SALT JOURNAL OF SCIENTIFIC RESEARCH IN HEALTHCARE
Oct 2024, Vol 4, Issue 1, Page No. 01-06

www.saltjsrh.in
ISSN 2583-3936 (Online)
https://doi.org/10.56735/saltjsrh.ms2404020106

# ANTIMICROBIAL AND ANTIOXIDANT PROPERTIES OF FERMENTED GUAVA JELLY

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**Article Type:** Research Article

**Article Citation:** Mayuri Rastogi, Bushra Shaida, Vandana Singh, Saleem Siddiqui. Antimicrobial and antioxidant properties of fermented guava jelly. SALT J Sci Res Healthc. 2024 Oct 04; 4(2): 01-06.

**Received Date:** Sep 20th, 2024
**Accepted Date:** Oct 03rd, 2024
**Published Date:** Oct 04th, 2024

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## ABSTRACT

The dietary diversity and increased food scarcity demands sustainable food system worldwide. Guava are widely cultivated throughout the tropical region and known for its significant nutritional contents. The present study was conducted with aim to develop a nutritionally rich fermented guava jelly to increase the antioxidants and antimicrobial properties of innovative food product. The pasteurized Guava pulp with sucrose was fermented with *Lactobacillus brevis* strain KU15152, for 72 hours, at 37°C. and further cooking was done followed by solidification of fermented guava jelly. The proximate analysis including moisture, ash, carbohydrate, protein and fats, along with functional properties such as antioxidants and antimicrobial was estimated of prepared fermented guava jelly. The results showed the presence of moisture (24.6%±1.20) in permissible limit. Ash and protein content was increased in comparison to fresh guava due to fermentation, while the total carbohydrate was decreased. The antioxidant potential has been increased after fermentation and demonstrates 20 µg/ml jelly extract showed potential radical scavenging activity, when compared with ascorbic acid. The antimicrobial properties showed a significant inhibition against gram positive and gram-negative bacteria. Thus the obtained results indicate that, *Lactobacillus brevis* strain KU15152 can be used as formulation of novel food product.

***Keywords:*** Antioxidants, antimicrobial, fermentation, lactobacillus, proximate analysis

## INTRODUCTION

Recent studies have showed the potential benefits of dietary modifications in addressing global issues like food scarcity, decreased biodiversity, and global warming. Researches conducted in developing countries suggest, the 70-80% less meat consumption and more vegetables and plant-based foods, will leads to sustainable food system[1]. Fruits and vegetables play a significant role in agricultural cropping systems via nitrogen fixation[2]. India is the fourth producer of Guava (*Psidium guajava*) worldwide and are grown all over the country. It is the cheapest and richest source of vitamin C and antioxidants that varies as per the area of cultivation. Since this is the perishable foods, and a large quantity is produced in India, a cost-effective method is required to preserve the fruit for future[3]. Although numerous guava products has been synthesized so far such as juices, jams, marmalades and jellies however, this accounts for 40-50% fruit wastage in terms of peel, seeds, pulp and lacking micronutrients[4].Thus, there is a need to utilize optimum fruit pulp and produce a healthy fruit product with special nutrient content.

Fermentation is one of the traditional and safe method to incorporate nutrients and enhance organoleptic properties of new product. For fermentation, generally recognised as safe (GRAS) microorganism like, Lactobacillus, pseudomonas, Propionibacterium, Saccharomyces etc are used by food industries to prepare innovative foods[5].The probiotic bacteria, uses carbohydrate as source of energy and convert it to lactic acid and acetic acid. Fermentation, using Lactobacillus (LAB) bacteria have been a successful practice that increase palatability, protein quantity and quality, and improve human immune system[6].

The fruit pulp jellies are widely enjoyed among children and adults. It is prepared by combing sugar and jellying agent with other ingredients and bring to the boil till sugar melts. Further, fruit flavour and colouring agents are added. The mixture is poured into the molds and solidified product are used as jelly. The present study aims to develop the guava pulp jelly fermented with *Lactobacillus brevis* strain KU15152, and further evaluate its proximate, antioxidant and antimicrobial properties.

# MATERIALS AND METHODS

## Procurement of Raw Material and Chemicals

The experiment was conducted in laboratory of Department of food Science and Technology, Sharda University, Greater Noida, India, during 2022-23. The fresh, ripe, undamaged, guava (*Psidium guajava*) was obtained from local market of greater Noida, India. The other materials such as, sucrose, glucose, citric acids, and chemicals were procured from used analytical grade. Guava were washed manually under tap water, cut in small pieces and blended to a thick pulp in mixer grinder. The pulp was sieved with muslin cloth to remove seeds and deseeded pulp packed in sealed glass bottles and stored at 4ºC for further use.

## Preparation of Inoculum

The pure bacterium culture of *Lactobacillus brevis* strain KU15152 in lyophilized form was procured from national Chemical Laboratory, Pune, India. The culture was re-activated in 200mL MRS broth, for 24 Hr at 37ºC. After repeated inoculum preparation, the broth culture was centrifuged and pellets were washed thrice with normal saline (0.85% NaCl) and cell count were measured at wavelength 600mn.

## Guava Pulp Fermentation and Preparation of Guava Jelly

500g Guava pulp with 50% sucrose and 200 mL distilled water was pasteurized under aseptic condition. The pH was maintained at 6 by adding 0.1N NaOH. After cooling the pulp, bacterial culture of 1.1 to 1.3 $\times 10^8$ CFU/g were added in guava pulp mix under aseptic conditions. The glass bottles were sealed tightly and kept static for fermentation for 72 hours at 37 ºC.

After 72 hours, the fermented guava pulp was used for jelly preparation. The fermented pulp was poured in steel container and added 20% sucrose, 10% glucose solution, 2.0g citric acid and 5g food grade Agar Agar to the mixture. The pulp was bring to boil for 5minutes with continuous stirring and poured immediately in silicon mold for cooling and solidification. The jellies were sprinkled with starch powder to prevent sticking followed by packed separately with butter-paper and stored in air tight sealed container.

## Analytical Determinants

### Proximate Analysis

Proximate analysis include determination of Ash, Moisture, protein, crude fats, and total carbohydrate were quantified as per the AOAC (2019) methods[7].

### Functional properties

Antioxidants Determination: Total antioxidants were analysed by 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radical scavenging activity[8], with modifications. A total of 18 milligrams of DPPH was dissolved in 80 ml chilled methanol to prepare stock solution. 100microliter of prepared sample extracts were dissolved in 3 mL DPPH stock solution and kept for 30 minutes in dark at room temperature. 100 microliter of stock solution was mixed with 3mL methanol, which served as standard. The final absorbance was checked at 517 nm comparable to control. Absorbance was recorded and calculation was done from following formulas:

$$\%\ of\ Antioxidant\ activity = Absorbance\ of\ Control - Absorbance\ of\ sample \times 100 \div Absorbance\ of\ Control$$

## Antimicrobial Properties

The antibacterial properties of prepared guava jelly was estimated by diffusion well plate agar method[9] The following microorganism were used for the study: *Staphylococcus aureus, Escherichia coli,* and *Pseudomonas aeruginosa.* Nutrient agar petri plates were prepared in aseptic conditions and 0.1ml of inoculum were evenly spread on nutrient agar plate. A sterile cork border of 8mm in diameter was used for making wells in agar plates. 100µL of fermented fruit jelly extract was poured into the well and kept to incubation at 37°C for 24 hours. The zone of inhibition was checked in millimetre (mm) and analysed with respect to each other.

## Sensory Evaluation

The fermented guava jelly was placed for sensory evaluation from 25 semi trained panel members with age between 18-40 years. The 9 point hedonic scale was used where, every point had its significance, such as, 9 for extremely liked, 8 was for liked very much, 6 for

liked slightly, 5 for neither liked nor disliked and 1 for extremely disliked.

### Statistical Analysis

All the analysis was done in triplets and data were recorded in excel sheet. The data was presented in Mean ± Standard Deviation and analysed by one way ANOVA, using SPSS version 22.0.

## RESULTS

### Proximate composition

The proximate analysis results have been shown in Table 1. The moisture content in fermented guava jelly was 24.6%±1.20, which was in ideal limit of prepared fruit jelly. The fresh guava pulp contains approximately 94% moisture and the loss in moisture in fermented jelly is due to heating process[10]. The duration of cooking also possesses a great impact on moisture content and thus shelf life of ready product[11]. Rana et al[12], has reported 26.78% to 29% moisture in pineapple coconut jam on different variations. Furthermore, Akelom et al[13], reported 31.5% moisture in cactus fruit jelly.

Ash content in fermented jelly was report at 3.05%. and it was increased from fresh guava ash content. Fruits and vegetables are poor sources of proteins, however, during fermentation, the protein content was increased to 4.02g/100mL, which was found a good source of proteins. Jellies are good source of carbohydrate, although, during fermentation, some amount of sugars are utilize by bacteria for energy production. Thus, the total carbohydrate in fermented jelly was reported at 70.34%±2.98. Total fats were found to be reduced to 0.64%±0.96.

Table 1: The proximate Analysis of Fermented Guava jelly

| Parameters | Fermented Guava Jelly |
|---|---|
| Moisture (%) | 18.21±1.20 |
| Ash (%) | 1.34±1.89 |
| Crude Fats(g/100g) | 0.12±0.68 |
| Protein (g/100g) | 7.12±0.54 |
| Total carbohydrates (g/100g) | 69.07%±2.98 |

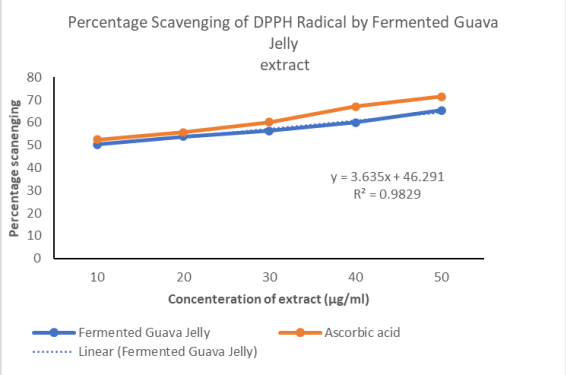
values are presented as Mean ± Standard Deviation

### Functional Properties

**Antioxidant Activity:** The Free radical scavenging activity was checked by DPPH method. After fermentation, the antioxidant activity was recorded at different concentrations and results were shown in Figure 1.

Results indicate that the antioxidant activity of Fermented Guava Jelly was concentration dependent but as at very high concentration activity declines when compared with ascorbic acid. Thus at 20 µg/ml of jelly shows a significant scavenging activity when compared with the standard ascorbic acid.

**Figure 1:** The Antioxidant property of fermented guava jelly




### Antimicrobial

Antimicrobial effect of developed product was studied against different microorganism strains and results are revealed in Table 2. Results indicated that guava jelly was found to be effective against these bacteria i.e. *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* with zone of inhibition 23±0.34mm, 21±1.12mm and 18±0.4mm respectively, while in comparison with standard drug, ampicillin, it was found effective. Thus, the fermented product shows antimicrobial properties and can be more effective against studied microorganisms.

Table 2: Antibacterial activity of Fermented Guava Jelly

| | Zone of inhibition diameter (mm) | |
|---|---|---|
| Microorganism | Fermented Guava Jelly | Ampicillin/ Clotrimazole (Sensitive) |
| Staphylococcus aureus | 23±0.34 | 22±0.93 |
| Escherichia coli | 21±1.12 | 24±0.56 |
| Pseudomonas aeruginosa | 18±0.4 | 21±0.76 |

Each value are presented in Mean ± Standard Deviation

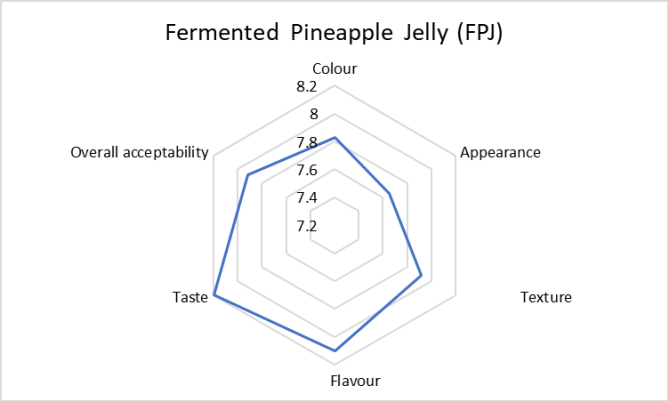
### Sensory Evaluation

The sensory score of fermented guava jelly showed a good acceptability in terms of colour, appearance, texture, flavour, taste and overall acceptability (Table 3). The mean overall acceptability was observed 7.92± 1.23, causing acceptable from panel members.

Table 3: Sensory Attributes of fermented guava jelly

| Sensory attributes | Fermented Pineapple Jelly (FPJ) |
|---|---|
| Colour | 7.83± 0.05 |
| Appearance | 7.65±0.08 |
| Texture | 7.92± 0.15 |
| Flavour | 8.1± 0.56 |
| Taste | 8.21± 0.07 |
| Overall acceptability | 7.92± 0.20 |

Figure 1: Sensory attributes of fermented guava jelly




# DISCUSSION

The moisture percentage in fermented guava jelly was found in advisable limit. In previous researches, the moisture content in fruits jams and jellies varies from 23.5% to 29.8% [14,15,16].The moisture content in present study was found in accordance with these researches. The water present in fruit products, adversely influence its shelf life. Thus, the fermented guava jelly, contain, permissible moisture, which, increases its shelf life. The ash content indicates the mineral and inorganic solids present in products. The previous researches recorded 0.77-0.89% ash content in fresh guava fruit, depending on location and soil nutrients[17]. However, in present study after fermentation, the ash content was increased, which was in line with the results recorded by Shahein[18]. The fermented guava jelly showed a higher ash content, due to evaporation of moisture during cooking and increased concentration of fruit matrixes. The total fats in fresh guava fruit was recorded between 1.2 to 1.6g/100g, while the fats present in fermented guava jelly was reduced to 0.12g/100g. The decrease in fats during fermentation is due to the utilization of oxidized lipids for energy production for growth and metabolic activities[19]. Fruits and vegetables are poor source of proteins. The fresh guava contain protein from 0.9 to 1.2g/100g. Due to addition of viable LAB, the protein content increases to 4.02g/100g. Thus, the recorded protein in fermented jelly is an excellent source of proteins. In the previous researches[19,20], also reported the increased protein in matrixes following LAB fermentation.

The total carbohydrate was reduced following fermentation, due to utilization of carbohydrate by Lactobacillus, for energy production and growth. However, the sensory appeal and acceptability of fermented guava jelly remain unaffected by the addition of glucose and sucrose during the jelly production.

The bioactive compounds present in food stuff is responsible for its antioxidant ability. Regular intake of antioxidants in diet, reduces oxidative stress, neutralized free radicals and boost up the immunity[10].The fermented jelly had showed the potential of antioxidants when compared to ascorbic acids (Figure 1). The present study findings are also supported by previous studies conducted by Hashemi[21] in sweet lime juice fermentation and Adenike et al[22,] during maize fermentation.

Table 2, shows the antibacterial potential of formulated jelly against gram positive *Staphylococcus aureus* and two gram negative *Escherichia coli* and *Pseudomonas aeruginosa* and it shows that, the fermented jelly inhibit E. coli and *Pseudomonas aeruginosa* by 21mm and 18mm diameter respectively, while with *Staphylococcus aureus*, it inhibit 23mm diameter in contrary to Ampicillin (22mm diameter). The zone of inhibition increases with increase in concentration of jelly extract. The jelly showed lowest resistance towards *Pseudomonas aeruginosa*, than other bacterial strain. The higher resistance of *Pseudomonas aeruginosa* is due to the presence of lipopolysaccharide (LPA) layer on outer membrane of gram-negative bacteria[23]. The current study findings are in agreement with of Pereira et al[24], where they claim that the fermented cashew apple juice can control the growth of other pathogenic bacteria. Thus, the guava jelly that has undergone fermentation contains antioxidants and has strong antibacterial activity.

The fermented guava jelly showed a satisfactory acceptability from consumers. Fermentation enhances flavour and taste of prepared product. Therefore, it can be considered as promising approach for healthy processes fruit product formulation.

## CONCLUSION

*Lactobacillus brevis* strain KU15152, which is used in the present study, showed a potential probiotic and demonstrated a useful strain for fermentation and producing value-added fruit product. The study estimates the proximate and functional properties of fermented jelly. the protein content was significantly increased after fermentation and showed a potential antioxidant ability and antibacterial ability. Overall, the study find the *Lactobacillus brevis* strain KU15152 suitable for fermentation and product development.

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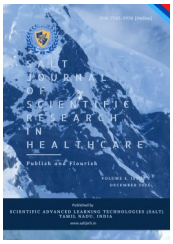

**ARTICLE TYPE:** Research Article; **ORCID ID:** Open Researcher and Contributor Identifier (ORCID) ID of corresponding author: https://orcid.org/0000-0001-6189-5707 ; **ETHICAL:** Institutional ethical committee and prior patient consent obtained; **ACKNOWLEDGEMENT:** The present study was supported by **SEED FUND Scheme (SU/SF/2022/14)** provided by Sharda University and Authors are thankful to the Department of Nutrition & Dietetics and Department of Food Science and Technology, Sharda University for providing authors necessary instruments and infrastructure to conduct research.; **FINANCIAL DISCLOSURE:** The authors declare that there was no financial aid received.; **CONFLICT OF INTEREST:** No conflict of interest associated with this research work.; **AUTHORS CONTRIBUTION:** M.R., Idea and Conceptualization & B.S.,V.S., Data Collection and Editing, S.S., Supervisor of the study.; **AUTHORS AFFILIATIONS:** Ms. Mayuri Rastogi, Ph.D. Scholar, School of Allied Health Sciences, Sharda University, Greater Noida, UP, India & Assistant Professor, School of Allied Health Sciences, Sharda University, Greater Noida, UP, India,; Ms. Bushra Shaida, Assistant Professor, Department of Food Technology, Jamia Hamdard University, New Delhi, India.; Ms. Vandana Singh, Assistant Professor, School of Allied Health Sciences, Sharda University, Greater Noida, UP, India, Dr Saleem Siddiqui, Professor, School of Agricultural Sciences, Sharda University, Greater Noida, Uttar Pradesh, India.; **CORRESPONDING AUTHOR EMAIL:** saleem.siddiqui@sharda.ac.in ; **ARTICLE CITATION:** Mayuri Rastogi, Bushra Shaida, Vandana Singh, Saleem Siddiqui. Antimicrobial and antioxidant properties of fermented guava jelly. SALT J Sci Res Healthc. 2024 Oct 04; 4 (2): 01-06.