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Assessment of the Microbial and Physicochemical Properties of Fermented and Commercial Jam

[1]Purvi Anand, [2]Ayushi Sood, [3]Puneet, [4]Gurinder Singh, [5]Harpreet Kaur
[1,2]Research Intern, [3]Senior Scientist, [4]Research Associate, [5]Centre Director
[1,2,3,4,5]Rapture Biotech Pvt. Ltd, Mohali, Punjab, India.

ABSTRACT
Fruit and vegetable processing provides a wealth of opportunities for value addition and waste reduction, which can lead to a substantial increase in revenue and employment in agrarian nations. Jam is made by boiling fruit pieces, pulp, or puree with nutritive sweeteners like sugar, dextrose, invert sugar, or liquid glucose until the desired consistency is reached. Jam can be made from ripe, fresh, dehydrated, frozen, or previously packed fruits, including fruit juices, fruit pulp, fruit juice concentrate, or dry fruit. We prepared Jam using apples, oranges, and candies. The study aimed to explore the physicochemical properties of both Fermented and commercial Jam like pH, total soluble solids, acid value and moisture content. The Phytochemical properties such as Vitamin C, phenolics content, anthocyanin and flavonoids content, antioxidant activity was analysed. The Antibacterial activity and Antibiotic susceptibility test were also carried out using a well diffusion method. The fermented jam has pH of acceptable range along with good quantities of Vitamin C, Phenolics, flavonoids and anthocyanin content. Whereas protein and tannin content were least in the commercial sample. Both fermented and commercial samples exhibit Antibacterial activity and sensitivity against antibiotics.
Keywords: Fruit Jam, Pectin, phytochemical, antibacterial activity, microbial analysis, biochemical test.

I. INTRODUCTION

Jam is a semi- solid food product which contains fruit or vegetables pulp, acid, pectin, and various other ingredients to a required consistency. It contains at least 45% pulp and 65% or more total soluble solids (TSS). There are typically two varieties of jams: one made from the pulp of a single fruit and another made by combining the pulp of two or more fruits. The sugar present in the jam inhibits the growth of microbes and keeps it from spoiling, sugar retains water, which increases the items' shelf life. Pectin added in the Jam improves the texture, thickening, and stabilizing properties of a variety of foods, including beverages, bread goods, confections, jam, and jelly. Citric acid is also added in the jam production. The accurate balance of citric acid is required in jellies preparation. Lemon juice and lime juice can be used as an alternative to citric acid [1]. The fruit used were apples, oranges, and candies. The Rosaceae family includes the apple. The Pomoideae subfamily includes the apple, pear, quince, medlar, and a few additional species. Two to five carpels encased in a fleshy covering are the distinguishing feature of pome fruits. Apples are a rich source of the beneficial nutrients that epidemiological studies have found to be associated with protection against staging diseases and cancers. Apples contain a good quantity of polyphenols, antioxidants, phytonutrients, flavonoids (epicatechin, procyanidin B_2 and quercetin) and tannins.[1] It has good quantities of Vitamin C, beta- carotene, B- Complex Vitamins such as riboflavin, thiamine, and pyridoxine (Vitamin B_6). Additionally, they also contain Tartaric acid which imparts flavour to the fruit. Oranges also contain good quantities of micronutrients like folate, Vitamin C and polyphenols (hesperidin) [2,3].

II. MATERIALS AND METHODS

A. Preparation of Jam

For the jam preparation, the fruits collected i.e. Apples and oranges were washed under running tap water which removes excess dirt and cut in half. The fruits and water were added in a clean container. Heat the mixture at 120°C for a few minutes until the consistency becomes soft. Once it starts boiling add pectin and citric acid (lemon juice) and sugar in the mixture and stir the mixture. The mixture is heated for 30 minutes till homogeneous mixture is obtained. The Jam was allowed to cool at room temperature and later on, it was stored in the refrigerator for further analysis [3].

B. Physicochemical Analysis

1. pH

A pH meter was used to analyze the pH, and pH 4.0 and pH 9.0 buffers were used to calibrate the meter. The electrode was washed and dried before data collection for each replication.

2. Titrable Acidity

The jam sample's titratable acidity was assessed. To create a clear extract, 1g of the material was combined with distilled water and filtered. 0.1N NaOH was used to titrate the mixture. The pale pink tint of the phenolphthalein, which is utilized as an indicator, serves as an endpoint. There will be three repetitions of this. The formula TA%=mL of NaOH used× normality of NaOH × equivalent weight of NaOH/weight of sample × volume of aliquot× 100 was used to measure acidity.

3. Total Soluble solids

The TSS was measured at room temperature using a digital handheld refractometer. One drop of the extracted juice was put on the prism of the dry refractometer for every sample (product), and the values were noted in °Brix.

4. Moisture content

The moisture content is measured using the standard method. A selection of watch glass dishes were dried for 20 minutes at 105°C in an oven. After drying, they were put in a desiccator, left to cool for five minutes, and then weighed. The dried and weighed dishes were carefully filled with around 2 g of the prepared jam sample. The plates and their contents were dried for 12 hours at 85°C in an oven. The dishes and their contents were dried, then allowed to cool in desiccators for 15 minutes to achieve room temperature before being weighed again. The amount of water present in the sample was determined by calculating the weight loss after drying it to a constant weight:

Moisture%= (W_3-W_2) /(W_1-W_2) ×100

Here W_2= Weight of empty plates

W_1= Weight of the sample

W_3= Weight of dry samples and plates

5. Antibacterial activity

The antibacterial activity was tested using the agar well plate method. The objective was to test the jam sample's antibacterial activity against two kinds of bacteria, Lactobacillus and Salmonella. Luria bertani agar medium was used as the growth medium. Bacterial cultures of Lactobacillus and Salmonella were streaked across the agar plates. For a whole day, the plates were incubated at 37°C. 50 microliters of extract were added to the wells created using sterile tips after incubation. By looking at the distinct zones of inhibition surrounding the wells, the antibacterial activity was determined. The sample's ability to suppress bacterial growth was demonstrated by the zones of inhibition. [4].

6. Antibiotic Susceptibility test

The susceptibility test was performed to determine the sensitivity of bacterial culture towards different antibiotics. The nutrient agar medium was used. The bacterial culture was streaked in the agar plate, let it dry for a few minutes. The minimum concentration of antibiotics was loaded into the wells. The plates are incubated for 24 hours at 37°C for bacterial growth. The positive result is indicated by the zone of inhibition around the wells [5].

C. Phytochemical Analysis

The phytochemical test was carried out to detect the presence of bioactive compounds present in the sample.

1. DETECTION OF PROTEINS

Ninhydrin test

The extract and 2 ml of 0.2%Ninhydrin solution was boiled, the appearance of Violet colour indicates the presence of amino acids and proteins [6].

2. DETECTION OF CARBOHYDRATES

Resorcinol test

In 2 ml of extract add few crystals of Resorcinol in equal volume of concentrated HCl and heat the solution. The appearance of rose colour indicates the presence of ketones in it.

3. DETECTION OF PHENOLICS

Potassium Dichromate test

In 2 ml of sample extract add a few drops of Potassium dichromate solution. A dark colour indicates the presence of Phenolic compound in it.

4. DETECTION OF TANNINS

Braymer's test

In 1 ml of sample add 3 ml of distilled water and a few drops of 10% $FeCl_3$ solution. The appearance of blue- green colour indicates the presence of tannin in it.

5. DETECTION OF FLAVONOIDS

Conc. H_2SO_4 Test

In Few ml of sample add conc. H_2SO_4. The appearance of yellow/ orange colour confirms the presence of flavonoids in it [7]

D. QUANTITATIVE PHYTOCHEMICAL ANALYSIS

1. Total anthocyanin content

A spectrophotometer (UV-400 spectrophotometer, Hamburg, Germany) and the pH dilution method were used to quantify the total anthocyanin content. A 15-minute equilibration period was followed by the preparation of two dilutions of the fruit and jam samples using potassium chloride buffer (pH 1.0) (0.5 mL sample extracts and 3.5 mL potassium chloride) and sodium acetate buffer (pH 4.5) (0.5 mL sample extract and 3.5 mL sodium acetate) against a blank. Each dilution's absorbance was measured with a UV–Vis spectrophotometer (UV-400 spectrophotometer, Hamburg, Germany) at 515 nm and 700 nm.

2. Antioxidant potential

The DPPH technique was used to assess the antioxidant activity. The extract was mixed with 1 mL of newly made DPPH (0.25 mM) and 1 mL of ethanol. After being well combined, the samples were allowed to sit at room temperature for half an hour in the dark. A spectrophotometer (UV-400 spectrophotometer, Hamburg, Germany) was then used to test each sample's DPPH radical scavenging activity at 517 nm. The activity was calculated using formula:

DPPH scavenging activity%= A_0- A_S/ A_0×100

3. Total phenolic content

A spectrophotometrically modified approach was used to estimate the total phenolic content of the jam sample. A 0.5 mL sample was added after 2.5 mL of 10% Folin-Ciocalteau's Reagent and 2 mL of a 7.5% sodium carbonate solution were mixed together to conduct the examination. A spectrophotometer set to 765 nm was used to measure the absorbance after the sample had been incubated for 40 minutes at 45°C.

4. Total flavonoids

A 0.1 g sample was combined with 0.1 M potassium acetate, then 2.8 mL of distilled water and 0.1 mL of aluminium chloride were added. After properly mixing the resultant solution, it was allowed to sit at room temperature for 30 minutes.

A UV-Vis spectrophotometer was then used to measure the reaction mixture's absorbance at 430 nm, using a blank as a reference. The quercetin curve was used to determine the results [4].

5. Vitamin C Content

The Vitamin C Content was checked using U.V- Vis spectrophotometer. The serial dilutions of sample are prepared which creates a calibration curve. The absorbance of sample is measured at 265nm.

E. Microbiological Analysis

1. Preparation of Media

The media used were Nutrient agar and Luria bertani agar medium. The media prepared was according to the manufacturer's instructions.

2. Preparation of cultures

The serial dilution of samples was carried out, appropriate dilution (dilution 4) was inoculated in the agar plate. Incubate the plates at 37℃ for 24 hours. The colonies on plates were counted and multiplied with dilution factor.

3. Identification of Isolates

Using a pour plate technique, the inoculation was aseptically moved from 10^{-4} into plates of the appropriate media and then gram stained. By repeatedly pouring the isolates onto their corresponding media, they were cleansed. Colony and cell morphology, as well as biochemical tests, were used to identify the bacteria plates at the genus level after they were incubated at 37℃ for 24 hours and 25℃ for 72 hours. For identification purposes, a 24-hour-old culture was made from each plate, and the bacteria isolates were recognized using a variety of identification tests, Gram staining reaction, and cultural features. The isolates were recognized by their physical and cultural traits, which were crucial to this procedure and were thus examined.

4. Gram Staining

Distilled water was dropped onto the center of a clean slide to create a smear of the organisms. A flame-sterilized loop was used to turn a loop full of bacterial colonies into a drop of water on the slide, which subsequently spread into a thin smear along the slide. The slide was placed over a Bunsen flame to allow the smear to air and fix with mild heat. After 30 to 60 seconds of flooding the smear with crystal violet solution, it was rinsed with tap water and treated with iodine, which was left to work for a minute.

After being decolorized with alcohol and allowed to sit on the slide for five to ten seconds, it was rinsed under running water. After that, a safranin solution was poured onto the slide, and it was left to work for two minutes. After that, it was washed with tap water and patted dry. Gram-positive and gram-negative bacteria were identified by examining it with oil immersion, noting the bacteria's colour and shape. [8]

F. Biochemical tests

1. Sugar test

This is a test to show the ability of an organism to ferment sugar indicating a change in pH of the medium. This include sucrose, lactose, maltose, dextrose, D- Mannitol test. The different Fermentation media was prepared, sterilised, and added a few drops of phenolphthalein indicator . The bacterial culture is inoculated in the media. The culture is incubated for 24 hours at 37℃ . The change in pH or colour of the sugar shows a positive result.[8]

2. Catalase test

After adding streaks of the isolate to a test tube, around 2 milliliters of hydrogen peroxide were added. When the organisms were added to the solution, bubbles appeared, indicating that they were catalase negative.

3. Urease test

The urea agar media was prepared, sterilised, phenol red indicator was added into the media. The bacterial culture was inoculated into the media, incubate the culture for 24 hours at 37℃.

4. Indole test

Tryptone broth was made using the specified ingredients.

After being poured into the tubes, the broth was sterilized. One tube was left uninoculated as a control, while the other tubes were filled with the test organisms. For 48 hours, the tubes were infected at 37°C.

One milliliter of KOVAC's reagent was added to each tube, including the control, following incubation. The tubes were shaken gently, they were left to stand for one to two minutes. The appearance of the cherry red ring confirms the positive result.

5. Methyl Red- Voges- Proskauer Test

The ability to carry out mixed-acid fermentation is tested using the Methyl-Red test. Peptone, glucose, and a phosphate buffer are all present in the nutrient broth. Methyl Red, a pH indicator, is added to the broth following incubation for 24 hours at 37℃. Methyl Red turns yellow at pH values above 6.0 and red at pH values below 4.4, which is a positive result. An intermediate pH is indicated by an orange colour, which would be regarded as a negative result.

Voges -Proskauer- It is used to detect acetoin in bacterial culture. Alpha naphthol and Potassium hydroxide is added to the broth which has been inoculated with bacteria followed by the addition of Indicator. A cherry - red colour indicates positive result whereas yellow colour indicates negative result [9]

6. Citrate test

When sodium citrate is the only source of carbon and energy, the citrate test is carried out by inoculating microorganisms into "Simons Citrate broth," an organic synthetic medium. When citric acid is metabolized, carbon dioxide is produced. This carbon dioxide interacts with sodium and water to make sodium carbonate, an alkaline substance that causes the colour to shift from green to blue and indicates a positive test. Bromothymol blue is used as an indicator [9].

III. RESULTS AND DISCUSSION

Table I. Physicochemical analysis

	Fermented Jam Sample	Commercial Jam Sample
p.H	4.1	5
Moisture content	0.49	0.008

Total soluble solids	2.08	1.020
Acid value	Positive	Positive

Physicochemical analysis

The pH is considered as one of the most crucial parameters that need to be controlled and monitored in jam for optimum gel consistency. p.H values of 4.1 and 5 were obtained. Low p.H in the sample prevents microbial growth. Fermented Jam sample has brix value whereas other sample has brix value. Sugar content in the sample contributes to the physical and chemical properties, thereby improving the appearance (colour and texture) of the jam [8].

Table II. Phytochemical Analysis

	Fermented Jam Sample	Commercial Jam Sample
Protein	Positive	Negative
Carbohydrate	Positive	Positive
Tannin	Positive	Negative
Phenolics	Positive	Positive
Flavonoids	Positive	Positive
Vitamin C	Positive	Positive

Table III. Biochemical test

	Fermented Jam Sample	Commercial Jam Sample
Gram staining	Positive	Negative
Shape	Coccus shaped	Rod shaped
Catalase test	Negative	Positive
Urease test	Positive	Positive
Dextrose test	Negative	Positive
Lactose test	Positive	Positive
Maltose test	Negative	Positive
Sucrose test	Positive	Positive
Citrate test	Negative	Negative
Indole test	Positive	Positive
Methyl Red-Voges-Proskauer test	Positive	Positive
Mannitol test	Positive	Positive

Biochemical Analysis

The Fermented jam sample showed positive result in almost all the biochemical test, could be glucose forming microbe (gram positive) such as bacillus spp., staphylococcus spp., pseudomonas spp. and streptococcus spp. which are all acid/gas forming bacteria. Whereas commercial Jam samples contain gram negative bacteria such as Escherichia coli (E.coli), Salmonella and Pseudomonas aeruginosa. The result indicates that physicochemical, phytochemical properties of our fermented Jam Sample is slightly comparable as mentioned in Rafique Nagina et al,2023 [4].

Microbial analysis

Both the samples showed antibacterial activity against Salmonella and Lactobacillus resulting in the zone of inhibition around the wells. The Fermented Jam sample has zone of inhibition ranging from 6mm to 9mm, whereas Commercial Jam sample has zone of inhibition around 5mm to 15mm as shown in fig 1 and fig 2. Alongside, in the Antibiotic Susceptibility test the antibiotics used were PENICILLIN, LEVOFLOXACIN, AMOXICILLIN, CEPHALEXIN. The experimental and commercial samples were highly sensitive towards all the four antibiotics, indicating that antibiotics inhibit microbial growth as shown in fig 3.

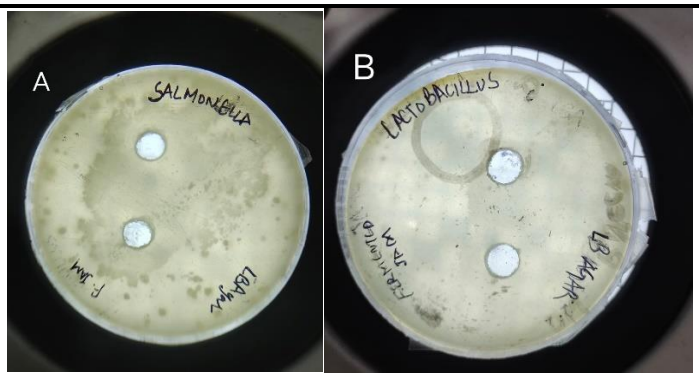


Fig. 1 (a) Antibacterial activity of Fermented Jam on Salmonella strain (b) Antibacterial activity of Fermented Jam on Lactobacillus strain.

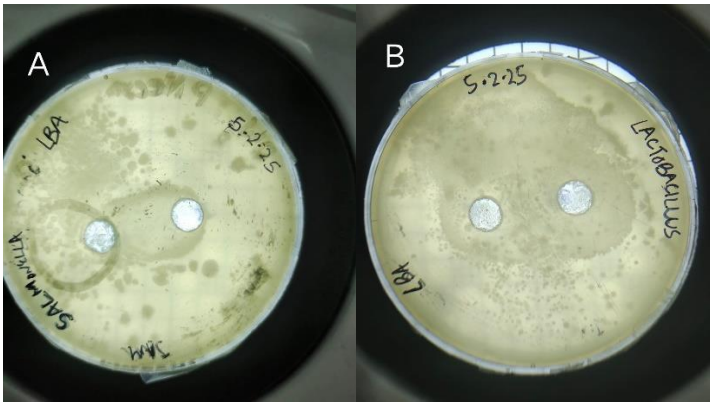


Fig. 2 (a) Antibacterial activity of Commercial Jam on Salmonella strain (b) Antibacterial activity of Commercial Jam on Lactobacillus strain.

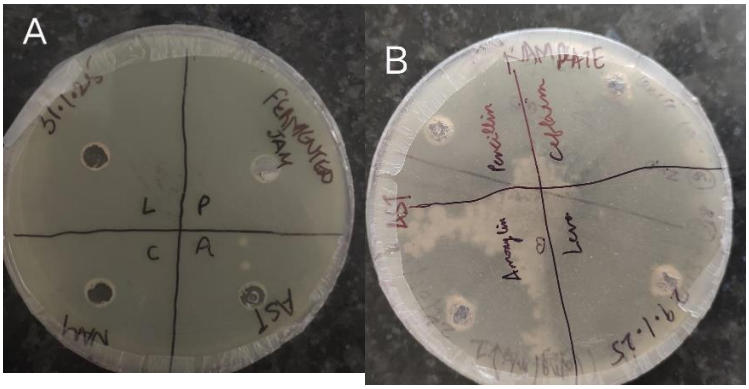


Fig. 3 (a) Antibiotic susceptibility test (AST) of Fermented Jam (b) Antibiotic susceptibility test (AST) of Commercial Jam

Antioxidant Activity test

The antioxidant potential of Fermented Jam Sample was 0.024% and Commercial Sample was 0.0173%.

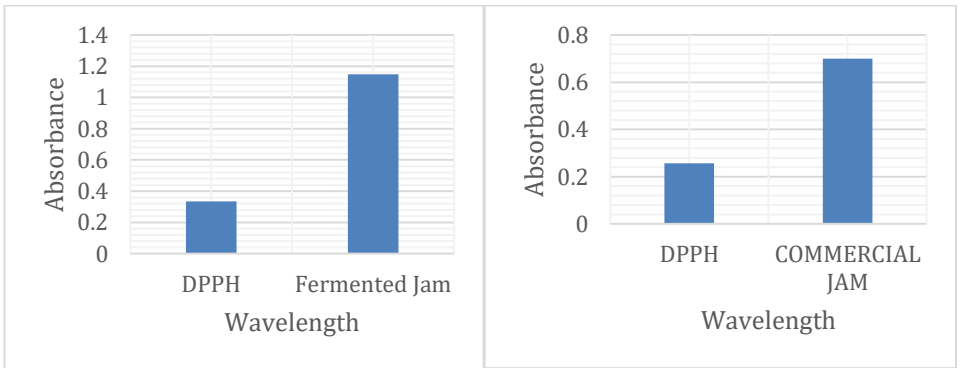


Fig. 4 (a) Antioxidant potential of Fermented Jam (b) Antioxidant potential of Commercial Jam

Phenolic content

The Phenolic content of Fermented Jam sample comes out to be 0.295 and commercial Jam sample was 0.491.

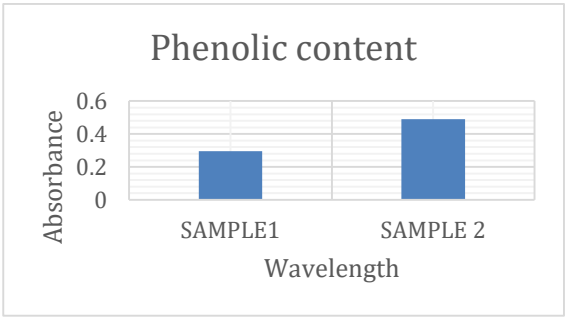
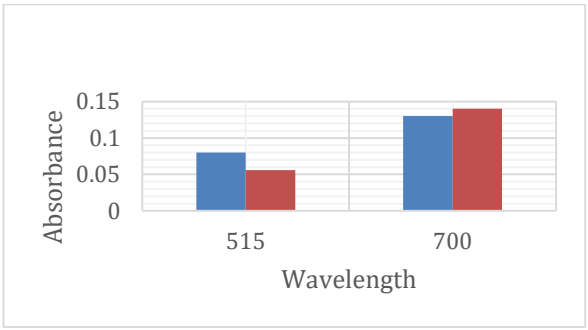


Fig. 5 Phenolic content (a) Sample 1- Fermented Jam (b) Sample 2- Commercial Jam

Anthocyanin content

The anthocyanin content of fermented sample ranges between 0.08 to 0.14 whereas in commercial jam sample ranges between 0.006 to 0.184.



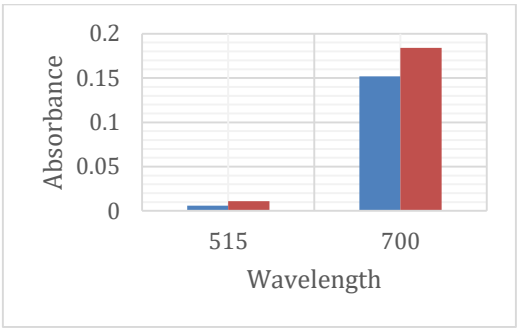


Fig.6 (a) Anthocyanin content of Fermented Jam (b) Anthocyanin content of Commercial Jam

Vitamin C content

The vitamin C content present in Fermented sample ranges from 0.003 to 0.606 whereas in commercial jam sample ranges from 0.032 to 1.857.

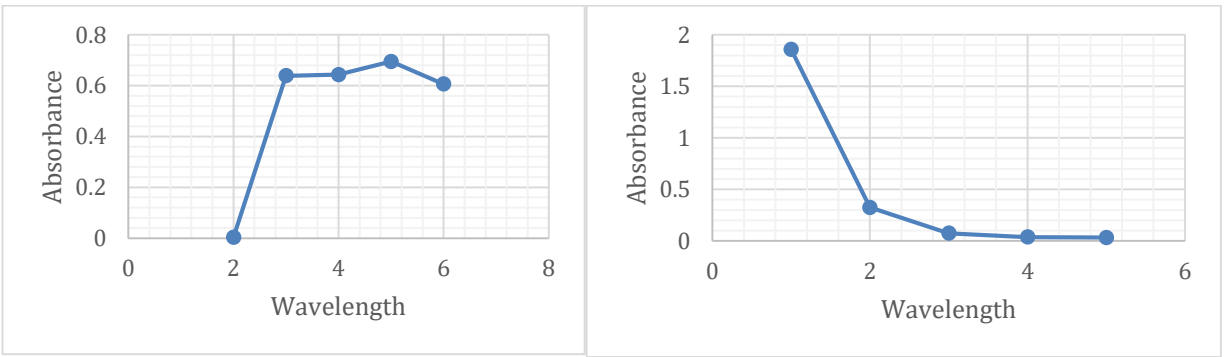


Fig. 7 (a) Vitamin C content of Fermented Jam (b) Vitamin C content of Commercial Jam

Flavonoids content

The vitamin P content in fermented jam sample comprises between 0.754 to 1.72 whereas in commercial jam sample ranges between 0.03 to 0.212.

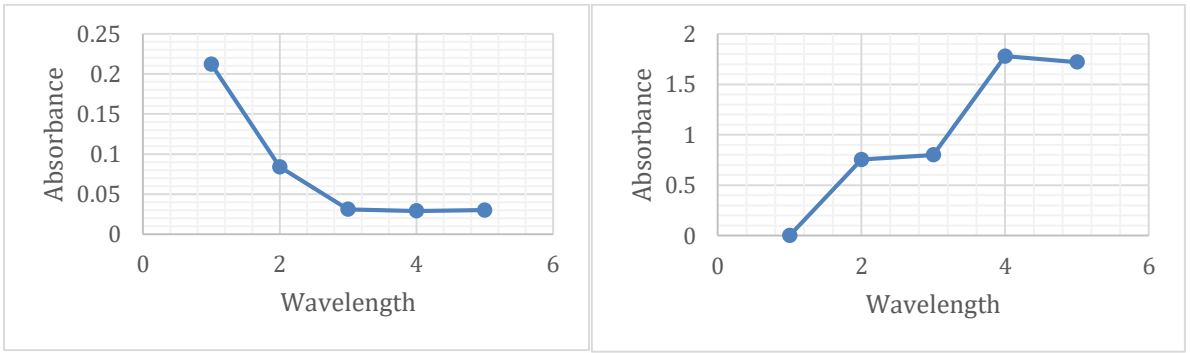


Fig. 8 (a) Flavonoids content of Commercial Jam (b) Flavonoids content of Fermented Jam

IV. CONCLUSION

In conclusion, the results of this study demonstrate that our sample holds significant potential as a source of nutrients and antioxidants. Its pH, vitamin C, total soluble solid content, anthocyanin content, phenolic and flavonoid content fall within the acceptable range.

Our fermented Jam sample scored average in almost all the parameters. It can be concluded that apples and oranges in combination with other fruits can be utilised for jam preparation which is nutritionally and microbiological safe for consumption. It is also a chance to investigate the possibilities of turning the fruit into other goods, like jam, to cut down on fruit losses after harvest.

V. CONFLICT OF INTEREST

There was no conflict of Interest.

VI. AUTHOR'S CONTRIBUTIONS

Experimentation was done by Purvi and Ayushi. The manuscript preparation was done by Purvi and Gurinder. The Proof reading was done by Dr. Puneet and infrastructure or lab facility was provided by Harpreet Kaur.

REFERENCES

[1] Awulachew, M. T. (2021). Fruit Jam Production. *International Journal of Food Science, Nutrition and Dietetics*, 532–537. https://doi.org/10.19070/2326-3350-2100092

[2] Musacchi, S., & Serra, S. (2018). Apple fruit quality: Overview on pre-harvest factors. In *Scientia Horticulturae* (Vol. 234, pp. 409–430). Elsevier B.V. https://doi.org/10.1016/j.scienta.2017.12.057

[3] Omolara, A., Nkechinyere, C., & Gbohunmi, L.-O. (2022). Comparison of Composite Jam Produced from Orange, Apple and Date Powder with Commercial Jam with Table Sugar "Comparison of Composite Jam Produced from Orange, Apple and Date Powder with Commercial Jam with Table. In *Research Article Sugar". Medicon Nutritional Health* (Vol. 1).

[4] Rafique, N., Mamoona, T., Ashraf, N., Hussain, S., Ahmed, F., Ali Shah, T., Salamatullah, A. M., Mekonnen, A. B., & Bourhia, M. (2023). Exploring the nutritional and sensory potential of karonda fruit: Physicochemical properties, jam production, and quality evaluation. *Food Science and Nutrition*, *11*(11), 6931–6944. https://doi.org/10.1002/fsn3.3619

[5] Gautam, D., Sharma, J., & Kaur, H. (2025). Issue 3 www.jetir.org (ISSN-2349-5162). In *JETIR2503375 Journal of Emerging Technologies and Innovative Research* (Vol. 12). www.jetir.org

[6] Yadav, R., & Agarwala, M. (2011). Phytochemical analysis of some medicinal plants. *Journal of Phytology*, *3*(12), 10–14. http://journal-phytology.com/

[7] Shaikh, J. R., & Patil, M. (2020). Qualitative tests for preliminary phytochemical screening: An overview. *International Journal of Chemical Studies*, *8*(2), 603–608. https://doi.org/10.22271/chemi.2020.v8.i2i.8834

[8] Moses, O., & Omotayo Aderonke, A. (2019). Microbial and physicochemical properties of date jam with inclusion of apple and orange fruits. In *International Journal of Food Science and Nutrition*. www.foodsciencejournal.com

[9] Hemraj, V., Diksha, S., & Avneet, G. (2013). A REVIEW ON COMMONLY USED BIOCHEMICAL TEST FOR BACTERIA.