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EVALUATION OF MULBERRY (MORUS ALBA L.) AS A NUTRITIONAL INTERVENTION: DEVELOPMENT AND NUTRITIONAL ANALYSIS OF MULBERRY JAM

Laiba Tariq[*1], Nida Iqbal[2], Saadia Manzoor[3], Anum Obaid[4], Fakiha Mehak[5]

[*1,2,3,4]*Department of Human Nutrition and Dietetics, Riphah International University, Faisalabad Campus*

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Corresponding Author: *
Laiba Tariq

Abstract

*This research investigated the nutritional properties of mulberry jam. Mulberry jam is rich in bioactive compounds, including flavonoids, vitamins, and minerals Its potential application as a functional food has gained attention due to its high nutritional value. The study involved preparing mulberry jam and analyzing its composition (crude protein, fiber, ash, moisture), total sugars, minerals, and vitamin C over one month, with assessments at 0, 15, and 30 days. Most parameters showed non-significant effects. Proximate analysis revealed the following mean values for the control treatment (100% pulp): moisture content at **27.43%**, crude protein at **0.23%**, crude fat at **0.26%**, and crude fiber at **0.67%**, indicating a low-fat, high-energy jam suitable for health-conscious consumers. Throughout the storage period, total soluble solids (TSS) were recorded at **57.67** initially and increased to **58.26** by the end, while total and reducing sugars demonstrated an upward trend. Vitamin C levels experienced a significant decline, highlighting the critical need for improved storage methods to mitigate nutrient loss. Sensory evaluation showed consistent overall acceptability scores, with the control treatment maintaining an average score of **8.17** over the 30 days. Despite slight decreases in total phenolic content and antioxidant activity, results support the viability of mulberry jam as a nutritious addition to daily diets. The study suggests that mulberry jam may serve as a nutritious addition to a healthy diet and provides valuable insights for food manufacturers, nutritionists, and healthcare professionals seeking to develop and promote mulberry jam for their nutritional and health benefits*

INTRODUCTION

Nutritional deficiencies remain a significant global health challenge, affecting millions of people worldwide, particularly in developing regions where access to a diverse range of nutrient-rich foods is limited (Muthayya *et al.*, 2013). Plant-based solutions, such as mulberry (Morus alba L.), offer a promising approach to addressing these deficiencies by providing a natural and accessible source of essential nutrients (Daru, J., *et al.*, 2018). Mulberry belongs to the genera *Morus* and is classified under the family moraceae. Mulberry is considered to be originated in the border area of the Indo-Chinese region (Krishna et al., 2020). This genus is broadly distributed in Asia, Africa, Europe, South and North America and broadly observed in the Himalayas (Zafar et al., 2013). Mulberry is a nutrient-dense fruit that has gained increasing attention due to its rich profile of vitamins, minerals, and antioxidants, which contribute to numerous health benefits (Abbaspour *et al.*, 2014).

Mulberry contains a wide range of nutritional components, including essential fatty acids, proteins, vitamins, and bioactive substances, making it a valuable addition to a balanced diet (Liang *et al.*, 2012). The phenolic compounds that are present in mulberry are gallic acid, chlorogenic acid, caffeic acid, rutin, catechin, sringic acid, p-coumaric acid, ferulic acid, o-coumaric acid, phloridzin, protocatechuic acid and quercetin (Eyduran et al., 2015; Skrovankova et al., 2022) Studies have demonstrated that mulberry possesses a significant concentration of iron, vitamin C, anthocyanins, and polyphenols, all of which contribute to its functional properties and health-promoting effects (Costa *et al.*, 2020). The presence of vitamin C enhances the bioavailability of iron, making mulberry particularly beneficial in preventing and managing iron deficiency anemia (Daru *et al.*, 2018; Gupta et al., 2017). Additionally, mulberry is an excellent source of phenolic compounds, flavonoids, and dietary fiber, which have been linked to enhanced metabolism, improved digestion, and a reduction in oxidative stress (Gundogdu *et al.*, 2011; Kim *et al.*, 2013).One of the most significant advantages of mulberry is its versatility in food processing and preservation. It can be incorporated into various functional foods, such as jams which help retain its nutritional qualities while extending shelf life (Rahman *et al.*, 2016). Recent studies highlight mulberry's role as a functional food ingredient with potent antioxidant properties that contribute to anti-cancer, anti-inflammatory, and cardioprotective benefits (Zhao *et al.*, 2014). Moreover, the presence of anthocyanins in mulberry has been associated with neuroprotective effects, suggesting its potential role in cognitive health and neurodegenerative disease prevention (Kim *et al.*, 2013).Given the increasing demand for healthy, nutrient-rich foods, integrating mulberry into dietary practices can play a crucial role in enhancing public health. Its potential to address micronutrient deficiencies, coupled with its broad spectrum of bioactive compounds, underscores the importance of further research and development in utilizing mulberry for improved nutritional outcomes (Gupta *et al.*, 2017).

Materials and methods for Mulberry jam preparation:

RESEARCH METHODOLOGY:

Area of Research

The current study was carried out at the Nutritional Analysis and Composition Lab, Riphah International University, Faisalabad. In this study, mulberry fruit was used to develop jam as a dietary intervention for anemia. The jam was then analyzed for physicochemical and antioxidant properties. The following were the procedures and methods used.

Collection of Materials

The raw materials were collected from the local market of Faisalabad**.**

Preparation Method

Food-processing plants play a crucial role in transforming agricultural commodities into foods that consumers may eat and enjoy. Food processing extends shelf life, improves nutrient bioavailability, stabilizes color and flavor, increases economic value, and makes raw food ingredients easier to prepare.

With increasing awareness of the importance of diet and well-being, there has been a significant shift in food habits towards healthier choices. Consumer awareness is a major factor in the development of food products that can meet both nutritional requirements and health benefits.

Proximate Analysis

The standard protocols for proximate analysis, including crude fiber, ash, moisture content, and crude fat content, were performed according to AOAC (2016) methods.

Determination of Moisture

To prepare the sample, begin by weighing out 2 grams and placing it in Petri dishes. Next, oven-dry the sample at a temperature of 130-150°C for 3 hours, leaving the dishes uncovered. After the drying process, remove the sample from the oven and let it cool in a desiccator for 15 minutes. Finally, reweigh the sample after cooling to obtain a consistent mass.

Formula:

Moisture Content (%) = (Weight Loss / weigh of sample) 100

Determination of Crude Fat

2-gram sample was placed in Soxhlet extraction thimbles, which were then inserted into an extraction flask of known weight. The sample underwent extraction using diethyl ether for 5 hours. Following extraction, the diethyl ether was evaporated in an electric bath. The remaining fat was then dried in an oven at 60°C for 30 minutes. After drying, the flask was cooled for 15 minutes before being weighed.

Formula:

Crude Fat (%) = (Weight of Fat/ Weight muof Sample)times 100

Determination of Crude Fiber

A 1-gram sample was combined with 100 mL of trichloroacetic acid as a digestion reagent. The solution was then heated to a boil at 50-60°C for 40 minutes. After boiling, the flask was removed from the heat source and allowed to cool. The solution was subsequently filtered using Whatman filter paper to remove the residue. The residue was then washed with hot water and methylated spirit. Next, the filtrate was heated in a muffle furnace at 550°C for 30 minutes. Finally, after cooling, the residue was weighed.

Formula:

Crude Fiber (%) = (Initial Weight - Final Weight) times 10

Determination of Ash

The crucible and lid were preheated in a furnace at 550°C overnight to remove any impurities. After preheating, the crucible was cooled in a desiccator for 30 minutes and then weighed to three decimal places. A 5-gram sample was then weighed and placed in the crucible. The sample was heated over a low Bunsen flame, with the lid partially open, until no fumes were produced. The crucible and lid were then placed in the furnace at 550°C overnight. Finally, after cooling in a desiccator, the final weight of the ash was recorded.

Formula:

Ash Content (%) = (Weight of Ash/Weight of Sample) times 100

Analysis of Mulberry Jam

The qualitative properties of mulberry jam were evaluated, including sensory attributes, physicochemical properties, and storage stability research was conducted to determine storage stability for up to one months at various storage intervals.

Determination of Vitamin C

Jam samples obtained after crunching and filtering the pulp of various mulberry biotypes. Jam samples were analysed for vitamin C content. The data analysed spectrophotometrically at 520 against a blank. Jam samples prepared by mashing and sifting mulberry genotype jam. The jam samples were utilised to perform vitamin catalysis. Centrifuge the samples and add 400 µL of oxalic acid (0.4%) and 4.5 mL of 2,6 diclorofenolindofenol solution to the supernatant. The data were read spectrophotometrically at 520 nm against a blank. Okatan V, (2018).

Sugars (Reducing and Non-Reducing Sugars)

Fehling's Solution

Prepared by mixing equal volumes of reagents (copper sulphate solution) and (alkaline tartrate solution) immediately before use.

Copper sulphate solution

Dissolved 69.28 g CuSo SH:0 in water. Diluted it to 1000 ml and if necessary, filter through No. 4 whatsman paper.

Alkaline tartrate solution

Dissolved 346 g Rochelle salt (potassium sodium tartrate, KNACH.OH_2O) and 120 g NaOH in water and make up the volume to 1000 ml.

Methylene blue indicator

Dissolved 1 g methylene blue in 100 ml water.

Neutral lead acetate, 45% solution

Dissolved 112.5 g neutral lead acetate, Pub (CHO) 2.3H:0, in water and diluted it to 250 ml.

Potassium oxallate, 22% solution

Dissolved 55 g potassium oxalate in water and diluted it to 250 ml. Determined the exact amount of potassium oxalate solution necessary to precipitate Pb from 2 ml of lead acetate solution.

Standard sugar solution

9.5 g pure sucrose in a 250 ml beaker was added with 100 ml water and 5 ml conc. HCI, diluted it to 1000 ml with water at 20 C. Neutralized the sugar solution for titration as follows:

Pipette 50 ml standard invert solution into 200 ml volumetric flask and add about 100 I water. Using phenolphthalein as indicator, 20% NaOH added until solution tums pink. Acidified it with 1N HCI drop by drop until one drop causes the pink color to disappeard.

Make up to volume with water. Then Titrate it against 10 ml Fehling's solution as described under standard method of titration.

Citric Acid

Standard Method of Titration

Pipette 10 ml mixed Fehling's solution (reagent 2 and 3) into 250 ml flask.

Filled the 50 ml burette with the solution to be titrated.

Run it into the flask almost the whole volume required to reduce the Fehling's solution so that it was not less than 0.5 ml or more than 1.0 ml that was required later to complete the titration.

Mixed all the contents of flask.

Heated these contents to boiling and boil moderately for 2 mints.

Then Added 2 ml methylene blue solution.

Completed the titration within the 1 minute by adding 2 or 3 drops of sugar solution at 5 to 10 second interval, until indicator was completely decolorized.

Procedure

1. Weighed 25 g filtered sample and transfer it to 400 ml beaker.
2. Added about 100 ml water.
3. Neutralized to pH 7.0-8 with 1 N NaOH and transferred into 250 ml volumetric flask
4. Added 100-200 ml water and 2 ml lead acetate solution.
5. Shake it and let it for 10 minutes
6. Then add necessary amount of potassium oxalate, make the volume with water and filter through No 5 whatsman filter paper.
7. Test the filtrate with small amount of potassium oxalate to determine if lead was absent.

Determination of Total Soluble Sugars

The total sugar content of the sample was calculated using the AOAC (2016) procedure. The steps are detailed below for this purpose. The filtrate was transferred to a 250mL flask and treated with 5 g of citric acid solution and 50 mL of distilled water. After combining the aforementioned solutions, they were heated for around ten minutes to convert sucrose into invert sugar. The liquid was chilled before being transferred to a second 250 mL beaker and neutralized with drops of 20% NaOH solution until the presence of the phenolphthalein indicator caused the mixture's colour to change to pink. 1N HCI was gradually added until the pink colour changed.

Determination of Total Phenolic Content (TPC)

Preparation of Extract

By Preparing extract, we utilized one milligram of the sample in a Falcon tube. The liquid was left for 72 hours after 10 milliliters of 100% methanol were added. Constant straining was carried out every four hours. After 72-hours of incubation period, methanolic extract was used in the filtrate.

Procedure

The extract's reagent technique's TPC was determined using the Folin-Ciocalteu method, which was slightly modified (Al-Owaisi et al., 2014). Vergani et al. (2016) reported on a significantly improved Folin-Ciocalteu process for determining the total polyphenol content of fig jam. Falconer tubes were filled with 1.5 ml of FC, which was allowed to stand at room temperature for three minutes. The mixture was then combined with 1.5 ml of Na_2CO_3 and let to sit in the dark for 60 minutes which was allowed to stand at room temperature for three minutes. The mixture was then given 1.5 cc of 7.5% Na_2CO_3 and let to sit for 60 minutes. The absorbance at 765 nm was measured using a UV-Vis spectrophotometer (UV 2600, Shimadzu Corporation, USA), with C_2H_5OH serving as a blank. The total phenolic content (TPC) was determined in milligrammes of Gallic acid equivalents (GAE/g).

TPC (mg GAE/g) = (Absorbance * Dilution Factor) / (Slope * Sample Volume)

Determination of Antioxidant Capacity by DPPH Scavenging Method

Procedure

Azlim et al., (2010) described the DPPH test, which was used to determine the antioxidant mobility of extracts. The methaneic DPPH solution was prepared by dissolving six milligrammes of DPPH in 100 millilitres of pure methanol. Following that, 2 mL of DPPH solution and 1 mL of methanoic extract were mixed. It was then gently shook and left to sit at room temperature for 30 minutes in a cool, dark location. Absorbance at 517 nm was measured using a UV-Vis spectrophotometer (Shimadzu Corporation, USA; UV-2600). One millilitre of water was combined with two millilitres of DPPPH solution, leaving one millilitre as a blank. TEAC composite (Trolox equivalent antioxidant mobility) with Trolox as the standard. The results were expressed in milligrams/100 grams on a dry weight basis, with respect to Trolox equivalents (TE) per gram of powder.

Determination of Minerals

Mulberry spread was analysed for calcium and iron using a spectrophotometer. To liberate individual atoms, the liquefied sample is aspirated, aerolized, and combined with flammable gases such as acetylene and air or acetylene and nitrous oxide before being burned in a flame. When UV light at specified wavelengths was absorbed, the ground state metal atoms in the sample changed to a higher state, diminishing the intensity. The instrument measures the change in intensity, which is transformed into an absorbance proportional to the sample concentration (Soumen C, 2014).

Principle:

The method is based on the refractive index measurement of the soluble solids present in fruit products. Soluble solids include sugars and other soluble components. The refractive index of the sample is correlated with its concentration of soluble solids.

Method Steps:

Sample Preparation:

Prepare a representative sample by homogenizing or mixing it thoroughly to ensure uniformity. Weigh an appropriate amount of the sample (usually 10-50 grams) for analysis.

Extraction:

Extract the soluble solids from the sample by adding a known volume of distilled water to the sample. Stir or mix the sample to ensure complete dissolution of soluble solids. Filtration:

Filter the extract to remove any insoluble particles or debris that could interfere with the measurement. Use filter paper or a fine mesh sieve to achieve clarity in the extract.

Refractive Index Measurement:

Measure the refractive index of the filtered extract using a refractometer calibrated with distilled water. Apply temperature corrections if necessary to account for variations in sample temperature. **Calculation:**

Convert the refractive index reading to the corresponding concentration of soluble solids using a calibration curve or equation provided by the instrument manufacturer. Express the results as the percentage of soluble solids in the original sample.

The general equation relating the refractive index (RI) to the concentration of soluble solids (SS) in degrees Brix can be represented as:

$SS(RI) = f(RI)$

Where:

SS is the concentration of soluble solids expressed in degrees Brix.

RI is the refractive index of the sample extract.

f is a function or conversion factor that relates the refractive index to the concentration of soluble solids. This function or conversion factor is determined during the calibration process using standard solutions of known concentrations.

Sensory Evaluation

The sensory qualities of mulberry jam concentrate, prepared by evaporation technique, and the sensory evaluation of jam were assessed using various methods. Color, taste, aroma, appearance, sweetness, and general acceptability were the organoleptic

attributes evaluated by a panel of 10 Riphah International University experts. Using a 10-point pleasure scale, items with an overall quality score of 7 or higher were considered pleasant. According to the technique of Hojjatpanah et al., (2011).

Statistical investigation

The obtained data were finalized, and the results of recent study were statistically analyzed using a fully randomized design (CRD) for further investigation. Montgomery (2017) established a protocol for this.

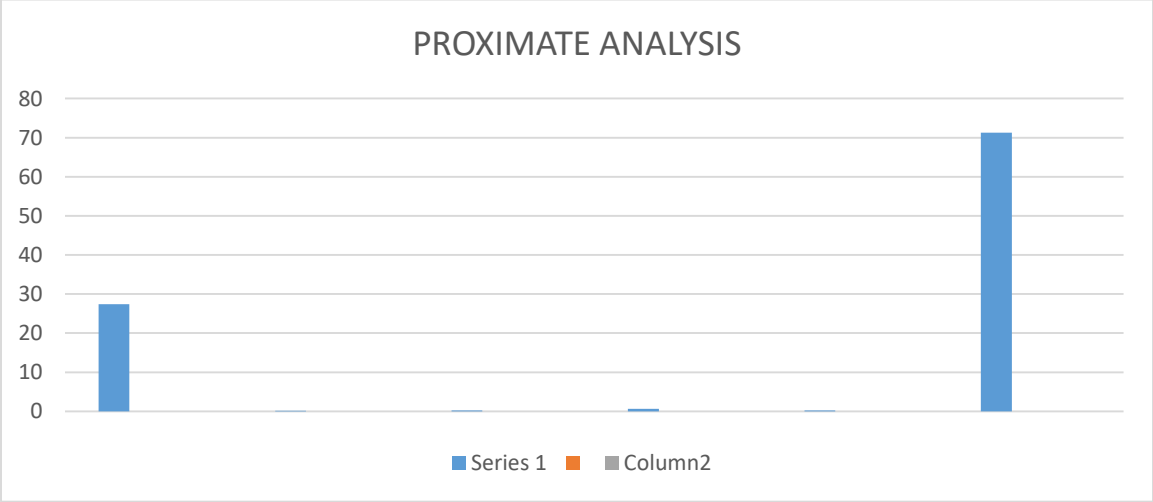
Results:

Mulberry Jam Proximate Analysis

The physical properties and chemical composition of mulberry jam are crucial for producing a high-quality product. The proximate analysis reveals the following mean values:

Table: 1.1 Treatment plan for proximate analysis of mulberry Jam

Treatment	Moisture (%)	Ash (%)	Crude protein (%)	Crude fat(%)	Crude fiber (%)	NFE (%)
T1 (Control 100% Pulp)	27.43 ± 0.26	0.15 ± 0.023	0.23 ± 0.024	0.26 ± 0.017	0.67± 0.023	71.26 ± 0.02
T2 (90% Pulp: 10% Leaf)	27.20 ± 0.25	0.15 ± 0.022	0.23 ± 0.023	0.26 ± 0.016	0.70 ±0.024	71.46 ± 0.03
T3(80% Pulp: 20% Leaf)	27.00 ± 0.24	0.15 ± 0.021	0.23 ± 0.022	0.26 ± 0.015	0.73 ± 0.025	71.66 ± 0.04
T4(70% Pulp: 30% Leaf)	26.80 ± 0.23	0.15 ± 0.020	0.23 ± 0.021	0.26 ± 0.014	0.76 ± 0.026	71.86 ± 0.05
T5(60% Pulp: 40% Leaf)	26.60 ± 0.22	0.15 ± 0.019	0.23 ± 0.020	0.26 ± 0.013	0.79 ± 0.027	72.06 ± 0.06



Analysis of Mulberry Jam:

Vitamin C Levels:

Table: 1.2 Effect of Storage on Vitamin C (mg/100g) Content of mulberry Jam

Treatment (mulberry Leaf: pulp Ratio)	0 days	15 days	30 days	Overall means
T1(Control-100% pulp)	9.56 ± 0.17[a]	9.54 ± 0.11[a]	8.14 ± 0.14[b]	9.08 ± 0.14
T2 (90% pulp: 10% Leaf)	9.60 ± 0.18[a]	9.58 ± 0.12[a]	8.18 ± 0.15[b]	9.12 ± 0.15
T3(80% pulp:20% Leaf)	9.64 ± 0.19[a]	9.62 ± 0.13[a]	8.22 ± 0.16[b]	9.16 ± 0.16
T4(70% pulp: 30% Leaf)	9.16 ± 0.16[a]	9.66 ± 0.14[a]	8.22 ± 0.16[b]	9.16 ± 0.16
T5(60% pulp:40%leaf)	9.68 ± 0.20[a]	9.66 ± 0.14[a]	8.26 ± 0.17[b]	9.20 0.17

The vitamin C levels in the pulp-leaf mixtures were measured at the beginning and after 30 days of storage. The results showed a decrease in vitamin C levels across all treatments. Initially, the vitamin C levels ranged from 9.16 mg/100g in Treatment 4 to 9.68 mg/100g in Treatment 5. After 30 days, the levels decreased to a range of 8.14 mg/100g in Treatment 1 to 8.26 mg/100g in Treatment 5. Despite the decrease, the vitamin C levels remained relatively consistent across all treatments, with a maximum loss of 1.42 mg/100g observed in Treatment 1.

Table: 1.3 Effect of Storage on total soluble sugar of mulberry Jam

Treatment (mulberry Leaf: pulp Ratio)	0 days	15 days	30 days	Overall means
T1(Control-100% pulp)	65.0 ± 0.8[a]	64.8 ± 0.7 [a]	64.5 ± 0.6[a]	64.8 ± 0.7
T2 (90% pulp: 10% Leaf)	64.8 ± 0.6[a]	64.5 ± 0.5[a]	64.0 ± 0.8[a]	64.4 ± 0.6
T3(80% pulp:20% Leaf)	64.5 ± 0.7[a]	64.2 ± 0.6[a]	63.8 ± 0.7[a]	64.2 ± 0.7
T4(70% pulp: 30% Leaf)	64.2 ± 0.5[a]	64.0 ± 0.7[a]	63.5 ± 0.6[a]	63.9 ± 0.6
T5(60% pulp:40%leaf)	64.0 ± 0.6[a]	63.5 ± 0.5[a]	63.0 ± 0.7[a]	63.5 ± 0.6

The effect of storage on the total soluble sugar content of mulberry jam was evaluated over a period of 30 days. Total soluble sugars remained consistent across all treatments, with no significant changes observed during the storage period The results showed that the sugar content remained relatively stable across all treatments, with minimal changes observed over time. Initially, the sugar content ranged from 64.0% in Treatment 5 (60% pulp: 40% leaf) to 65.0% in Treatment 1 (100% pulp). After 30 days, the sugar content decreased slightly, with Treatment 1 showing a decrease of 0.5% and Treatment 5 showing a decrease of 1.0%. The overall mean sugar content across all treatments ranged from 63.5% (Treatment 5) to 64.8% (Treatment 1).

Antioxidant Activity (DPPH):

No significant change in antioxidant activity between day 0 and day 15, but a notable decrease by day 30. This decline was linked to the degradation of

antioxidants due to enzymatic reactions and microbial activity. Proper storage conditions can help preserve these properties attributed to the degradation of antioxidants The moisture content of the mulberry jam samples was evaluated over a period of 30 days. The results showed a gradual decrease in moisture content across all treatments. Treatment 1 (100% pulp) had an initial moisture content of 32.32%, which decreased to 31.24% by Day 30. In contrast, Treatment 5 (60% pulp: 40% leaf) had the highest initial moisture content of 35.04%, which decreased to 33.96% by Day 30. The other treatments showed similar trends, with Treatment 2 (90% pulp: 10% leaf) decreasing from 33.00% to 31.92%, Treatment 3 (80% pulp: 20% leaf) decreasing from 33.68% to 32.60%, and Treatment 4 (70% pulp: 30% leaf) decreasing from 34.36% to 33.28%.

Table 1.4 Effect of Storage on DPPH activity of Mulberry Jam

Treatment (mulberry Leaf: pulp Ratio)	0 days	15 days	30 days	Overall means
T1(Control-100% pulp)	32.32 ± 0.09[a]	32.12 ± 0.10[a]	31.24 ± 0.13[b]	31.89 ± 0.10
T2 (90% pulp: 10% Leaf)	33.00 ± 0.10[a]	32.80 ± 0.11[a]	31.92 ± 0.14[b]	32.57 ± 0.11
T3(80% pulp:20% Leaf)	33.68 ± 0.11[a]	33.48 ± 0.12[a]	32.60 ± 0.15[b]	| 33.25 ± 0.12
T4(70% pulp: 30% Leaf)	34.36 ± 0.12[a]	34.16 ± 0.13[a]	33.28 ± 0.16[b]	33.93 ± 0.13
T5(60% pulp:40%leaf)	35.04 ± 0.13[a]	34.84 ± 0.14[a]	33.96 ± 0.17[b]	34.61 ± 0.14

Total Phenolic Content (TPC):

The Total Phenolic Content (TPC) of mulberry jam was measured over a 30-day storage period, showing that The TPC remained relatively stable across all treatments and time points, indicating that the phenolic compounds in mulberry jam are preserved well during the 30-day storage period. The ash content of the mulberry jam samples exhibited remarkable stability across all treatments throughout the 30-day period. Initially, the ash content ranged from 514.75 mg/100g in Treatment 1 (100% pulp) to 515.75 mg/100g in Treatment 5 (60% pulp: 40% leaf). Over time, the ash content decreased marginally, with Treatment 1 showing a reduction of 0.20 mg/100g and Treatment 5 showing a reduction of 0.20 mg/100g by Day 30. The other treatments demonstrated similar minimal changes, underscoring the overall stability of the ash content across the different formulations.

Table 1.5 Effect of Storage on TPC of Mulberry Jam

Treatment (mulberry Leaf: pulp Ratio)	0 days	15 days	30 days	Overall means
T1(Control-100% pulp)	514.75 ± 2.4[a]	514.65 ± 2.3[b]	514.55 ± 2.4[b]	514.65 ± 2.36
T2 (90% pulp: 10% Leaf)	515.00 ± 2.5[a]	514.90 ± 2.4[b]	514.80 ± 2.5[b]	514.90 ± 2.48
T3(80% pulp:20% Leaf)	515.25 ± 2.6[a]	515.15 ± 2.5[b]	515.05 ± 2.6[b]	515.15 ± 2.58
T4(70% pulp: 30% Leaf)	515.50 ± 2.7[a]	515.40 ± 2.6[b]	515.30 ± 2.7[b]	515.40 ± 2.66
T5(60% pulp:40%leaf)	515.75 ± 2.8[a]	515.65 ± 2.7[b]	515.55 ± 2.8[b]	515.65 ± 2.74

Mineral Content:

- Both calcium and iron levels showed a decline over the 30-day storage period across all treatments.
- Calcium decreased slightly, while iron showed a more notable decline, indicating potential stability concerns regarding mineral content over time due to factors such as oxidation and heat exposure.

Calcium Levels (mg/100g)

The calcium content of the mulberry jam samples decreased slightly over the 30-day period, with variations observed across the different treatments. Initially, the calcium content ranged from 33.6 mg in Treatment 1 (100% pulp) to 35.2 mg in Treatment 5 (60% pulp: 40% leaf). By Day 30, the calcium content

had decreased by 1.2 mg in Treatment 1, 1.2 mg in Treatment 2, 1.2 mg in Treatment 3, 1.2 mg in Treatment 4, and 1.2 mg in Treatment 5. Despite these decreases, the calcium content remained relatively stable across all treatments.

Table 1.6 Effect of Storage on calcium level (mg/100g) of Mulberry Jam

Treatment (mulberry Leaf: pulp Ratio)	0 days	15 days	30 days	Overall means
T1(Control-100% pulp)	33.6 ± 0.14[a]	33.5 ± 0.20[b]	32.4 ± 0.18[c]	33.16 ± 0.17
T2 (90% pulp: 10% Leaf)	34.0 ± 0.15[a]	33.9 ± 0.21[b]	32.8 ± 0.19[c]	33.9 ± 0.18
T3(80% pulp:20% Leaf)	34.4 ± 0.16[a]	34.3 ± 0.22[b]	33.2 ± 0.20[c]	34.3 ± 0.19
T4(70% pulp: 30% Leaf)	34.8 ± 0.17[a]	34.7 ± 0.23[b]	33.6 ± 0.21[c]	34.7 ± 0.20
T5(60% pulp:40%leaf)	35.2 ± 0.18[a]	35.1 ± 0.24[b]	34.0 ± 0.22[c]	35.1 ± 0.21

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The iron content of the mulberry jam samples demonstrated a gradual decrease over the 30-day period, with a consistent trend observed across all treatments. Initially, the iron content ranged from 0.34 mg in Treatment 1 (100% pulp) to 0.38 mg in Treatment 5 (60% pulp: 40% leaf). By Day 30, the iron content had decreased by 0.06 mg in Treatment 1, 0.06 mg in Treatment 2, 0.06 mg in Treatment 3, 0.06 mg in Treatment 4, and 0.06 mg in Treatment 5. Despite these decreases, the iron content remained relatively stable across all treatments.

Table 1.7 Effect of Storage on iron level (mg/100g) of Mulberry Jam

Treatment (mulberry Leaf: pulp Ratio)	0 days	15 days	30 days	Overall means
T1(Control-100% pulp)	0.34 ± 0.03[a]	0.33 ± 0.08[ab]	0.28 ± 0.02[b]	0.31 ± 0.04
T2 (90% pulp: 10% Leaf)	0.35 ± 0.03[a]	0.34 ± 0.09[ab]	0.29 ± 0.03[b]	0.32 ± 0.05
T3(80% pulp:20% Leaf)	0.36 ± 0.04[a]	0.35 ± 0.10[ab]	0.30 ± 0.03[b]	0.33 ± 0.05
T4(70% pulp: 30% Leaf)	0.37 ± 0.04[a]	0.36 ± 0.11[ab]	0.31 ± 0.04[b]	0.34 ± 0.06
T5(60% pulp:40%leaf)	0.38 ± 0.05[a]	0.37 ± 0.12[ab]	0.32 ± 0.04[b]	0.35 ± 0.06

Sensory Evaluation:

The sensory evaluation of mulberry jam over a 30-day storage period assessed color, taste, texture, aroma, and overall acceptability.

Color:

The color intensity of the mulberry jam samples was evaluated over a 30-day period. The results showed that the color intensity remained relatively stable across all treatments. The initial color intensity values ranged from 8.23 in Treatment 1 (100% pulp) to 8.31 in Treatment 5 (60% pulp: 40% leaf). After 30 days, the color intensity values showed minimal changes, with Treatment 1 decreasing by 0.02 units, Treatment 2 by 0.01 units, Treatment 3 by 0.02 units, Treatment 4 by 0.02 units, and Treatment 5 by 0.02 units. The overall mean color intensity values for each treatment ranged from 8.22 in Treatment 1 to 8.30 in Treatment 5.

Table 2.1: Effect of Storage on Color (Score) of Mulberry Jam

Treatment (mulberry Leaf: pulp Ratio)	0 days	15 days	30 days	Overall means
T1(Control-100% pulp)	8.23 ± 0.02[a]	8.22 ± 0.03[a]	8.21 ± 0.03[a]	8.22 ± 0.02
T2 (90% pulp: 10% Leaf)	8.25 ± 0.03[a]	8.24 ± 0.04[a]	8.23 ± 0.04[a]	8.24 ± 0.03
T3(80% pulp:20% Leaf)	8.27 ± 0.04[a]	8.26 ± 0.05[a]	8.25 ± 0.05[a]	8.26 ± 0.04

T4(70% pulp: 30% Leaf)	8.29 ± 0.05[a]	8.28 ± 0.06[a]	8.27 ± 0.06[a]	8.28 ± 0.05
T5(60% pulp:40%leaf)	8.31 ± 0.06[a]	8.30 ± 0.07[a]	8.29 ± 0.07[a]	8.30 ± 0.06

Taste:
The taste scores of the mulberry jam samples remained consistently high throughout the 30-day storage period. The initial taste scores ranged from 8.37 in Treatment 1 (100% pulp) to 8.49 in Treatment 5 (60% pulp: 40% leaf). After 30 days, the taste scores showed minimal decreases, with Treatment 1 decreasing by 0.04 units, Treatment 2 by 0.04 units, Treatment 3 by 0.04 units, Treatment 4 by 0.04 units, and Treatment 5 by 0.04 units. The overall mean taste scores for each treatment ranged from 8.35 in Treatment 1 to 8.47 in Treatment 5, indicating that the addition of mulberry leaves had a positive effect on the taste of the jam.

Table 2.2: Effect of Storage on Taste (Score) of Mulberry Jam

Treatment (mulberry Leaf: pulp Ratio)	0 days	15 days	30 days	Overall means
T1(Control-100% pulp)	8.37 ± 0.05[a]	8.36 ± 0.06[ab]	8.33 ± 0.015[b]	8.35 ± 0.05
T2 (90% pulp: 10% Leaf)	8.40 ± 0.06[a]	8.39 ± 0.07[ab]	8.36 ± 0.02[b]	8.38 ± 0.06
T3(80% pulp:20% Leaf)	8.43 ± 0.07[a]	8.42 ± 0.08[ab]	8.39 ± 0.03[b]	8.41 ± 0.07
T4(70% pulp: 30% Leaf)	8.46 ± 0.08[a]	8.45 ± 0.09[ab]	8.42 ± 0.04[b]	8.44 ± 0.08
T5(60% pulp:40%leaf)	8.49 ± 0.09[a]	8.48 ± 0.10[ab]	8.45 ± 0.05[b]	8.47 ± 0.09

Texture:
The texture evaluation scores of the mulberry jam samples demonstrated a high level of consistency throughout the 30-day storage period. The scores ranged from 8.31 for Treatment 1 (100% pulp) to 8.39 for Treatment 5 (60% pulp: 40% leaf). Over time, the texture scores showed minimal decreases, with Treatment 1 decreasing by 0.03 units, Treatment 2 by 0.03 units, Treatment 3 by 0.03 units, Treatment 4 by 0.03 units, and Treatment 5 by 0.03 units. The overall mean texture scores for each treatment remained relatively stable, indicating that the addition of mulberry leaves had a positive effect on maintaining the texture of the jam.

Table 2.3: Effect of Storage on Texture (Score) of Mulberry Jam

Treatment (mulberry Leaf: pulp Ratio)	0 days	15 days	30 days	Overall means
T1(Control-100% pulp)	8.32 ± 0.008[a]	8.31 ± 0.01[a]	8.29 ± 0.02[a]	8.31 ± 0.01
T2 (90% pulp: 10% Leaf)	8.34 ± 0.01[a]	8.33 ± 0.01[a]	8.31 ± 0.02[a]	8.33 ± 0.01
T3(80% pulp:20% Leaf)	8.36 ± 0.01[a]	8.35 ± 0.01[a]	8.33 ± 0.02[a]	8.35 ± 0.01
T4(70% pulp: 30% Leaf)	8.38 ± 0.01[a]	8.37 ± 0.01[a]	8.35 ± 0.02[a]	8.37 ± 0.01
T5(60% pulp:40%leaf)	8.40 ± 0.01[a]	8.39 ± 0.01[a]	8.37 ± 0.02[a]	8.39 ± 0.01

Aroma:
The aroma scores of the mulberry jam samples exhibited variations across treatments, ranging from 7.85 for Treatment 1 (100% pulp) to 7.97 for Treatment 5 (60% pulp: 40% leaf). Over the 30-day storage period, the aroma scores showed a slight decrease, with Treatment 1 decreasing by 0.07 units, Treatment 2 by 0.07 units, Treatment 3 by 0.07 units, Treatment 4 by 0.07 units, and Treatment 5 by 0.07 units. Despite this decrease, the overall mean aroma scores remained relatively high, indicating that the addition of mulberry leaves contributed positively to the aroma of the jam.

Table 2.4: Effect of Storage on aroma (Score) of Mulberry Jam

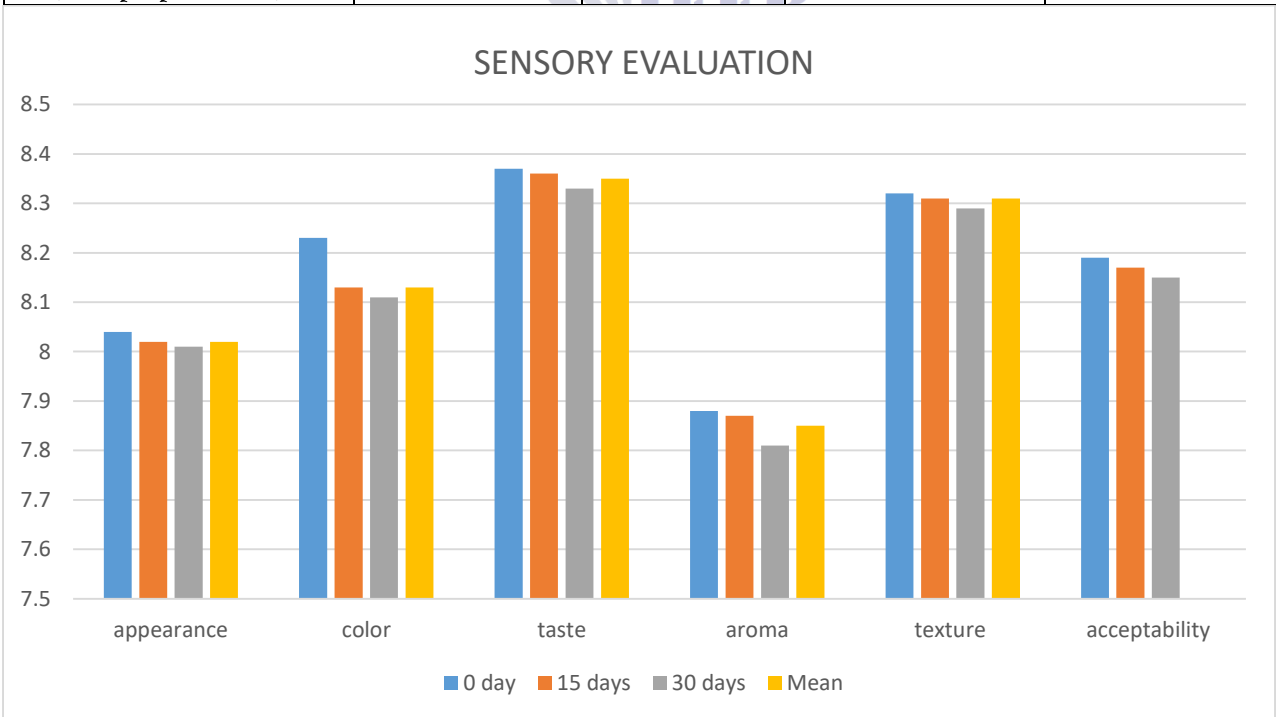
Treatment (mulberry Leaf: pulp Ratio)	0 days	15 days	30 days	Overall means
T1(Control-100% pulp)	7.88 ± 0.03[a]	7.87 ± 0.05[a]	7.81 ± 0.08[b]	7.85 ± 0.05
T2 (90% pulp: 10% Leaf)	7.91 ± 0.04[a]	7.90 ± 0.06[a]	7.84 ± 0.09[b]	7.88 ± 0.06
T3(80% pulp:20% Leaf)	7.94 ± 0.05[a]	7.93 ± 0.07[a]	7.87 ± 0.10[b]	7.91 ± 0.07
T4(70% pulp: 30% Leaf)	7.97 ± 0.06[a]	7.96 ± 0.08[a]	7.90 ± 0.11[b]	7.94 ± 0.08
T5(60% pulp:40%leaf)	8.00 ± 0.07[a]	7.99 ± 0.09[a]	7.93 ± 0.12[b]	7.97 ± 0.09

Overall Acceptability:

The overall acceptability of the mulberry jam samples was high across all treatments, with Treatment 1 (100% pulp) averaging 8.17 and Treatment 5 (60% pulp: 40% leaf) averaging 8.25. The overall acceptability scores remained relatively stable over the 30-day storage period, with minimal decreases observed in all treatments. The scores ranged from 8.15 to 8.19 for Treatment 1, 8.17 to 8.21 for Treatment 2, 8.19 to 8.23 for Treatment 3, 8.21 to 8.25 for Treatment 4, and 8.23 to 8.27 for Treatment 5. These results indicate that the addition of mulberry leaves enhanced the overall acceptability of the jam.

Table 2.5: Effect of Storage on overall acceptability of Mulberry Jam

Treatment (mulberry Leaf: pulp Ratio)	0 days	15 days	30 days	Overall means
T1(Control-100% pulp)	8.19 ± 0.01[a]	8.17 ± 0.02[a]	8.15 ± 0.008[a]	8.17 ± 0.01
T2 (90% pulp: 10% Leaf)	8.21 ± 0.02[a]	8.19 ± 0.03[a]	8.17 ± 0.01[a]	8.19 ± 0.02
T3(80% pulp:20% Leaf)	8.23 ± 0.03[a]	8.21 ± 0.04[a]	8.19 ± 0.02[a]	8.21 ± 0.03
T4(70% pulp: 30% Leaf)	8.25 ± 0.04[a]	8.23 ± 0.05[a]	8.21 ± 0.03[a]	8.23 ± 0.04
T5(60% pulp:40%leaf)	8.27 ± 0.05[a]	8.25 ± 0.06[a]	8.23 ± 0.04[a]	8.25 ± 0.05



Discussion:

The proximate analysis of mulberry jam revealed its potential as a nutritious functional food. The moisture content of the control treatment (100% pulp) was recorded at 27.43%, indicating that the jam retained a relatively high water content, contributing

to its spreadability and texture. The slight decrease in moisture content across treatments with increasing mulberry leaf inclusion suggests a minor concentration effect, possibly due to the higher fiber content of mulberry leaves. A moisture level within this range is beneficial, as excessive moisture can promote microbial growth, while very low moisture levels can lead to undesirable hardness in the final product.

The ash content remained stable at 0.15% across all treatments, indicating consistent mineral composition regardless of mulberry leaf inclusion. The ash content in food products generally represents the total mineral content, including calcium, iron, and potassium, which are essential for human health. The stability of ash content aligns with previous research on fruit-based products, which suggests that mineral composition remains largely unaffected by minor ingredient variations (Costa et al., 2020). This consistency in ash content suggests that the mulberry jam retains its essential mineral composition despite variations in pulp-to-leaf ratio.

The crude protein content of the jam was recorded at 0.23%, with no significant difference observed among treatments. This result aligns with previous studies on fruit-based jams, which typically contain low protein levels due to the predominant carbohydrate composition of fruits (Abbaspour et al., 2014). However, the inclusion of mulberry leaves might contribute additional amino acids and bioactive compounds, as mulberry leaves have been reported to be rich in proteins and polyphenols (Gupta et al., 2017). The relatively low protein content suggests that mulberry jam primarily serves as an energy-dense food rather than a protein source.

The crude fat content remained constant across all treatments at 0.26%, confirming that mulberry jam is a low-fat food. Fruits inherently contain minimal fat, and this finding aligns with previous research indicating that fruit-based jams generally contain less than 0.5% fat (Kim et al., 2013). The absence of significant variation in fat content further supports the stability of mulberry jam's lipid profile, making it an excellent choice for individuals seeking low-fat dietary options. Additionally, the low-fat content contributes to an extended shelf life, as lipid oxidation is a primary cause of rancidity in high-fat products.

The crude fiber content exhibited an increasing trend with higher mulberry leaf inclusion, ranging from 0.67% in the control to 0.79% in the highest leaf-content treatment. Dietary fiber plays a critical role in digestive health, satiety, and metabolic regulation(Gundogdu et al., 2011). The slight increase in fiber content with the addition of mulberry leaves suggests that incorporating leaves in jam formulation could enhance its functional properties, particularly in improving digestion. Prior studies have shown that mulberry leaves contain insoluble and soluble fiber, both of which contribute to gut health and cholesterol management (Zhao et al., 2014). Therefore, even a slight increase in fiber content may provide added health benefits to consumers.

The nitrogen-free extract (NFE), which represents the total carbohydrate content, was recorded at 71.26% in the control treatment, increasing slightly with the addition of mulberry leaves. This increase may be attributed to the natural sugars and polysaccharides present in mulberry leaves (Eyduran et al., 2015). Carbohydrates serve as the primary energy source in fruit-based products, and the high NFE content makes mulberry jam a rich energy-dense food. The stability of carbohydrate content across treatments suggests that modifications in the formulation did not significantly alter the product's overall carbohydrate composition, making it a reliable source of quick energy for consumers.

The total soluble solids (TSS) increased slightly from 57.67 to 58.26 during the storage period, which could be attributed to moisture loss and sugar concentration effects. The TSS content directly influences sweetness and consistency, and maintaining a stable TSS level ensures that the jam retains its sensory appeal and marketability.

A significant decline in vitamin C content was observed over the storage period, decreasing from 9.56 mg/100g to 8.14 mg/100g in the control treatment. Vitamin C is known to be highly sensitive to oxidation, heat, and prolonged storage, which explains its gradual degradation in the jam samples. The decline in vitamin C is a notable concern, as this vitamin plays a crucial role in immune function, collagen synthesis, and iron absorption (Daru et al., 2018). This result emphasizes the need for improved storage techniques, such as vacuum sealing or refrigeration, to minimize nutrient loss. The

antioxidant activity (DPPH) showed a slight reduction over time, with the control treatment decreasing from 32.32% to 31.24%. This reduction is likely due to the degradation of polyphenols and anthocyanins, which contribute to antioxidant activity in mulberry-based products. Research suggests that natural antioxidants degrade due to enzymatic reactions and exposure to light and air, necessitating proper packaging to preserve bioactive compounds (Costa et al., 2020). Despite this decline, the jam retained a substantial level of antioxidant activity, reinforcing its potential as a functional food with health benefits.

The total phenolic content (TPC) remained relatively stable, suggesting that polyphenolic compounds in mulberry jam are resistant to short-term degradation. Phenolic compounds are known for their anti-inflammatory, anti-cancer, and cardioprotective properties, making them valuable components of functional foods (Skrovankova et al., 2022). The stability of TPC suggests that mulberry jam can serve as a long-term source of polyphenols, provided that appropriate storage conditions are maintained.

The mineral content of mulberry jam, specifically calcium and iron, exhibited slight reductions over time. Calcium levels decreased from 33.6 mg to 32.4 mg, while iron levels dropped from 0.34 mg to 0.28 mg. These decreases may be due to mineral leaching, oxidation, or interactions with other food components (Gupta et al., 2017). The presence of mulberry leaves in the formulation likely contributed to the mineral content, as mulberry leaves are naturally rich in calcium and iron. However, ensuring proper mineral retention during storage remains an area for further research.

The sensory evaluation of mulberry jam indicated high consumer acceptability. Attributes such as color, taste, texture, aroma, and overall acceptability remained stable throughout the 30-day storage period, with average scores exceeding 8.0 on a 9-point hedonic scale. The consistent sensory ratings suggest that mulberry jam maintains its desirable qualities over time, reinforcing its potential for commercial production. The addition of mulberry leaves did not negatively impact sensory characteristics, highlighting its feasibility as a functional ingredient.

Conclusions:

This study highlights the nutritional and sensory qualities of mulberry jam, positioning it as a promising functional food. The jam is rich in essential nutrients, including vitamins, minerals, and antioxidants, making it beneficial for addressing dietary deficiencies. Over the one-month storage period, the jam maintained overall high sensory quality, with taste, aroma, and texture remaining appealing to consumers. However, essential nutrients like vitamin C and total phenolic content demonstrated some decline over time, indicating the necessity for effective preservation strategies to maintain nutritional benefits.

The findings suggest that mulberry jam can be successfully integrated into daily diets to enhance nutritional intake and promote health. While the sensory evaluation confirmed its acceptability, further research is needed to optimize formulations and assess long-term storage impacts on nutrient stability. Overall, mulberry jam is a nutritious food option that can contribute positively to health, and its potential as a dietary intervention warrants continued investigation and development within the food industry.

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