Original Article

Immunity Boosting Role of Low Caloric Amla-Moringa Jam Against Covid-19

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Abstract

Aim: A functional food, herbal jam, developed using Phyllanthus emblica (Amla fruit), Moringa oliefera (Moringa) leaves powder and Stevia rebaudiana (natural sweetener) used as sugar replacer to make low caloric jam that can boost up immunity against COVID-19. Stevia present in the jam made it suitable for diabetic patients. **Experimental:** A control was developed by replacing stevia with sugar with same composition. The ratios of amla and moringa were altered and the best one was selected on the bases of sensory evaluation using 9-point headonic scale. The jams were characterized at different storage days to check the stability and nutritional composition by carrying proximate analysis and mean values were compared. **Results:** The energy value calculations showed significant difference between the control jam (sugar based) & optimized jam (stevia based). Sensory evaluation concludes high score value of sugar based jam. Microbial analysis was done using Total Plate Count (TPC) and Yeast Mold Count (YMC). Moreover, studies are required to improve the textural attributes of stevia based jam.

Keywords: Malnutrition, Functional Foods, Moringa Oliefera, *Phyllanthus Emblica, Stevia Rebaudiana.*

Introduction

With the emerging trend of healthy lifestyle, people are more conscious about their dietary requirements. Moreover, due to rapid increase in the population, everyone is facing problem of malnutrition. It refers to individuals who either over-or- under nourished.[1] Individuals who have compromised immune systems are more susceptible to recurrent infections and serious sequelae. They might be more vulnerable to illnesses like pneumonia. For an individual whose immune system is impaired, bacteria and viruses—including the virus that causes COVID-19 can be fatal.

A pneumonia outbreak of unclear cause was documented in Wuhan, Hubei Province, China, in December 2019. The World Health Organization declared a pandemic on March 12, 2020, due to the thousands of deaths caused by the coronavirus disease (COVID-19) and the spread of disease.[2] Recently, for severe malnutrition, although treatment protocols becoming efficient yet patients still have no access to such settings. The best way to address such problems are food based strategies. In the existing health services, different strategies i.e., micronutrient supplementation, food fortification and food enrichment etc. can be followed for the provision of nutrients.[3]

Plants are known to be the richest source of traditional drugs, pharmaceutical intermediates, nutraceuticals and food supplements etc.[4] Consumption of natural products obtained from plant source have lesser side effects and more acceptance. However, botanist, microbiologist, chemist and food technologist are trying to use the medicinal benefits of plants in medicines and food fortification.[5] Functional foods are defined as any food having an additional function as contains nutrients beyond traditional constituents.[6] Such foods are designed to have benefits or/and minimizes risk of disease beyond traditional benefits, and in appearance similar

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to conventional food may consumed as part our daily diet.[7] Since there is currently no evidence based treatment for COVID-19, it is more important than ever to fortify our defense system against it. Numerous researches are there that encourages foods and herbs can prevent COVID-19 and may have antiviral action against COVID-19.

Natural plant products with less negative effects have grown in popularity over time. Among all these natural sources, that can be the best source of nutrients and can be used to add functionality or act as functional food is *Moringa oliefera* against COVID-19. Moringa oliefera is a viable food source in the tropics because the Moringa leaves, which are high in protein, calcium, iron, and vitamins, have been used to combat malnutrition. It has the ability to increase food security, improve nutrition and support zero hunger. Also has anti-fungal and anti-bacterial property.[8]

The new study recommends the possibility of the food industry implementing and using moringa leaves more vigorously. Moringa oliefera has anti-oxidant properties which helps to boost up the immunity, prevention of cancer.[9] Role of Moringa as anticipation for covid-19 makes it more popular ingredient for functional foods as it contains kaempferol, pterygospermin, morphin, quercetin & apigenin-7-O-rutinoside. Among these, apigenin plays key role against Covid-19.[10]

"Phyllanthus emblica" is also an important and beneficial medicinal plant having "Fruit" with five tastes including sweet, sour, bitter, astringent and pungent but "sour and astringent" are most dominant.[11] The plant contains tannins, alkaloids and phenols but fruit contain 28% of the total tannins. It contains most of vitamin C than any food; fruit juice contains 478.56mg/100ml which is more than citrus fruits and used as rejuvenating herb.[12]

The fruit primarily contain carbohydrates and amino acids, phytochemicals like Gallic acid, flavonoids and alkaloids. Amla is used as a laxative, diuretic, anti-pyretic, hair tonic, and ulcer preventative in the Ayurveda school of medicine. Analgesic, adaptogenic, and anti-cancer characteristics have also been discovered via research and studies.[13]

It also possesses anti-oxidant, anti-inflammatory, and free radical scavenging properties. It aids in regulating blood sugar level, maintains functioning of liver, lowers the cholesterol, strengthen the functioning of brain.

Sweetness preference could encourage our ancestral primates to choose energy dense foods and foods hunger can be avoided by storing extra calories in the body. Recently in the world of food and nutrition, there is a trend of using artificial sweeteners. Consumers are facing with a range of contrasting views and studies on artificial sweeteners' effectiveness, safety and security as they may lead to migraines or headaches, stress, weight gain, liver and kidney problems, muscle dysfunction. Use of natural sweetener is safe and don't cause health issues. One such natural sweetener is *Stevia rebaudiana. Stevia rebaudiana* is a tiny perennial shrub contains Steviol glycosides (mainly stevioside and rebaudioside), which are 250-300 times sweeter of sugar, are active ingredients and are heat-stable, pH-stable and not fermentable.[14] Glycosides in stevia is not metabolized by body so contains zero calories. In comparison to sugar, stevia has slower onset and longer duration.[15]

Recently, food scientists and nutritionists are trying to use the herbs in developing food products which can be consumed as part of our routine diet. These herbs can be used to make ready to serve beverages, juices, jellies, jams, marmalades, biscuits, muffins, tea powder etc.[16]

Herbal jam manufactured using i.e., *Moringa oliefera and Emblica officinal* contain high concentrations of nutrients, minerals and vitamins. The attractive feature of the jam is its "low caloric property" as sugar is replaced by natural sweetener, "*Stevia rebaudiana".* The main herb based ingredients made it healthy and nutritious while use of Stevia enhanced its health benefits.

Hence, the objective of this study was introducing innovative idea to develop an immunity boosting herbal jam having beneficial role against Covid-19 as fruit based jams like mango jam, orange jam and strawberry jam etc. are common in the market.

Materials and Methods

The whole study includes; collection of raw materials (Moringa, Amla, Stevia, Pectin, Citric acid), preparation of jam, study of physico-chemical and chemical characters and sensory evaluation executed at research lab of Chemistry Department, UET, Lahore.

The selection of materials was based on their therapeutic effects. Amla was used as base of the jam, moringa leaves powder added in the jam having compounds like apigenin that boost up the immunity. Stevia was added to make it low caloric and suitable for diabetic patients while a control was run with same ingredients, using sugar instead of stevia. Pectin, a gelling agent, was added to give texture and helps in setting of jam. Citric acid was used to enhance the flavor and preservative properties of the jam and helps to avoid microbial growth.

Preparation of Jams

Ripen, healthy and fresh amla fruit of uniform shape and color was selected from local market of Baghbanpura, District Lahore. The fruit was cleaned & washed to remove impurities. The fruits were blanched in hot water for 5 minutes to soften and to stabilize the color. The fruit pulp was prepared after removing seeds.

Fresh Moringa leaves stripped off from leaf petiole, washed in clean potable water to remove dirt. To remove microbes, leaves were soaked into 1% saline solution (NaCl) for 5 min. Leaves were then further washed with 70% ethanol followed by washing twice with distilled water. Leaves were dried in a well ventilated room and milled in a stainless steel hammer mill. The leaf powder was sieved through 0.5mm-1.0mm pore size screen.[17]

Stevia powder available in the market was added as sugar replacer in the jam and cooked until desired consistency. A control jam was prepared with same composition using sugar instead of stevia.

All the ingredients were mixed in the given proportion as shown in the Table 1 and Table 2 for jams. Cooked it with continues stirring until desired consistency was obtained.

The jams were cooled and stored in hot sterile glass bottles.[18]

Table 1: Recipe of Amla-Moringa Jam with Sugar (Control Jam).

Ingredients	Quantity in Grams (g)
Amla	300g
Moringa leaves powder	10g
Sugar	250g
Citric acid	1g
Pectin	2g

Table 2: Recipe of Amla-Moringa Jam with Stevia (Optimized Jam).

Ingredients	Quantity in Grams (g)
Amla	300g
Moringa leaves powder	10g
Stevia	40g
Citric acid	1g
Pectin	2g

Endpoint Determination

When the jam has reached a suitable consistency, dip a spoon into it so that the jam slides off the spoon's side. The jam has reached the end point if, upon cooling, it falls off as a unit rather than freely flowing in a single stream. By spreading a small amount of jam across a dry sheet or the jar cover's surface, then quickly flip the sheet or cover over. The finish point has been achieved if it doesn't run off.[19]

Proximate Analysis

Samples of both jams were evaluated for following parameters such as proximate analysis, physico- chemical analysis, microbial analysis and sensory evaluation.

Determination of Moisture Content

To determine moisture content of samples, AOAC (2000) method was followed.

An empty dish with lid was dried in an oven at 105ºC for approximately 3 hours before being moved to a desiccator to cool and weigh. Spread 3g of jam sample evenly throughout the plate. Place the dish with the sample in the oven for 3 hours at 105ºC to dry. Then, to cool, placed partially covered dish in the desiccator.

Moisture (%) = $W_i - W_f / W_i$
W_i = Weight of sample before drying
W_f = Weight of Sample after drying

Determination of Ash

A crucible with a cover was placed in a furnace at 550ºC overnight to allow impurities to settle on the crucible's surface and cool. In a crucible, 5g of sample was weighed and heated it over a low Bunsen flame with a half-covered lid. Placed it in the furnace at 550ºC overnight when there are no more smells. Placed it in a desiccator to cool. When the sample turned grey, following formula was used to weigh the ash, crucible, and lid;

$$\text{Ash (\%)} = \frac{W2-W1}{W} \times 100$$

Where,
W = Weight of sample
W_1 =Weight of Sample + Crucible after ashing
W_2 = Weight of Sample + Crucible after ashing

Determination of Protein

The protein content of the jam was determined using the Kjeldahl method, as recommended by AOAC (2000).

In a digestion flask, 0.5-1.0g sample was taken. 5g catalyst and 200ml conc.H_2SO_4 added to the mixture. A blank without a sample was run using the ingredients listed above. The flask in an inclined position was placed and heated the solution gently until it clears. When the mixture cooled, carefully poured in 60ml distilled water. The flask was connected to the condenser's digesting bulb right away, with the tip of the condenser immersed in acid and 5-7 drops of mix indicator in the receiver. The flask was heated and rotated until all of the NH_3 distilled. Then, the receiver was removed, condenser tip was cleaned, and used a regular NaOH solution to titrate the excess acid.

% Nitrogen content = (S-B) × 0.1 N × 14.01 × 100 / weight of sample
% Protein = %N × 6.25

Here:
6.25 is a nitrogen factor that was used to get the protein content
S = titer value of sample
B = titer value of blank

Determination of Fat Content

Fat content of the jam was measured using Soxhlet apparatus as per AOAC, 2000.

3-5g sample was taken and wrapped in a filter paper. Transferred it to the extraction thimble and Soxhlet. The bottle was filled with around 250 mL of petroleum ether and placed it on the heating mantle. Turned on the heating mantle after attaching it to the Soxhlet apparatus and turning on the water to cool. It was heated for around 14 hours. The solvent was evaporated with a vacuum condenser, incubate until sample evaporates bottle dried and transferred to desiccator.

$$\text{Fat (\%)} = \frac{W2-W1}{W} \times 100$$

W = Weight of sample
W_1 =Weight of empty beaker
W_2 = Weight of empty beaker + ether extract

Determination of Fiber Content

A round bottom flask was filled with 200ml of 1.25 percent H_2SO_4 and 2g of weighed sample. Filter it through filter paper using a hot plate that has been boiling for 30 minutes. To remove the acid, washed the remnants with hot distilled water. Then either transferred the contents to a beaker and add 200ml of 1.25 percent sodium hydroxide, or digest for half an hour. To remove the alkali, washed it again with hot distilled water. The leftovers were then moved to crucibles, weighed, and dried overnight at 105ºC in a hot air oven before being sent to a muffle furnace for 3 hours at 550-600ºC. The weight loss following burning represents the fiber content of the sample. The crude

fiber content as a percentage was computed as follows:
% Crude Fiber = Loss in weight / Sample weight × 100

Determination of Ascorbic Acid

A titration approach using a 2, 6- dichlorophenol indophenol dye solution was used to quantify the quantity of ascorbic acid in the samples. The 2, 6 dichlorophenol indophenol dye is reduced to a colorless state for testing using ascorbic acid in an alkaline solution. In order to calculate the dye factor, the dye solution was first normalized against normal ascorbic acid. The dye solution was titrated against the phosphoric acid extract until a pink hue developed, which lasted only a few seconds. The following formula was used to calculate the amount of ascorbic acid in each mL:
Ascorbic acid % = Titer volume × dye factor × volume made up/ aliquot of sample taken × vol. of sample × 100
Dye factor = 0.5 / titer volume

Analysis of Physico-chemical Properties
Determination of pH

pH of the jam was measured by pH meter. Electrodes of the pH meter was calibrated by dipping into Buffer solution (7.0). Sample solution was prepared in distilled water. Dipped the calibrated electrode in to sample solution. Reading on pH meter was noted.

Determination of Titrable Acidity

The AOAC technique was used to determine the sample's titrable acidity (2000).
Before being titrated with 0.1 NaOH and phenolphthalein as an indicator, the sample was diluted to 20ml with distilled water. The ultimate result was a pink color that lasts a few seconds, allowing you to calculate the titrable acidity as follows:

$$\text{Titrable Acidity \%} = \frac{\text{volume of titrant} \times N \times 90}{\text{weight of sample} \times 1000} \times 100$$

B = Burette reading
W = Weight of Sample

Determination of Total Soluble Solids (TSS)

5g of sample was weighed and added 100-140 ml of distilled water. Then heated for 2-3 minutes, stirrer it, cooled and mixed thoroughly. After 15-20 min, filtered it through filter paper and weighed 0.01g sample. The filtrate was used to determine TSS of sample using hand refractometer (0-80º Brix).

Sensory Analysis

For sensory evaluation of jam, 9-point hedonic scale was used which widely used for measuring food acceptability. A taste testing panel (15 individuals) evaluated the product by scoring hedonic scale, 1to 9 ranging from "dislike extremely" to "like extremely".

Microbial Analysis
Total Plate Count (TPC)

To analyze the microbial quality of the jam samples, 1ml of diluted sample was transferred into sterile plates. Then transferred sterilized media into petri plates and allowed them to cool and solidify. Incubated the plates in an incubator in inverted position at 37ºC for 48 hrs.

Yeast and Mold Count

After 48 hours, number of colonies were counted and recorded results of bacterial count. For fungal count, place the plates in an upright position for up to 5 days at 25ºC. Colonies were counted and expressed as cfu/g.

Shelf life Study

Active compounds present in fruits may largely reduce during processing and storage. Studies have been conducted relevant to texture and sensory assessment of jam. Physico- chemical stability and sensory profile at different time interval was evaluated.

Energy Calculation

The caloric energy value of the jam was calculated using following formula;
Energy Kcal = 100 - (Carbohydrates% × 4) + (total fat% × 9) + (total protein % × 4)

Statistical Analysis (ANOVA)

Standard deviation and analysis of variance were used to assess the efficiency of the data gathered and its importance in relation to different elements, as well as to examine the variation in the means of all the specified parameters. The results obtained were represented by P ≤0.05 as a criterion for determining the significance of differences. An attempt has been made to develop a nutritious and healthy and immunity boosting functional product against COVID-19, also best for diabetic patients as well as for weight maintaining persons. The final products as shown in Figure 1 and Figure 2 was subjected to characterization, proximate analysis, microbial analysis which concluded that optimized jam containing stevia has almost the same composition but with low calories i.e., 150kcal as compared to jam based on sugar having high calories i.e., 265.67kcal. Sensory evaluation shows more acceptance of control jam as sugar gives a good texture and taste and glossiness to the jam.

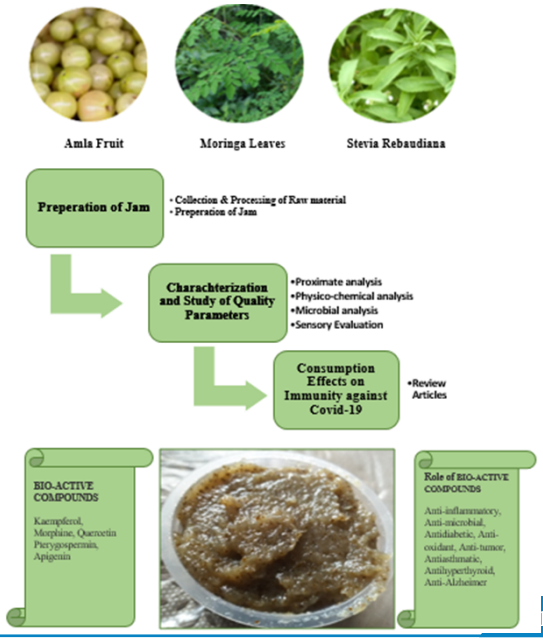


Figure 1: Graphical Representation.

Figure 2: Amla-Moringa Jam with Sugar.

Proximate Composition

The proximate composition of Amla-Moringa jams incorporated with sugar and stevia were analyzed and the results are as shown in the Table 3.

Table 3: Results of Proximate Analysis (Control Jam & Optimized Jam) w.r.t Shelf Life Studies.

Parameters	Control Jam			Mean	Optimized Jam			Mean
	T1	T2	T3		T4	T5	T6	
Moisture	34.2±1.7	32.98±1.6	32.08±1.5	33.0±1.06	62±1.4	61.5±1.3	60.9±1.5	61.9±0.70
Ash (%)	1.41±0.03	1.33±0.04	1.26±0.02	1.33±0.07	1.18±0.03	1.13±0.05	1.09±0.07	1.15±0.04
Protein (%)	1.35±0.03	1.43±0.05	1.5±0.01	1.42±0.07	1.72±0.02	1.67±0.04	1.59±0.01	1.66±0.06
Fat (%)	0.74±0.03	0.69±0.07	0.63±0.04	0.71±0.09	0.59±0.01	0.54±0.09	0.51±0.3	0.57±0.10
Crude Fiber(%)	1.51±1.1	1.71±1.7	1.63±1.4	1.67±0.10	1.21±0.06	1.42±0.9	1.31±0.3	1.36±0.10
NFC	62.3	63.57	64.53	63.4±1.11	34.51	35.16	33.91	34.52±0.62

T1, T4= 0 Days; T2, T5= 15 days; T3, T6= 30 Days
The values are expressed as mean ±SD.

Figure 3: Amla-Moringa Jam with Stevia.

Table 4: Results of Physico-chemical Parameters (Control Jam & Optimized Jam) w.r.t Shelf Life Studies.

Parameters	Control Sample				
	T1	T2	T3	Mean	
pH	4.00±0.04	3.97±0.06	3.9±0.06	4.09±1.06	4.
Titrable Acidity(%)	0.61±0.04	0.66±0.03	0.69±0.06	0.63±0.04	0.
Total Soluble Solids (ºBrix)	63.5±1.2	64.2±0.03	65.5±0.06	64.8±0.04	2.
Ascorbic Acid (mg/100g)	245±4.80	239±4.9	234±5.01	236.5±5.5	2

T1, T4= 0 Days; T2, T5= 15 days; T3, T6= 30 Days, The values are expressed as mean

The moisture content is an important parameter as it affects

the freshness and shelf life of the jam. High contents of moisture reduces the shelf life of product. It was observed that there was decrease in moisture content during storage may be due to reopening of the jar for sample analysis or due to permeability of packaging.[20]

Ash content represents the mineral content of the food products, obtained after destruction of organic matter. The decrease in ash content refers the reduction of mineral content. It was observed that decrease in ash content may be due to the increased microbial activities by using minerals for growth.[21]

Protein content of the control and sample jam was determined by Kjeldahl method**.** Similar observations were found by AH Reddy and V Chikkasubbanna while studies on the storage behavior of amla jam.[22]

The results obtained from fat determination using Soxhlet extraction method are as: The mean value obtained from the results for control jam was 0.71 % in optimized jam was recorded 0.57%.

The percentage crude fiber and NFE content of the optimized jam and control jam were also determined**.**

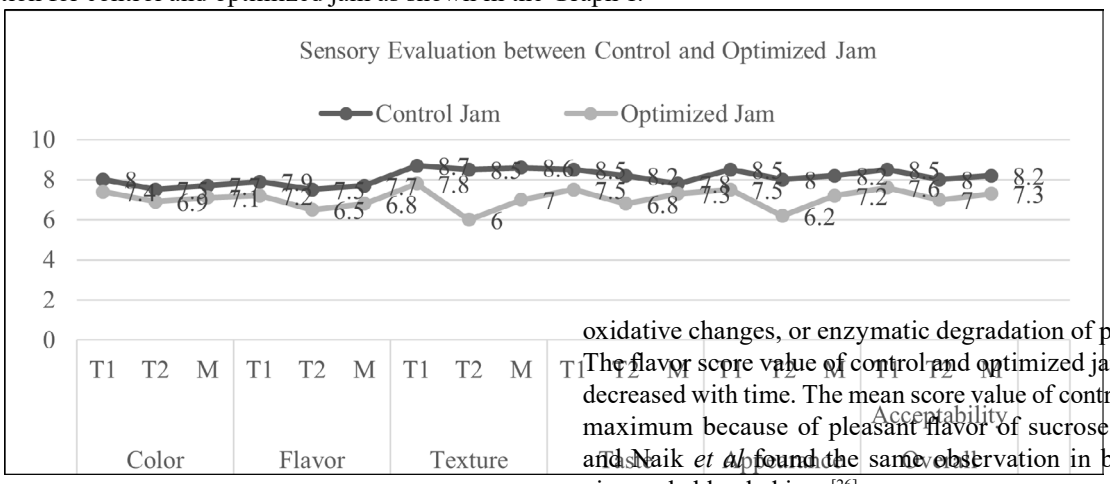
Physico-Chemical Parameters

pH is an important factor in jams that play role to obtain optimum gel condition. The mean value obtained from the results for control jam was 4.0 and pH in control jam was recorded 4.19. Decrease in pH may be due to the degradation of sugar content or may be due to hydrolysis of pectin. Ehsan *et al*. also reported similar observation in grape fruit apple marmalade.[23]

The mean value obtained from the results for control jam was 1.35 and pH in optimized jam was recorded **0.6.** The increase in the values of titrable acidity was noticed, might be due to degradation of polysaccharides resulting the formation of organic acids. Wasif S *et al* observed increase in acidity of apple and olive blended jam.[24]

Sensory Evaluation

Different parameters of jam were evaluated on the basis of 9-point Hedonic scale. Following are the results of sensory evaluation for control and optimized jam as shown in the Graph 1.



Graph 1: Results of Sensory Evaluation for Control and Optimized Jam.
T1 = 0 days; T2= 30 Days; M=Mean

Color of the control jam had maximum color score value because it contained sugar that gives glossiness to the jam. The color of optimized jam was changed with time during storage. These changes were may be due to ascorbic acid degradation, and polymerization of color pigments. Khan *et al* found decreasing trend in apple jam during storage.[25]

The flavor of both jams was decreased, may be, due to oxidative changes, or enzymatic degradation of phenol. The flavor score value of control and optimized jam was decreased with time. The mean score value of control was maximum because of pleasant flavor of sucrose. Patel and Naik *et al* found the same observation in banana pineapple blended jam.[26]

The texture of control jam was not so affected with time but there was a major reduction in texture of optimized (low calorie) jam. The texture of freshly prepared control and optimized jam was reduced. The texture of jam is dependent on network developed by pectin with sugar and acid. In optimized jam, stevia did not form the network with pectin or may be pectin undergoes to hydrolysis. Abolila *et al* found there was decrease in the textural properties of low caloric orange jam.[27]

The appearance of the jams was reduced may be due to storage conditions or reactions occurs in jam. Bhople *et al* found similar observation studying the effects of storage on organoleptic properties.[28]

The overall acceptability was reduced due to change in texture, color and flavor. Muhammad *et al* found similar observations in diet apple jam.[29]

Optimized Sample			
T4	T5	T6	Mean
.22±0.06	4.16±0.07	4.09±0.06	4.19 ±0.06
.32±0.04	0.36±0.05	0.42±0.06	0.35±0.05
5.0±0.04	26.5±0.05	28±0.06	27.2±0.05
28±4.10	223±3.99	219±4.1	221±4.5

SD.

Microbial Analysis

The microbial load of the Amla-Moringa jam in both control and optimized was analyzed and the results were as shown in Table 5:

Table 5: Results of Microbial Analysis at Different Time Intervals.

Treatments	Stor	
	0days	1
Sugar based Amla-Moringa Jam	0	
Stevia based Amla Moringa Jam	0	5×10^3	13×10^3

The fresh sample was free from any microbial activity but in the samples analyzed on 15th day and 30th day of storage shows increase in the yeast and mold count. But the results ranged in acceptable limits. The control jam also shows growth on media.

Nutritional Value and Energy Calculation of Jams

Nutritional facts were studied as shown in Table 6 & Table 7:

Table 6: Energy Calculations Amla-Moringa Jam with Sugar (Control Jam).

Calories Per 100g	265.67kcal
	%D.V
Total Fat	0%
Total Carbohydrates	26%
Dietary Fiber	5%
Protein	3%
Vitamin C(mg/100g)	134

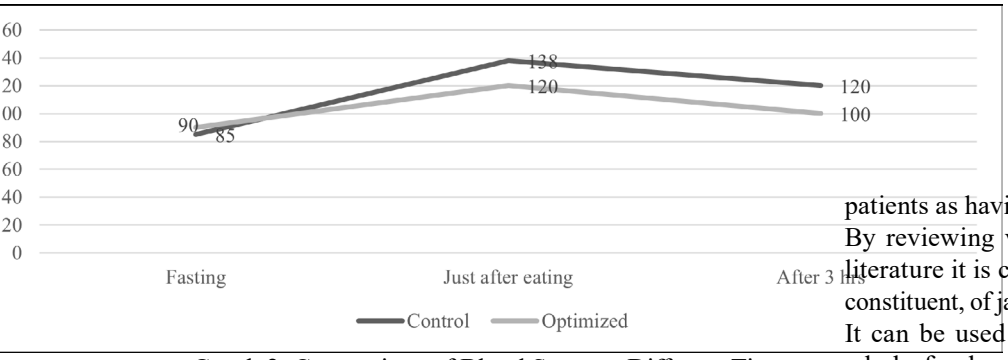
Table 7: Energy Calculations Amla-Moringa Jam with Stevia Sweetener (Optimized Jam).

Calories Per 100g	150kcal
	%D.V
Total Fat	0%
Total Carbohydrates	24%
Dietary Fiber	5%
Protein	3%
Vitamin C(mg/100g)	134

Consumption Effects of Jams on Blood Sugar Level of Diabetic Patient

A study was conducted to check the effects of control (sugar based jam) and optimized jam (based on stevia) to check the effects on blood sugar level. Both the jam samples were consumed by diabetic patient, in different intervals, and sugar level was checked on fasting, just after consumption and 3 hrs. after eating.

There was a significant difference in the blood sugar level of diabetic patient as shown in the graph 2.



Graph 2: Comparison of Blood Sugar at Different Time Intervals.

Summary and Conclusion

The investigation was based on the development of immunity boosting low caloric jam based on herbal ingredients (Amla fruit and Moringa leaves powder) and Stevia as sugar replacer, a healthy and nutritious product against COVID-19. The product can be utilized by diabetic patients as having low caloric values.

By reviewing various journals and the availability of literature it is concluded that Moringa oliefera, a major constituent, of jam has medicinal and nutritional potential. It can be used as immunity stimulator as it contains whole food source of vitamins, proteins, minerals, phytochemicals and amino acids that keeps our body healthy against COVID-19 patients.

Conflict of Interest

The authors have no conflicts of interest to declare. All co-authors have seen and agree with the contents of the manuscript and there is no financial interest to report. We certify that the submission is original work and is

not under review at any other publication.

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