

Original Research Paper

## Comparative effect of different sugars instigating non-enzymatic browning and Maillard reaction products in guava fruit leather

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### ABSTRACT

**Browning is a major quality deterioration process affecting both visual colour and nutritional value of guava leather. The aim of the study was to determine the role of different sugars viz., sucrose, fructose, glucose and sorbitol in non-enzymatic browning and antioxidant activity of guava fruit leather. The total free amino acids, ascorbic acid and antioxidant activities were at significantly lower levels in glucose and fructose treated guava leather, while the sorbitol added samples had all of above parameters at the highest level; while a reverse trend was observed in browning index and non-enzymatic browning. Among the browning intermediate products, Hydroxymethylfurfural was present at higher concentration (12.80-32.32 ng/g) than furfural (0.29-0.95 ng/g) in guava leather samples. Among the treatments, hydroxymethylfurfural was found lowest in sorbitol (12.8 ng/g) and highest in fructose (32.3 ng/g). In brief, this paper describes a novel effort in bringing the *in-vitro* studies related to sugars and total free amino acids, influencing the biochemical and nutritional attributes which are responsible for browning in guava fruit leather.**

**Keywords:** Total free amino acids, ascorbic acid, browning, furfural, hydroxymethylfurfural, non-enzymatic and sugars

### INTRODUCTION

Guava (*Psidium guajava* L.) a species of *Myrtaceae* family is cultivated widely around tropical and subtropical regions. It is known for pleasant flavour, refreshing taste and nutritional value. Guava is abundant in vitamins, especially vitamin C (ascorbic acid) other vitamins include vitamin A, thiamine, riboflavin, niacin, and pyridoxine (Kumari *et al.*, 2017). Dietary fibres and bioactive compound contribute to prevention of chronic degenerative diseases (Blancas-Benitez *et al.*, 2015). The fruit is also rich in considerable amounts of minerals *i.e.*, phosphorus, calcium, iron (Kumari *et al.*, 2017).

Guava fruits are often consumed fresh and are also suitable for processing into jelly, jam, juice, nectar, wine and fruit leather among other products (Kumari *et al.*, 2017). Guava fruit leather is one among the popular processed products. Fruit leather

is a dehydrated fruit-based confectionery dietary product which is often eaten as a snack or dessert. Fruit leathers are made by combining fruit puree with other ingredients such as sugar, pectin, acid, glucose syrup, colour, and potassium metabisulphite, then dehydrating them under controlled conditions.

Browning is an important biochemical reactions taking place during processing and storage of fruit leather. Browning not only affects the sensory attributes (colour; off flavour) but also deplete the nutritional quality. Decline in quality and color due to browning was the major hindrance in production of guava fruit leather (Singh *et al.*, 2019). Similar claims were done for apple leather (Demarchi *et al.*, 2013). Non-enzymatic browning is primarily caused by the Maillard reaction, caramelization, and ascorbic acid degradation at the product development stage by production of hydroxymethylfurfural (HMF) and



furfural (FUR) (Akyildiz *et al.*, 2021). HMF and FUR could be used as the non-enzymatic browning indicators in dehydrated products (Kus *et al.*, 2005). Specific sugars and amino acids, as well as their concentrations, play an important role in the Maillard reaction, determining the severity of browning, which is a reflection of the product's nutritional quality (Murata, 2021). In this regard, the role of different sugars (sucrose, fructose, glucose, and sorbitol) and their interactions with biomolecules in determining non-enzymatic browning in guava fruit leather was investigated.

## MATERIALS AND METHODS

### Raw material

This study employed firm ripe guava (cv. Arka Poorna) fruits produced from a guava plantation at the ICAR-Indian Institute of Horticultural Research in Bengaluru.

### Preparation of leather

The selected guava fruits were washed thoroughly using potable water. Fruits were subjected to manual peeling, cut into halves, pulp was extracted in a laboratory grade pulper and seeds were removed by passing the pulp through a sieve. The extracted pulp without pasteurizing was incorporated directly with 15% sugars *viz.*, sucrose, fructose, glucose and sorbitol in separate lots (treatments) followed by addition of 0.3 % citric acid and 700 ppm potassium metabisulphite to maintain the desirable acidity and as a preservative respectively. Further, the mixture was stirred gently for five minutes. The mixtures were spread on a tray and dried at  $60 \pm 5$  °C in a cabinet dryer. The drying process continued till the moisture content reached ~15%. The guava leather sheets were cut into 8 x 4 cm bars and later subjected to various analyses.

### Physico-chemical analysis

Moisture content was analyzed in a thermo-ventilated oven gravimetrically to obtain a consistent weight consecutively in three measurements at 12 h interval. Water activity was measured using an electric water activity meter (Rotronic Hydrolab, UK) at  $25 \pm 2$  °C. Titratable acidity was estimated by titrating against 0.1N NaOH with phenolphthalein as an indicator (AOAC, 1990). Reducing and total sugars were estimated as suggested by Lane and Eynon (1923) as

reported by Ranganna (1986). Non-reducing sugars was calculated from the difference between of total sugars and reducing sugars. Total free amino acid was estimated using ninhydrin reagent (Moore and Stein, 1948) and expressed as mg leucine/100g. The 2, 6-dichlorophenol indophenol dye technique was used to determine the vitamin C content suggested by Johnson (1948) and described by Ranganna (1986). The total phenolic content was estimated as per Folin - Ciocalteu spectrophotometric method and expressed in gallic acid equivalent (mg GAE/100g) (Yilmaz *et al.*, 2017). Ferric Reducing Antioxidant Potential (FRAP) was used to determine antioxidant activity (Ndou *et al.*, 2019) and expressed in ascorbic acid equivalents (mg AAE/100g). Non-enzymatic browning was recorded by submerging the samples in 60 per cent ethanol overnight and reading the OD values at 440 nm (Ranganna, 1986).

### Color

The color ( $L^* a^* b^* C^* h^\circ$ ) was measured using colorimeter (Model: Colour Reader, CR-10, Konica Minolta, Japan). Browning Index was calculated based on  $L^* a^* b^*$  co-ordinates. The browning index is generated using the following equation to capture this variance in a single index that is associated to a brown color. (Pathare *et al.*, 2013)

$$BI = 100 \frac{(X - 0.31)}{0.17}$$

$$X = \frac{(a^* + (1.75 \times L)) \times a^*}{((5.645 \times L) + a^* - (3.012 \times b^*))}$$

### Furfural and hydroxymethylfurfural

To extract furfural (FUR) and hydroxymethylfurfural (HMF), 2g of material was homogenized in 15 ml of HPLC grade water. The extract was filtered using 0.45 µm nylon filters. The HPLC studies were carried out on a Shimadzu Series LC-20AT system (Shimadzu, Kyoto, Japan), which included a liquid chromatograph coupled to a UV-VIS detector (SPD-10A), binary pump (LC-10AT), auto sampler (SIL-20A HT), and LC solution Workstation software, Kinetex, column of dimension 250 x 4.6 mm, 5µm C18 (Phenomenex, USA) was used, along with a security guard column made of the same material. Samples were injected using the auto sampler. At 32°C, the column and guard column were thermostatically controlled. The flow rate was 1 ml/min, and the mobile phase was 0.3

percent tetrahydrofuran. The instrument was operated in isocratic mode and elutants were detected at 280 nm. The retention time for HMF was 10.80 minutes, whereas the retention time for FUR was 11.64 minutes (Zhong-Fu *et al.*, 2016). The values were expressed in ng/g.

### Statistical analysis

The analysis was done in triplicates and the results were presented in Mean  $\pm$  SE (standard error). One-way ANOVA was used to determine the CD of means and variance among different sugars. Duncan multiple range test (DMRT) was performed at  $\alpha = 0.05$  level of significance of using R software.

## RESULTS AND DISCUSSION

### Physico-chemical composition of guava pulp

**Table 1. Physico-chemical composition of fresh guava pulp**

Colour	<i>L</i> *	57.07
	<i>a</i> *	3.20
	<i>b</i> *	12.48
	<i>C</i> *	12.89
	<i>h</i> <sup>o</sup>	75.61
Moisture (%)		84.15
Water activity		0.824
TSS ( $^{\circ}$ Brix)		12.5
Titrateable acidity (%)		0.4
Reducing sugar (%)		5.53
Total sugar (%)		9.77
Non-reducing sugar (%)		4.24
Total free amino acids (mg Leu/100g)		1.06
Ascorbic acid (mg/100g)		206.62
Total Phenols (mg GAE/100g)		591.67
Antioxidant Activity (mg AAE/100g)		1574.19

The Physico-chemical composition of the fresh guava (cv. Arka Poorna) pulp is given in Table 1.

### Effect of different sugars on the properties of guava leather

#### Moisture content and water activity

The moisture content and water activity did not show any significant ( $p > 0.05$ ) difference among guava leather developed using different sugars (Table 2). The moisture content and water activity was  $\sim 15$  and  $\sim 0.6$  respectively. Moisture content in guava leather was in agreement with food safety and standards regulations, 2011 *i.e.*, not more than 20%. That moisture contents at 15% and water activity of 0.6 is found to be safe with respect to microbiological activity and adverse biochemical and deteriorative reactions (Suna *et al.*, 2014). In this regard the guava leather developed had acceptable moisture content and water activity levels.

#### Titrateable acidity

The titrateable acidity in guava leathers did not vary significantly among different sugars ( $p > 0.05$ ). The values ranged from  $1.62 \pm 0.02$  % to  $1.70 \pm 0.03$  % (Table 2).

#### Sugar

The sugar composition of guava leather is presented in Table 2. Total sugars values in guava leather ranged from  $29.15 \pm 0.31$  to  $71.30 \pm 1.19$ %. The highest total sugar was on par in sucrose ( $71.12 \pm 0.84$ %), fructose ( $70.26 \pm 0.57$ %) and glucose ( $71.30 \pm 1.19$ %), and the lowest was found in sorbitol ( $29.15 \pm 0.31$ %). As sorbitol is a sugar alcohol its addition even at 15% did not contribute to the total sugar content (Choi *et al.*, 2013). Reducing sugars content varied significantly ( $p > 0.05$ ) in guava leather as the base material used was different sugars. Guava leather with fructose ( $41.99 \pm 0.86$ %) reported to have a highest reducing sugar which was statistically on par with glucose ( $41.21 \pm 0.21$ %) and the lowest was recorded in sorbitol ( $13.07 \pm 0.60$ %). Reducing sugars are capable of producing reactive carbonyl species (RCS) which aid in development of Maillard reactions products (Picouet *et al.*, 2009) which further cause non-enzymatic browning. The highest non-reducing sugar was found in guava leather with sucrose ( $53.79 \pm 0.49$ %) and the lowest in sorbitol ( $16.08 \pm 0.51$ %). Sucrose has an acetal structure with anomeric carbons combined together by a glycosidic bond. This is a stable structure that cannot be oxidised.

#### Total free amino acids

Incorporation of different sugar in guava leather had a significant ( $p > 0.05$ ) impact on total free amino acids (TFAA) (Table 2). Guava leather with sorbitol ( $2.91 \pm 0.02$  mg/100g), which was on par with sucrose

( $2.86 \pm 0.05$  mg Leu/100g), had the highest TFAA, while fructose ( $2.26 \pm 0.02$  mg Leu/100g), which was on par with glucose ( $2.32 \pm 0.09$  mg Leu/100g), had the lowest. The decline in TFAA was found to be higher in guava leather incorporated with fructose and glucose; this is due to differential reaction between amino acids and RCS, resulting in the production of a variety of Maillard reaction products depending on the affinity and reactivity of individual amino acids. Among the amino acids, leucine, glutamic acid, tryptophan and lysine contributed more for Maillard reaction. Leucine, alanine, aspartic acid, glutamic acid and glycine was comparatively found high in guava fruit (Chen *et al.*, 2007).

### Ascorbic acid

Ascorbic acid (Vitamin-C) plays an important role in human nutrition due to its antioxidant nature (Cruz *et al.*, 2009). It is thermo-labile and considered as a quality indicator in dehydration process (Ali *et al.*, 2016). Guava leather developed using different sugars showed significant ( $p > 0.05$ ) difference in of ascorbic acid levels (Table 3.) The highest ascorbic acid level was found in sorbitol ( $136.13 \pm 3.27$  mg/100g) which was statistically on par with sucrose ( $132.47 \pm 2.38$  mg/100g), while the lowest was found in fructose ( $116.7 \pm 1.50$  mg/100g) and glucose ( $119.64 \pm 0.60$  mg/100g). Ascorbic acid would have been degraded to dehydroascorbic acid, then hydrolyzed to 2,3-diketogulonic acid, and lastly polymerized as a result of the Maillard reaction product, which is catalysed by multiple oxidation and reduction processes involving reducing sugars (Chuah *et al.*, 2008) Mango juices with the highest glucose: fructose ratio showed decreased ascorbic acid concentration (Pithava and Pandey, 2018). Furthermore, amino acids have the ability to act as catalytic agents in the decomposition of ascorbic acid (Shinoda *et al.*, 2005). According to Yu *et al.* (2017), the interaction of ascorbic acid with lysine, arginine, and histidine was more important in the synthesis of browning pigments.

### Total phenols

The total phenols content of guava fruit leathers showed significant ( $p > 0.05$ ) difference among different sugar source (Table 3). The highest total phenols were found in Sorbitol ( $436.23 \pm 12.2$  % mg GAE/100 g) and sucrose ( $427.95 \pm 6.61$  mg GAE/100g). whereas, fructose, and glucose significantly reported low values for total phenol content of 392.09

$\pm 2.85$  and  $410.87 \pm 2.11$  mg GAE/100g respectively. The degradation of total phenols was high in samples with fructose and glucose. Phenols are also common substrates for Maillard reaction (Amaya-Farfan and Rodriguez-Amaya, 2021). This browning reaction also involves various oxidation and reduction process which will degraded the total phenol content severely. In addition to this, the RCS formed by reducing sugars bind to phenols and make them biologically unavailable.

### Antioxidant activity

Varying the sugar forms had significantly different antioxidant activity in guava fruit leathers ( $p > 0.05$ ) (Table 3). Sorbitol ( $1,146.20 \pm 41.02$  mg AAE/100g) had the highest antioxidant activity, which was on par with sucrose ( $1,086.35 \pm 35.13$  mg AAE/100g). The samples with fructose ( $935.97 \pm 9.81$  mg AAE/100g) and glucose ( $949.36 \pm 6.30$  mg AAE/100g) significantly deprived the antioxidant activity. Ascorbic acid and phenolics contribute the lion share of antioxidant activity (Eyiz *et al.*, 2020). It can be inferred that guava leather processed using fructose and glucose resulted in highest degradation of ascorbic acid and loss of phenols and thus adversely affected the antioxidant activity of the guava leather.

### Color:

#### $L^* a^* b^*$

The colour values of guava fruit leather are presented in Table 4. The lightness ( $L^*$ ) values varied significantly among the different guava leather developed using different sugar sources. The highest lightness was reported in samples containing sorbitol ( $61.40 \pm 0.78$ ) and the lowest values were reported in sucrose ( $58.90 \pm 0.72$ ), which was on par with glucose ( $58.70 \pm 0.66$ ), and fructose ( $58.27 \pm 0.35$ ). The decrease in the  $L^*$  values indicates the product is comparatively darker, this occurred in the samples with reducing sugars (fructose and glucose) and the highest luminance was reported in guava leather containing sorbitol. The redness ( $a^*$ ) values varied significantly among the different guava leather developed using different sugar sources. Redness indicates the occurrence of browning in the product. The highest redness was reported in samples containing fructose ( $4.63 \pm 0.46$ ) and the lowest values were reported in sorbitol ( $3.13 \pm 0.12$ ). The highest yellowness ( $b^*$ ) was reported samples containing fructose ( $34.37 \pm 0.25$ ) which was found on par with

**Table 2. Physico-chemical composition of guava leather**

Treatment	Moisture (%)	Water activity	Reducing Sugar (%)	Non - Reducing Sugar (%)	Total Sugar (%)	Titratable acidity (%)	Total free amino acids (mg Leu/100g)
Sucrose	15.23 <sup>a</sup> ± 0.22	0.672 <sup>a</sup> ± 0.01	17.32 <sup>b</sup> ± 0.13	53.79 <sup>a</sup> ± 0.86	71.12 <sup>a</sup> ± 0.84	1.62 <sup>a</sup> ± 0.04	2.86 <sup>a</sup> ± 0.05
Fructose	15.38 <sup>a</sup> ± 0.12	0.677 <sup>a</sup> ± 0.01	41.99 <sup>a</sup> ± 0.86	28.27 <sup>b</sup> ± 1.43	70.26 <sup>b</sup> ± 0.57	1.65 ± 0.02	2.26 <sup>b</sup> ± 0.02
Glucose	15.08 <sup>a</sup> ± 0.17	0.665 <sup>a</sup> ± 0.01	41.21 <sup>a</sup> ± 0.36	30.09 <sup>b</sup> ± 1.19	71.30 <sup>b</sup> ± 1.19	1.70 <sup>a</sup> ± 0.05	2.32 <sup>b</sup> ± 0.09
Sorbitol	15.28 <sup>a</sup> ± 0.03	0.675 <sup>a</sup> ± 0.01	13.07 <sup>c</sup> ± 0.60	16.08 <sup>c</sup> ± 0.51	29.15 <sup>b</sup> ± 0.31	1.65 <sup>a</sup> ± 0.35	2.91 <sup>a</sup> ± 0.02
C.D.	NS	NS	1.07	2.01	1.52	NS	0.18
SE(m)	0.09	0.004	0.32	0.61	0.46	0.10	0.05

Note: Mean values followed by different letters in the same column differs significantly ( $\alpha = 0.05$  level).

**Table 3. Functional attributes of guava leather**

Treatment	Ascorbic Acid (mg/100g)	Total Phenols (mg GAE/100g)	Antioxidant Activity (mg AAE/100g)
Sucrose	132.47 <sup>a</sup> ± 2.38	427.95 <sup>a</sup> ± 6.61	1,086.35 <sup>b</sup> ± 35.13
Fructose	116.70 <sup>b</sup> ± 1.50	392.09 <sup>c</sup> ± 2.85	935.97 <sup>c</sup> ± 9.81
Glucose	119.64 <sup>b</sup> ± 0.60	410.87 <sup>b</sup> ± 2.11	949.36 <sup>c</sup> ± 6.30
Sorbitol	136.13 <sup>a</sup> ± 3.27	436.23 <sup>a</sup> ± 12.2	1,146.20 <sup>a</sup> ± 41.02
C.D.	4.16	13.72	52.71
SE(m)	1.26	4.14	15.92

Note: Mean values followed by different letters in the same column differs significantly ( $\alpha = 0.05$  level).

**Table 4. Color ( $L^* a^* b^* C^* h^\circ$ ) and non-enzymatic browning (NEB) in guava leather**

Treatment	Color					NEB	
	L	a	b	C	H		Browning Index
Sucrose	58.90 <sup>a</sup> ± 0.72	3.43 <sup>b</sup> ± 0.15	34.20 <sup>a</sup> ± 0.26	34.00 <sup>a</sup> ± 1.75	84.53 <sup>a</sup> ± 0.47	84.61 <sup>b</sup> ± 1.97	0.193 <sup>c</sup> ± 0.01
Fructose	58.27 <sup>a</sup> ± 0.35	4.63 <sup>a</sup> ± 0.46	34.37 <sup>a</sup> ± 0.25	34.43 <sup>a</sup> ± 0.58	83.00 <sup>b</sup> ± 0.36	90.58 <sup>a</sup> ± 0.82	0.232 <sup>a</sup> ± 0.01
Glucose	58.70 <sup>a</sup> ± 0.66	3.83 <sup>b</sup> ± 0.06	34.13 <sup>a</sup> ± 0.41	34.27 <sup>a</sup> ± 0.46	84.07 <sup>a</sup> ± 0.25	89.40 <sup>a</sup> ± 0.98	0.211 <sup>b</sup> ± 0.01
Sorbitol	61.40 <sup>b</sup> ± 0.78	3.13 <sup>b</sup> ± 0.12	33.07 <sup>b</sup> ± 0.15	32.93 <sup>b</sup> ± 0.21	84.47 <sup>a</sup> ± 0.23	77.72 <sup>c</sup> ± 1.74	0.181 <sup>d</sup> ± 0.01
C.D.	1.24	0.48	0.54	1.01	0.66	1.99	0.011
SE(m)	0.37	0.15	0.16	0.30	0.20	0.60	0.003

Note: Mean values followed by different letters in the same column differs significantly ( $\alpha = 0.05$  level); Browning index was estimated using average values of  $L^*$ ,  $a^*$ ,  $b^*$

sucrose ( $34.20 \pm 0.26$ ) and glucose ( $34.13 \pm 0.41$ ) and lowest values were reported in sorbitol ( $33.07 \pm 0.15$ ). Lower  $L^*$  values and high  $a^*$  and  $b^*$  values will indicate the intensity of browning. Decreasing  $L^*$  values in combination with decreasing  $b^*$  values, indicating the occurrence of mild browning due to non-enzymatic browning (Korley *et al.*, 2015). In guava leather yellowness indicate the undesirable colour change towards browning. In addition to it  $a^*$  values also significantly contribute to non-enzymatic browning.

### **$C^*$ and $h^\circ$**

Chroma values indicate the purity of color, in guava leather fructose had the highest value indicating more browning attributes (low-lightness; high-redness; high-yellowness). The Chroma ( $C^*$ ) values varied significantly among the different guava leather developed using different sugar sources (Table 4). The highest Chroma was reported samples containing fructose ( $34.43 \pm 0.58$ ) which was found on par with sucrose ( $34.00 \pm 1.75$ ) and glucose ( $34.27 \pm 0.46$ ) and lowest values were reported in sorbitol ( $32.93 \pm 0.21$ ). The Hue ( $h^\circ$ ) values varied significantly among the different guava leather developed using different sugar sources. The highest hue value was reported in samples containing sucrose ( $84.53 \pm 0.47$ ) which was found on par with sorbitol ( $84.47 \pm 0.23$ ) and glucose ( $84.07 \pm 0.25$ ) and lowest values were reported in fructose ( $83.00 \pm 0.36$ ). Lower hue value indicates redder colour of the product (Korley *et al.*, 2015). The shift in hue values from  $90$  to  $0^\circ$  indicate change in color from yellow to red, which was predominant in fructose followed by glucose containing samples. Hue angle  $\sim 90^\circ$  suggests that the product has more yellowness than redness (Pedisic *et al.*, 2009)

### **Browning index (BI)**

To determine the change in visual quality, colour coordinates ( $L^* a^* b^*$ ) were utilized to derive browning index. BI aid in determining the degree of brown colour occurred during dehydration. BI changed between  $77.72 \pm 1.74$  and  $90.58 \pm 0.82$  with different sugars (Table 4). The highest BI in leather was recorded in sample containing fructose ( $90.58 \pm 0.82$ ) and glucose ( $89.40 \pm 0.98$ ). As discussed earlier and supported by literature, reducing sugars play an important role in determining the colour of the final product as they are the potential source of reactive carbonyl species which contribute significantly to

Maillard reaction (Fu *et al.*, 2020; Calin-Sanchez *et al.*, 2020). The total free amino acids also decreased in guava leather containing fructose ( $2.26 \pm 0.02$ ) and glucose ( $2.32 \pm 0.09$ ) (Table 3). As a result, these reactive carbonyl species and amino acids are likely to have interacted to form various Maillard reaction products, resulting in greater BI in fructose and glucose samples. Furthermore, ascorbic acid degradation in guava leather has contributed to the creation of HMF, which in turn produces Maillard reaction product which caused the browning. Yu *et al.* (2017) reported that the degree of browning was only related to the total amount of L-ascorbic acid in the reaction system. Similar results were observed in citrus and apple juices (Burdurlu *et al.*, 2006).

### **Non-enzymatic browning (NEB)**

The absorbance at 440 nm is commonly used to determine the degree of browning in a non-enzymatic browning reaction, often caused by Maillard reaction (Paravisini and Peterson, 2016). NEB indicates the intensity of browning in processed product through spectrometric OD values. NEB values were reported between  $0.232 \pm 0.01$  and  $0.181 \pm 0.01$  in guava fruit leather (Table 4). Among different sugars investigated, highest NEB values were recorded in fructose ( $0.232 \pm 0.01$ ) and glucose ( $0.211 \pm 0.01$ ) treated samples and the lowest was reported in sorbitol ( $0.181 \pm 0.01$ ) and sucrose ( $0.193 \pm 0.01$ ). Degradation of ascorbic acid (Table 3) and production of reactive carbonyl groups from the reducing sugars (Table 2) contributed to higher NEB values in guava leather. Browning is complex biochemical reaction which involves numerous biological compounds to take part in the reaction to yield varied degree of browning in processed products. Our results were in confirmation with, Paravisini and Peterson, (2016) who reported decomposition of sugars under acidic conditions to form reactive intermediates. Major mechanisms, being ascorbic acid degradation, acid-catalyzed sugar degradation, and Maillard reactions, have been identified as the main reaction pathways responsible for NEB (Bharate and Bharate, 2014). Maillard reaction rate is highest in intermediate moisture foods with water activity range of 0.5 - 0.7 (Malec *et al.*, 2002). The physico chemical composition of guava leather mentioned in Table 2, 3 and 4 shows that in this product all above mentioned favorable environment for browning reactions were present.

### Furfural and hydroxymethylfurfural

Maillard reaction products such as Furfural (FUR) and Hydroxymethylfurfural (HMF) are considered as the biochemical markers for non-enzymatic browning (ErtekinFiliz and Seydim, 2018). Among the two Maillard products, HMF (32.3 -12.8 ng/g) content was found to be higher than FUR (0.95-0.29 ng/g) in all guava leather samples (Fig. 1; Table 5). HMF production occur in product high

**Table 5. Biochemical markers of non-enzymatic browning in guava leather**

Treatment	Furfural (ng/g)	Hydroxymethylfurfural (ng/g)
Sucrose	0.33	14.32
Fructose	0.95	32.3
Glucose	0.73	29.3
Sorbitol	0.29	12.8

Note: The values presented are mean values of two replicates

in reducing sugar *i.e.*, fructose and glucose, whereas FUR production occur in xylose and arabinose rich product (Machado *et al.*, 2016). Among the treatments, guava leather with fructose and glucose reported remarked higher HMF of 32.3 and 29.3 ng/g respectively than sucrose (14.32 ng/g) and sorbitol (12.8 ng/g) treated samples. The ascorbic acid degradation in guava fruit leather containing fructose and glucose (Table 3) and production of RCS for maillard reaction has also contributed to HMF formation (Chen *et al.*, 2022). Similar results were found in apple leather (Ruiz *et al.*, 2012) reporting degradation of ascorbic acid caused higher levels of HMF which in turn produced brown pigments (Helyes *et al.*, 2006).

Besides being identified as thermal processing indicator, HMF is instrumental in imparting certain typical flavors to the food products. However, the toxicity of compound has been much discussed as a carcinogen (Severin *et al.*, 2010). The estimates of HMF for human daily intake range from 2 to 150 mg/person (Capuano and Fogliano, 2011). It is understood from this study that HMF generation couples with loss of nutrients such as ascorbic acid and so the antioxidant activity of the guava leather. Therefore, it is advisable to treat HMF as a nutritional quality indicator in guava leather and to lay down a permissible limit as a part of implementation of food standards.

### CONCLUSION

This study revealed the effects of different sugars (Fructose, glucose, sucrose and sorbitol) and their role in non-enzymatic browning and antioxidant activity in guava leather. The application of different sugars during the product development affected the colour ( $L^*$ ,  $a^*$ ,  $b^*$ ,  $C^*$ ,  $h^\circ$ , Browning Index), total free amino acids, ascorbic acid, total phenols, antioxidant activity, NEB, furfural and hydroxymethylfurfural. Highest losses in nutritional attributes such as total free amino acids, ascorbic acid, total phenol and antioxidant activity was found in guava leather incorporated with fructose and glucose and the least in sorbitol which was followed by sucrose. While, the colour values *i.e.*, highest  $L^*$  and  $h^\circ$ , lowest  $a^*$ ,  $b^*$ , and  $C^*$  values, lowest browning index and lowest NEB were found superior in sorbitol and sucrose followed by fructose and glucose. Among the biochemical markers for NEB, HMF was found to be predominant than FUR and was found in high level in fructose followed by glucose, sucrose and sorbitol. Therefore, from this study it was evident that sugar composition and its concentration

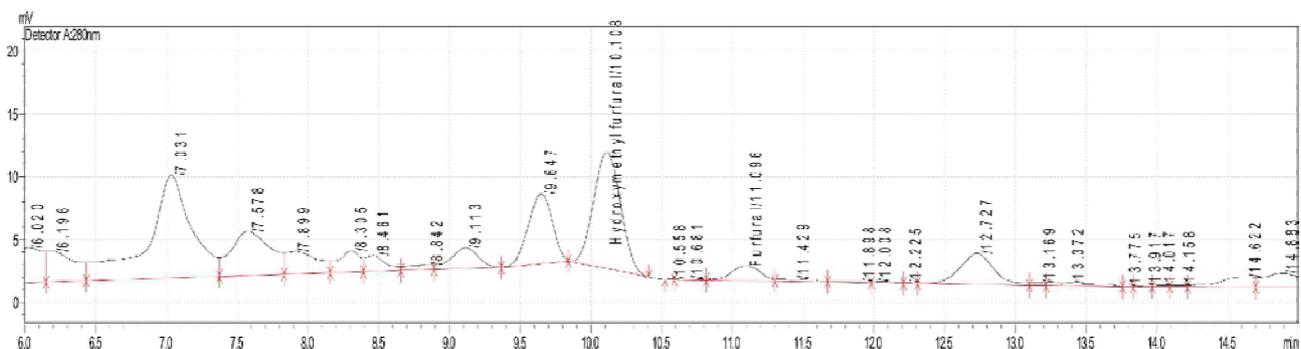


Fig. 1. Chromatogram showing hydroxymethylfurfural (10.32) and furfural (11.38) as biochemical markers in NEB in guava leather

in guava leather play a significant role in non-enzymatic browning. Use of optimal non reducing sugar, least reducing sugar and their combinations will aid in minimizing the browning and preserving functional attributes of dehydrated product.

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### Conflict of Interest

The authors have declared no conflicts of interest for this article.

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