In vitro antioxidant potential of Rose-Roselle (Rosa-Hibiscus rosa-sinensis) jam sweetened with Beetroot (Beta vulgaris)

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***Abstract*—Antioxidants help neutralize free radicals, preventing cellular damage, lowering the risk of oxidative stress-related diseases, and thereby improving overall health. In this study, the *in vitro* antioxidant potential of a jam prepared using Rose (*Rosa*) and Roselle (*Hibiscus rosa-sinensis*) flower was investigated. Two variations of the jam were formulated, differing in the proportions of the ingredients used. The nutrient and phytochemical composition of the jam was analyzed using standard methods. Bioactive compounds present in the jam were identified through Gas Chromatography–Mass Spectrometry (GC-MS). Antioxidant activity was assessed using DPPH and FRAP assays. Sensory evaluation was conducted using a 9-point hedonic scale with 50 semi-trained panelists. The results shows that both variations of Rose-Roselle jam exhibited promising antioxidant potential, with Variation 2 containing slightly higher bioactive compound content. However, Variation 1 achieved greater overall acceptability in sensory evaluation, suggesting that consumer preference may be influenced by taste, while Variation 2 may be preferred for its functional benefits rather than taste.**

***Index Terms*—Antioxidants, bioactive compounds, *in vitro*, jam, Nutrient, Oxidative stress, phytochemicals, Rose, Roselle, sensory evaluation.**

I INTRODUCTION

Oxidative stress is a phenomenon caused by an imbalance between the production and accumulation of oxygen reactive species (ROS) in cells and tissues and the ability of a biological system to detoxify these reactive products [1]. Oxidative stress is said to be excess levels of free radicals in the body.Numerous diseases, such as diabetes mellitus, neurodegenerative disorders (Parkinson's disease, Alzheimer's disease, and multiple sclerosis), cardiovascular diseases (atherosclerosis and hypertension), respiratory diseases (asthma), cataract development, rheumatoid arthritis, and various cancers (such as breast, colorectal, bladder, prostate, and lung cancers), are linked to oxidative stress caused by free radicals [2]. Excessive exposure to UV radiation, long-term stress conditions, lack of exercise, improper diet (modern lifestyle associated with processed food), exposure to a wide range of chemicals and stimulant usage lead to increased formation of free radicals [3],[4].

Antioxidants play a crucial role in preventing oxidative stress by neutralizing or removing free radicals. Recent studies have shown that antioxidants can help prevent oxidative damage by either stopping the spread of free radicals or inhibiting their formation altogether. This can lower oxidative stress, boost immunity, and lengthen healthy life [5]. A variety of plant materials are known to be natural sources of antioxidants, such as herbs, spices, seeds, fruits and vegetable. These are particularly rich in phenolic compounds, vitamins and carotenoids. Research findings support the consumption of edible flowers as functional foods and their use as sources of natural antioxidants [6],[7]. Phenolic substances with various chemical structures, primarily phenolic acids, flavonols, and anthocyanins, are found in edible flowers. These chemicals have been positively linked to human metabolism and offer antioxidant capacity and protection against damage caused by free radicals [8]. Polyphenols, carotenoids and vitamin C (ascorbic acid) are the important compounds that are present in flowers that exert antioxidant and anti-inflammatory properties [9].

Rose (*Rosa*) is a beautiful and fragrant plant which has many medicinal and substance uses. Various parts of rose such as fruits, flowers, leaves, and bark can be used in various product development, in the food, pharmaceuticals and cosmetics industries. Rose petals are rich source of carbhohydrates and also contains chemical compounds like terpenes, aroma alcohols, flavonoids, anthocyanin, phenolic acids, polyphenols, aldehydes, ketones, tannins, vitamin C (ascorbic acid), carotenoids, minerals that exert certain properties like analgesic, anticonvulsive, hypnotic, cardiovascular, laxative, antioxidant properties, and it is also used as natural colorant. Therefore, these properties of Rose flower aids in the treatment of inflammation, diabetes, dysmenorrhea, depression, stress, seizures [10],[11].

Roselle (*Hibiscus rosa-sinensis*) is a source of bioactive compounds such as polyphenols, carotenoids, ascorbic acid, and tannins [12]. The main constituent of Roselle in context of therapeutic importance are a polysaccharide, organic acid and flavonoids mainly anthocyanins that exert potent antioxidant-antiradical activity, anti-inflammatory action, antiobesity, antihyperlipidemic, antihypertensive, antimicrobial, anticancer activity [13].

Beetroot (*Beta vulgaris)* has health promoting and disease preventing functions [14]. Beetroot is a good source of betalains (betanin), nitrates, proteins, carbohydrates, amino acids, fatty acids, phytosterols, phytosterols, fibers, minerals such as iron, zinc, phosphorous, potassium, calcium, sodium, magnesium, manganese, and copper and vitamins, such as vitamin A, E, B6, and C. Betalains present in beetrrot have been proven to eliminate oxidative and nitrative stress by scavenging free radicals, preventing DNA damage, and reducing LDL [15]. Researches have shown that the bioactive compounds present in beetroot exhibit antioxidant activity, anti-inflammatory activity, antitumor activity, hepato-protective activity, cognitive improvement, and regulation of blood pressure [16].

Dates (*Phoenix dactylifera*) belong to the palm family Arecaceae and are one of the oldest cultivated plants, with a history of human consumption spanning over 6,000 years [17]. The date palm fruit and seeds are rich in of phytochemicals. These include phenolics, anthocyanin, carotenoids, tocopherols, tocotrienols, phytosterols, and dietary fiber, all of which contribute to the date palm's exceptional nutritional value and potential health benefits [18]. Dates and their components have been found to play a role in preventing various diseases due to their antioxidant, anti-inflammatory, antibacterial, anti-tumor, and anti-diabetic effects [19],[20].

Raisins are dried grapes derived from various cultivars of *Vitis vinifera L.* and are widely consumed globally [21]. Raisins are a rich source of essential nutrients, including potassium, and bioactive compounds that can provide health benefits. They contain a variety of polyphenols, such as phenolic acids, flavonols, and anthocyanins, which can vary in concentration depending on the raisin variety. The specific polyphenols found in raisins include caftaric acid, coutaric acid, quercetin, kaempferol, and rutin. Additionally, raisins contain other phytochemicals, including triterpenoids and tartaric acid, which can work together with fiber to support a healthy digestive system [22],[23]. raisins have demonstrated exceptional antioxidant properties in both laboratory and human studies. The antioxidant and antibacterial effects of raisins are primarily attributed to their high content of phenolic compounds [24].

This study focused on formulating a jam using rose and roselle flowers, along with beetroot, dates, and raisins, and evaluating its synergistic *in vitro* antioxidant potential as a food-based approach to addressing oxidative damage, highlighting its antioxidant capacity and potential as a unique alternative to commercial jams.

II MATERIALS AND METHODS

This study employed an *in vitro* experimental research design to evaluate the antioxidant potential of Rose-Roselle jam. Lyophilization was performed at the Centralized Instrumentation Laboratory, Madras Veterinary College, Vepery, Chennai. The analysis was carried out at Affyclone Laboratories Pvt. Ltd., an ISO-certified and NABL-accredited laboratory in Chromepet, Chennai. Sensory evaluation was conducted in the Food Science Laboratory, Department of Home Science, Women's Christian College, Chennai. The study was approved by the Institutional Ethics Committee of the Department of Home Science, Women's Christian College, Chennai.

Two variations of the jam were formulated, differing in the proportions of the ingredients used. Variation 1 contained 20 g of Rose flower, 2.5 g of Roselle flower powder, 30 g of beetroot, 20 g of dates, 10 g of raisins, and 2 g of pectin, while Variation 2 included 20 g of Rose flower, 2.5 g of Roselle flower powder, 40 g of beetroot, 10 g of dates, 20 g of raisins, and 2 g of pectin.

1. Procurement and preparation of the Rose-Roselle jam

Fresh rose flowers were collected from the home garden. Beetroot was procured from the vegetable market at Chennai, Tamil Nadu. Organic roselle flower powder, dates, and raisins were procured from an organic shop in Chennai, Tamil Nadu. The jam was prepared by weighing fresh rose petals and beetroot according to the specified amounts for each variation, followed by pulverization. The pulverized samples were then subjected to lyophilization. Additionally, other ingredients, such as dates and raisins, were ground into a paste. The lyophilized rose and beetroot powder, date and raisin paste, and roselle flower powder were combined. This mixture was then added to 100 mL of water, along with 2 grams of pectin, and heated over medium heat for 10 to 20 minutes until thickened. A plate test was conducted to determine if the jam had reached the desired consistency.

2. Nutritional and phytochemical analysis of the Rose-Roselle jam

The nutrient composition, such as carbohydrates, protein, fats, dietary fiber, vitamin C, iron, and calcium, was analyzed using standard methods. Bioactive compounds in the Rose-Roselle jam were identified through Gas Chromatography–Mass Spectrometry (GC-MS). The mass spectra of unknown components in the Rose-Roselle jam were compared with those of known compounds stored in the NIST database.

3. Assessment of *in vitro* antioxidant potential of the Rose-Roselle jam

The *in vitro* antioxidant potential of both variations of Rose-Roselle jam was assessed using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay and the Ferric Reducing Antioxidant Power (FRAP) assay, following standard protocols. The DPPH and FRAP activities were measured at concentrations ranging from 20 µL to 100 µL, and the IC50 values for both assays were determined.

4. Sensory evaluation of the Rose-Roselle jam

The sensory evaluation of both variations of Rose-Roselle jam was conducted using a 9-point hedonic rating scale to assess sensory attributes, such as appearance, flavour, colour, odour, spreadability, and overall acceptability. The evaluation was carried out by 50 semi-trained panellists from the Department of Home Science, Women's Christian College.

5. Data analysis

The data collected in this study were analyzed using Microsoft Excel to determine the mean, standard deviation, and correlation.

III RESULTS AND DISCUSSION

1. Quantification of specific nutrients present in the Rose-Roselle jam

The results of nutrient analysis of Variation 1 and Variation 2 of the Rose-Roselle jam are represented in table 1.

Table 1: Specific nutrient content of Rose-Roselle jam (Variations 1 and 2)

Nutrients	Variation 1	Variation 2
Carbohydrate (g/100mg) (Mean ± SD)	1.3456 ± 0.12	1.3888 ± 2.26
Protein (g/100mg) (Mean ± SD)	0.7067 ± 1.54	0.7525 ± 2.31
Vitamin C (g/100g) (Mean ± SD)	0.6561 ± 1.20	0.8693 ± 0.69
Fat %	2.03 %	2.53 %
Dietary fibre %	27.57 %	40.29 %
Iron (mg/100g)	2.05	1.489
Calcium (mg/100g)	0.00258	0.00288

From table 1, it is evident that Variation 2 had a slightly higher mean value for carbohydrate, protein, fat, vitamin C, calcium and dietary fibre than Variation 1. Iron was however found to be high in Variation 1 compared to Variation 2 owing to the additional amount of dates used in this preparation. The dietary fibre was found to be in appreciable levels in Variation 2 which can be attributed to the slightly higher amount of beetroot used in its preparation. Besides this, the dates and raisins would have also contributed to the dietary fibre content. It can be noted here that, though the mean values were higher in Variation 2, they were not significantly different from Variation 1.

This jam which is low in calories could serve as a excellent choice for individuals who want to restrict their sugar intake while still enjoying the sweetness and flavour of fruit spreads. Thus, this jam may find its use amongst weight watchers and health-conscious individuals who aim to limit their consumption of table sugar. This jam being high in dietary fibre may help in alleviating constipation. Besides this, the absence of artificial flavour and colour enhancers makes it a better choice compared to commercial jams that are loaded with sugar and synthetic chemicals to enhance their shelf life and sensory appeal.

2. Identification of bioactive compounds present in the Rose-Roselle jam using the Gas Chromatography - Mass Spectrometry (GC-MS) method.

The first five bioactive compounds which had highest peaks for Variation 1 and Variation 2 of the Rose-Roselle jam are represented in table 2 and 3. The chromatogram for both variations are shown in figs. 1 and 2.

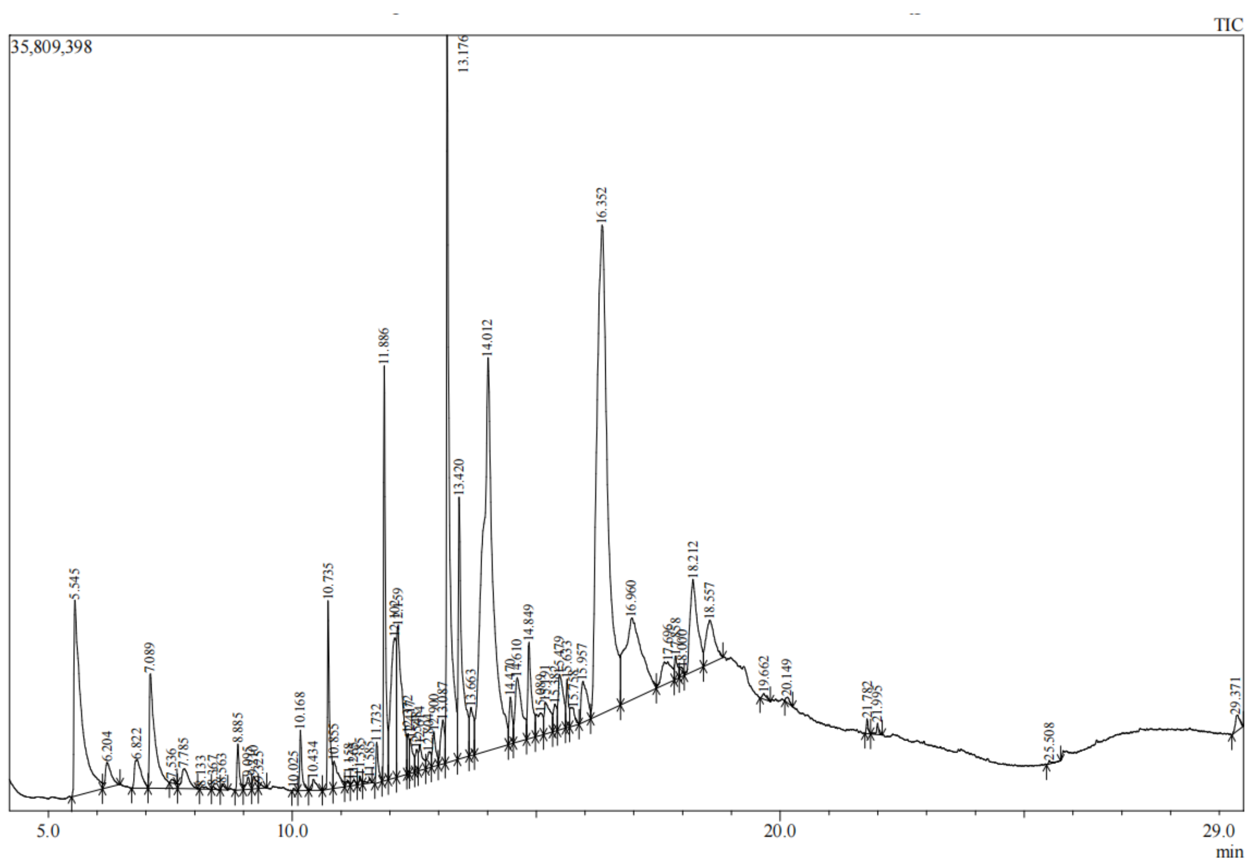


Fig. 1: Chromatogram of bioactive compounds of Variation 1 of Rose-Roselle jam

Table 2: Compounds identified in Variation 1 the Rose-Roselle jam

S.No	Retention time	Area %	Compound name
1	16.352	21.56	1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-
2	14.012	16.43	Acetoxyacetic acid, nonyl ester
3	13.176	8.72	5-Hydroxymethylfurfural
4	5.545	5.85	Glyceraldehyde
5	16.96	5.66	3,4-Altrosan

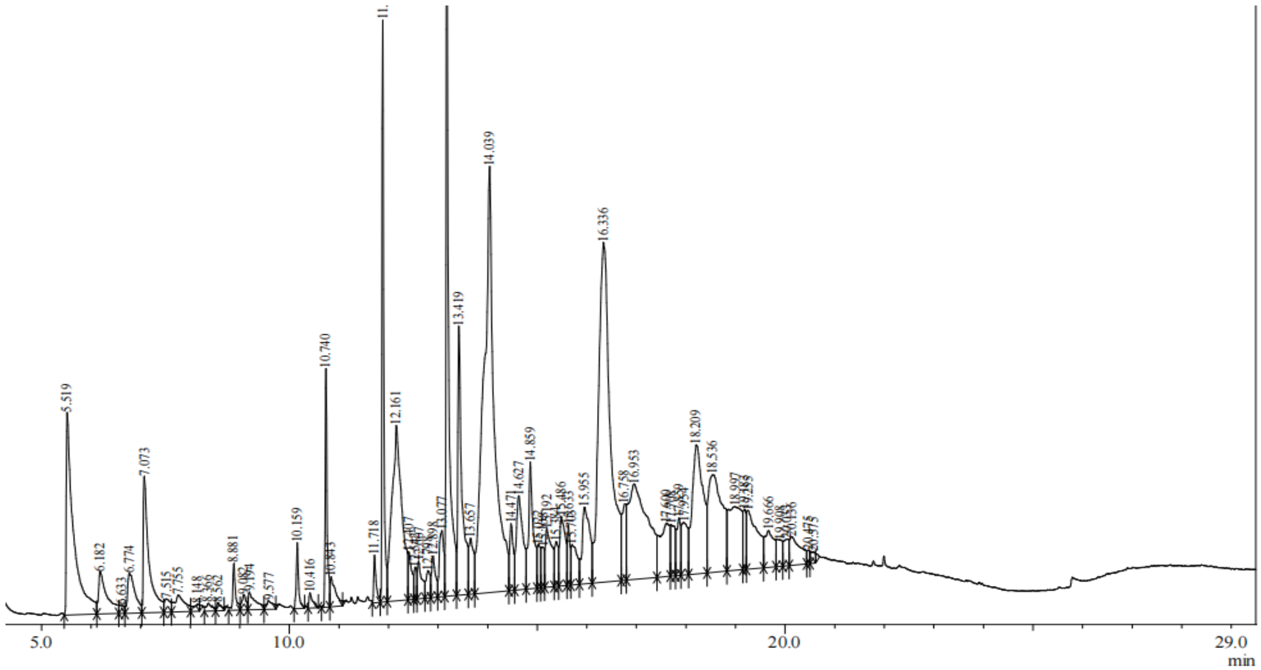


Fig. 2: Chromatogram of bioactive compounds of Variation 2 of Rose-Roselle jam

Table 3: Compounds identified in Variation 2 the Rose-Roselle jam

S.No	Retention time	Area %	Compound name
1	14.039	13.63	Acetoxyacetic acid, nonyl ester
2	16.336	12.11	1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-
3	13.176	5.69	5-Hydroxymethylfurfural
4	16.953	5.62	d-Glycero-d-ido-heptose
5	18.209	4.37	3-Deoxy-d-mannoic lactone

Among the first five bioactive compounds of Variation 1 and 2 of Rose-Roselle jam all the bioactive compounds possess antioxidant activity except Glyceraldehyde. Thus, the bioactive compounds that possess antioxidant activity identified in Variation 2 were more than those in Variation 1. Therefore Variation 2 could possess greater antioxidant potential than Variation 1.

3. Quantification of phytochemicals present in the Rose-Roselle jam

The results for quantification of phytochemicals for Variation 1 and Variation 2 of the Rose-Roselle are represented in table 4.

Table 4: Quantification of phytochemicals present in Rose-Roselle jam

Phytochemicals	Variation 1 Mean ± SD	Variation 2 Mean ± SD
Total phenolic content (mg GAE/100g)	25.715± 3.15	12.65 ± 2.03
Total Flavonoid content (mg QE/100g)	111.19 ± 2.29	189.29 ± 1.88
Anthocyanin content (mg /100g)	40.58 ± 17.70	88.39 ± 65.78
Total antioxidants (mg AAE/100g)	46.75 ± 62.08	132.73 ± 112.61

Total Flavonoid content, Total Anthocyanin content and Total Antioxidant content was found to be high in Variation 2 than Variation 1, whereas Total Phenol content was found to be high in Variartion 1 than Variation 2. This indicates that the presence of phenolic compounds such as 2- Furanmethanol and Maltol (identified only in Variation 1) along with 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, and Resorcinol which is identified in both variations 1 and 2 could have contributed to the higher phenolic content in Variation 1.

4. Antioxidant activity of the Rose-Roselle jam using DPPH and FRAP assay

Table 5: Radical Scavenging activity of Rose-Roselle jam assessed by DPPH assay

Variation 1 (20g rose:2.5g Roselle: 30g beetroot: 20g Dates: 10g raisins)			Variation 2 (20g rose:2.5g Roselle: 40g beetroot: 10g Dates: 20g raisins)		
Concentration	Percentage of inhibition (percent)	IC_{50}	Concentration	Percentage of inhibition (percent)	IC_{50}
20 µl	37.42	58.01	20 µl	49.96	20.15
40 µl	43.12		40 µl	55.32	
60 µl	50.76		60 µl	61.70	
80 µl	56.28		80 µl	68.26	
100 µl	62.76		100 µl	73.06	

The DPPH assay was performed to assess the antioxidant capacity of the Rose-Roselle jam by evaluating its ability to scavenge free radicals. The radical scavenging activity of Rose-Roselle jam increases with increase in the concentration of the sample. The percentage of DPPH radical inhibition of variation 1 of Rose-Roselle jam was found to be 62.76 percent at 100 µl concentation and for the Variation 2 of Rose-Roselle jam it was found to be 73.06 percent at 100 µl concentration. The IC_{50} value represents the concentration of a sample needed to inhibit half the amount of DPPH radicals. A lower IC_{50} value indicates a higher antioxidant capacity of the sample. The IC_{50} value of Variations 1 and 2 of Rose-Roselle jam was found to be 58.01 and 20.15 respectively. When compared to Variation 1, Variation 2 of Rose-Roselle jam showed higher antioxidant activiy indicating that it could exhibit radical scavenging activity at lower concentrations compared to variation 1. Therefore, the DPPH assay showed that Variation 2 of Rose-Roselle jam had a higher antioxidant potential with a low IC_{50} value when compared to the Variation 1. The results for 2,2-Diphenyl – 1 – picrylhydrazyl (DPPH) assay is represented in fig. 3.

Fig.3 Radical Scavenging activity of Variations 1 and 2 assessed by DPPH assay

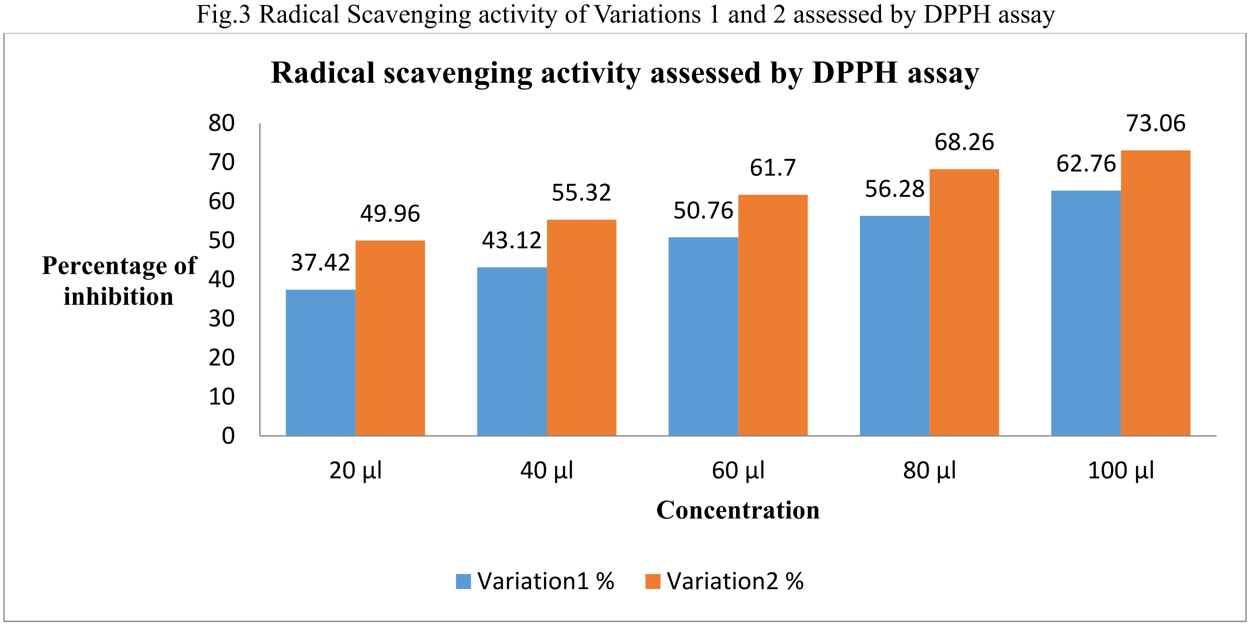
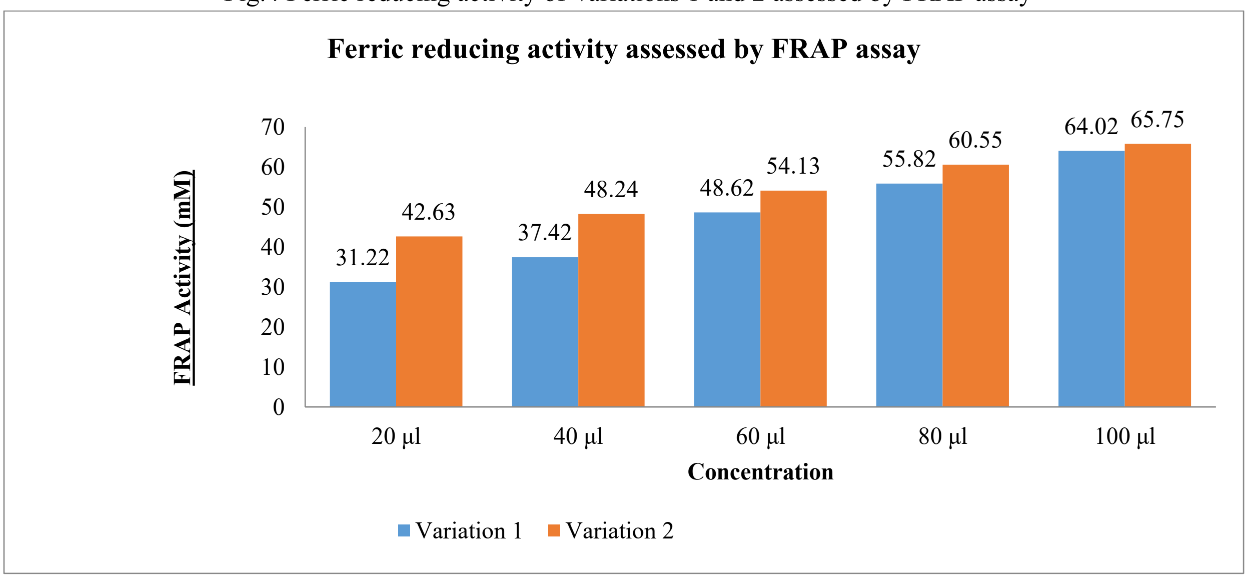


Table 6: Ferric reducing activity of Rose-Roselle jam assessed by FRAP assay

Variation 1 (20g rose:2.5g Roselle: 30g beetroot: 20g Dates: 10g raisins)			Variation 2 (20g rose:2.5g Roselle: 40g beetroot: 10g Dates: 20g raisins)		
Concentration	FRAP Activity (mM)	IC_{50}	Concentration	FRAP Activity (mM)	IC_{50}
20 µl	31.22	63.83	20 µl	42.63	45.98
40 µl	37.42		40 µl	48.24	
60 µl	48.62		60 µl	54.13	
80 µl	55.82		80 µl	60.55	
100 µl	64.02		100 µl	65.75	

The FRAP assay was performed to assess the ferric reducing power of the Rose-Roselle jam. The ferric reducing activity of the Rose-Roselle jam increases with increase in the concentration of the sample. The percentage of ferric reduction activity of Variation 1 of Rose-Roselle jam was found to be 64.02 percent and for the variation 2 of Rose-Roselle jam was found to be 65.75 percent at 100 µl concentration of sample. IC_{50} value represents the concentration at which a sample reduces 50 percent of ferric ions. A lower IC_{50} value represents greater reducing potential at a low concentration. The IC_{50} value of Variations 1 and 2 of Rose-Roselle jam was found to be 68.83 and 45.98 respectively (from table 12). When compared to Variation 1, Variation 2 showed higher antioxidant activiy at lower concentrations. Therefore, the FRAP assay showed that variation 2 was more potent in reducing ferric ions when compared to the variation 1 of Rose-Roselle jam. The results for FRAP assay is represented in fig. 4

Fig.4 Ferric reducing activity of Variations 1 and 2 assessed by FRAP assay



Thus, the antioxidant assays clearly indicate that Variation 2 exhibited better radical scavenging and ferric reducing potential (that is greater antioxidant activity) than Variation 1 of the Rose-Roselle jam.

5. Sensory evaluation of the Rose-Roselle jam

The sensory evaluation of Rose-Roselle jam was conducted using a scorecard with a 9-point hedonic rating scale by 50 panel members. The mean sensory scores for all attributes, including appearance, flavor, color, odor, spreadability, and overall acceptability, were calculated using a t-test. The results of the sensory evaluation are presented in Table 7.

Table 7: Sensory evaluation of thes Rose-Roselle jam

Sensory parameters	Variation 1 mean ± sd	Variation 2 mean ± sd	"t" value	"p" value
Appearance	8.36 ± 0.72	8.2 ± 0.83	1.026	0.307
Flavour	8.16 ± 0.71	7.9 ± 0.78	1.731	0.086
Colour	8.54 ± 0.54	8.36 ± 0.69	1.446	0.151
Odour	8.18 ± 0.80	7.94 ± 0.93	1.379	0.171
Spreadability	8.42 ± 0.67	8.26 ± 0.77	1.100	0.273
Overall acceptability	8.36 ± 0.72	8 ± 0.83	2.309	0.023

There was no significant difference in appearance, flavour, colour, odour and spreadibility between Variations 1 and 2. However, Variation 1 demonstrated a significantly higher overall acceptability score compared to Variation 2. Therefore Variation 1 is considered the more accepted variation. This may be because of slightly higher amount of dates used in Variation 1 of Rose-Roselle jam which would have imparted an additional sweetness to the Variation 1. Variation 2 had a higher amount of raisins compared to Variation 1, which the panellists opined that it gave a tangy flavour to the jam.

IV CONCLUSION

Based on the findings, Variation 2 demonstrated a significantly higher nutrient content, phytochemical profile, and antioxidant properties (as assessed by DPPH and FRAP assays), as well as superior GC-MS analysis results, compared to Variation 1. However, although the values for Variation 2 were higher, they did not differ significantly from those of Variation 1. Thus, both variations of Rose-Roselle jam exhibited notable antioxidant potential, with Variation 2 showing a slightly greater potential than Variation 1. In terms of sensory attributes, no significant differences were observed between Variations 1 and 2 in terms of appearance, flavor, color, odor, and spreadability. However, Variation 1 exhibited a significantly higher overall acceptability score. Therefore, the choice of Rose-Roselle jam is ultimately dependent on individual preference and taste. Thus Rose-Roselle jam can be included in the diet in the quantities mentioned in this study and can be a suitable alternative for jams loaded with sugars. It can be considered as a good source of natural antioxidants.

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