85±0.35)^{abc}
25 % Jaggery	HF	(8.20±0.27)^{a}	(8.40±0.55)^{a}	(8.50±0.61)^{a}	(8.70±0.27)^{a}	(8.45±0.35)^{a}
	CF	(8.20±0.27)^{a}	(8.10±0.42)^{ab}	(8.00±0.79)^{abc}	(8.40±0.65)^{a}	(8.18±0.42)^{ab}
50 % Jaggery	HF	(7.80±0.27)^{ab}	(7.70±0.57)^{abcd}	(7.40±0.42)^{abc}	(7.60±0.65)^{abcd}	(7.63±0.44)^{abcd}
	CF	(7.80±0.27)^{ab}	(7.40±0.55)^{abcd}	(7.30±0.45)^{abc}	(7.40±0.55)^{abcd}	(7.48±0.43)^{abcd}
75 % Jaggery	HF	(7.30±0.27)^{bc}	(7.50±0.79)^{abcd}	(7.00±0.79)^{abc}	(6.70±1.04)^{bcd}	(7.13±0.56)^{bcd}
	CF	(7.20±0.27)^{bc}	(7.00±1.00)^{bcd}	(6.80±1.15)^{bc}	(6.60±1.08)^{bcd}	(6.90±0.78)^{cd}
100 % Jaggery	HF	(6.70±0.67)^{c}	(6.70±0.67)^{cd}	(6.40±0.96)^{c}	(6.30±0.84)^{d}	(6.53±0.68)^{d}
	CF	(6.70±0.67)^{c}	(6.50±0.71)^{d}	(6.40±1.29)^{c}	(6.40±0.96)^{cd}	(6.50±0.81)^{d}

Data is represented as mean value±S.D. (N=3). Values with different superscripts (a,b,c,….) depict statistical differences between samples ($p \leq 0.05$) column wise. HF=hot filled, CF=cold filled

Table S4. Optimization of different level of date syrup in guava nectar based on organoleptic evaluation

Level of sugar substitution/%	Processing treatment	Parameter				
		Colour	Odour	Mouthfeel	Flavour	Overall acceptability
Control	HF	(7.50±0.50)^{a}	(7.90±0.22)^{ab}	(7.80±0.45)^{a}	(7.60±0.55)^{a}	(7.70±0.19)^{ab}
	CF	(7.60±0.55)^{a}	(7.80±0.27)^{abc}	(7.90±0.22)^{a}	(7.50±0.50)^{ab}	(7.70±0.29)^{ab}
20 % Date syrup	HF	(8.00±0.35)^{a}	(8.10±0.42)^{a}	(8.00±0.35)^{a}	(7.90±0.55)^{a}	(8.00±0.32)^{a}
	CF	(7.90±0.22)^{a}	(8.10±0.22)^{a}	(8.20±0.57)^{a}	(8.10±0.22)^{a}	(8.08±0.11)^{a}
30 % Date syrup	HF	(7.90±0.42)^{a}	(8.50±0.50)^{a}	(8.30±0.45)^{a}	(8.60±0.42)^{a}	(8.33±0.37)^{a}
	CF	(7.90±0.42)^{a}	(8.30±0.45)^{a}	(8.10±0.55)^{a}	(8.40±0.22)^{a}	(8.18±0.27)^{a}
40 % Date syrup	HF	(7.80±0.84)^{a}	(7.70±0.84)^{abc}	(7.60±0.65)^{ab}	(7.60±0.55)^{a}	(7.68±0.67)^{ab}
	CF	(7.80±0.84)^{a}	(7.50±0.50)^{abc}	(7.50±0.71)^{abc}	(7.50±0.50)^{ab}	(7.58±0.60)^{abc}
50 % Date syrup	HF	(6.80±1.10)^{a}	(6.60±1.34)^{bc}	(6.30±0.97)^{bc}	(6.30±0.97)^{b}	(6.50±1.08)^{bc}
	CF	(6.80±1.10)^{a}	(6.40±0.96)^{c}	(6.20±1.04)^{c}	(6.30±1.04)^{b}	(6.43±0.99)^{c}

Data is represented as mean value±S.D. (*N*=3). Values with different superscripts (a,b,c,….) depict statistical differences between samples ($p \leq 0.05$) column wise for a particular sweetener substituted. HF=hot filled, CF=cold filled