

Original Research

From Herb to Dessert: Unveiling *Swertia chirata*'s Impact on Orange Jelly Dynamics

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ABSTRACT

Background: Foods had a distinctive medicinal role as a remedy for illness for many centuries. The threat of Antimicrobial Resistance (AMR) in all over the world is growing extremely due to the reason that any use of antimicrobials, however appropriate and justified, results in accumulation of resistance. Thus, researchers all over the world are toiling hard to introduce foods that possess therapeutic effects as medicine. Citrus fruits were the first foods to provide a true cure of any illness. The present study was undertaken to develop a value-added product that was hypothesized to bestow numerous dietary properties, to meet the ever growing demands of the consumers. **Methods:** A strategy was developed to formulate good quality jelly from Orange & *Swertia chirata*. To develop the jelly different proportions of orange juice & *S. chirata* namely T1 (95:05), T2 (90:10), T3 (85:15) was formulated and T0 was utilized as control (100% orange jelly). In addition to microbial analysis, phytochemical analysis, physicochemical properties, and sensory evaluation were conducted. **Results:** The physicochemical properties of the formulated jellies were in alignment with previous studies. The results of the microbiological analysis were excellent. As, there were no detectable levels of *E. coli* and *Salmonella* in the formulation for a period of up to 2 months. The sample T3 exhibited maximum level of total phenolic content (168.3 mg GAE/gm), total flavonoid content (49.1 mg QE/gm) and antioxidant activity (135.63 mg/100gm). Therefore, the bioactive compounds had a high precedence on the sample T3 than the control one. **Conclusion:** After evaluating all the factors, it can be concluded that creating a value-added product will make it easier to market a product that not only attracts consumers but also possesses concealed medicinal properties.

Keywords: Antimicrobial resistance; citrus; value-added product; *Swertia chirata*; physico-chemical; phytochemical evaluation

1. INTRODUCTION

A big risk to people's well-being is the development of resistance to antimicrobial.⁽¹⁾ Antimicrobial resistance (AMR) poses a significant risk to the well-being of the general population, leading to significant challenges in preventing and treating chronic illnesses. The primary factors contributing to this problem are the inappropriate and excessive use

of antibacterial medicines.⁽²⁾ Functional food can combat antimicrobial resistance by decreasing the utilization of antimicrobials in animals, so mitigating the emergence of foodborne bacteria that are resistant to these drugs and can potentially be passed to humans as contaminants in food.⁽³⁾ Functional food refers to a type of food that goes beyond providing basic nutrition and offers additional benefits due to specific components that provide physical or biological advantages.⁽⁴⁾ Furthermore, functional foods have a significant impact on promoting a healthy lifestyle and mitigating the risk factors associated with a range of diseases, including cancer, cardiovascular diseases, gastrointestinal tract disorders, and neurological diseases.⁽⁵⁾

Humans have been consuming citrus fruits since prehistoric times. Citrus fruits were once used to treat digestive ailments, skin diseases, gout, scurvy, poison, and plague outbreaks.⁽⁶⁾ Citrus fruits are among the most widely consumed fruits, second only to mangoes, tomatoes, and bananas.⁽⁷⁾ The unique flavor and health benefits of these fruits explain their high consumption. Without cholesterol or sodium (Na), they are low in fat. Furthermore, no health risks have been reported.⁽⁸⁾ They comprise a sufficient quantity of vitamin C, fibre, potassium, folate, calcium, thiamin, and niacin.⁽⁹⁾ There are many secondary metabolites, including alkaloids, flavonoids, phenolic acids, carotene, coumarins, and essential oils. These constituents exhibit anti-cancer, anti-inflammatory, antioxidant, and cardiovascular protective properties. Furthermore, citrus fruits have been utilized as traditional medicinal herbs in a number of Asian nations, including China, Japan, and Korea.⁽¹⁰⁾ The seasonal availability and short storage life of citrus necessitate the identification and production of citrus-derived products (such as jam, jelly, and marmalade) that preserve dietary micronutrients and bioactive compounds.⁽¹¹⁾

Swertia chirata (*S. Chirata*), a herbaceous plant classified within the Gentianaceae family, is widely recognized and utilized in traditional medical systems such as Ayurveda, Siddha, Unani, and others.⁽¹²⁾ There are about 135 species of *Swertia* in the world, and 40 of them are native to Indian subcontinent. Out of these, Chirata is known for having the most important health benefits. As a result, it can be used to treat a wide range of illnesses, such as skin problems, high blood pressure, malaria, gastritis, sores, liver problems, and anemia.⁽¹³⁾ Bioactive substances like anthocyanins, flavonoids, and polyphenols have been shown to have antioxidant

qualities.⁽¹⁴⁻¹⁶⁾ This plant, *Swertia chirata*, is a herb that is very high in flavonoids, anthocyanins, and polyphenols.⁽¹⁷⁾ Pharmacological studies on *S. chirata* have been initiated due to its diverse ethnobotanical uses. Previous research has evaluated *S. chirata*'s pharmacological properties, including antibacterial, antifungal, anti-hepatitis B virus, anti-inflammatory, hypoglycemic, and antidiabetic properties, using various in vitro and in vivo test systems.^(12,18-24) Due to its harsh flavor, no research has been done on adding this herb to our diet. Only adding this herb to a food product that covers its awful taste while keeping its nutritional value will remedy this. Formulating food products with *S. chirata* may provide a natural supply of bioactive chemicals with health benefits and traditional uses.

Therefore, this study aimed to develop a confectionery product that appeals to both infants and adults, who have a notable preference for confectionery items⁽²⁵⁾ that include orange and *S. chirata*. The study also aimed to assess consumer approval of this product.

2. METHODS

2.1 Sample Accumulation

All raw materials used in this experiment were purchased from local supermarket. The dried *S. chirata* powder (50 gm) and oranges (5 Kg) were bought from Chawkbazar, Chattogram, Bangladesh. In addition, sucrose (commonly known as sugar) was collected in anticipation of the study. Throughout the process, a digital refractometer, agar agar powder, a digital glass pH meter, a weighing balance, a knife and plates were all used.

2.2 Formulation of Extracts

To obtain fruit extract, orange fragments were pulverized in a mixer grinder. Subsequently, the liquid was filtered and preserved for subsequent utilization. A *S. chirata* solution was prepared by combining 2 grams of *S. chirata* powder with 100 milliliters of boiling water. After letting it to steep, the water was cooled, filtered through muslin linen, and kept for later use. The final juice extract was produced following proportions used by⁽²⁶⁾ with slight modifications by combining orange juice and *S. chirata* extract in the following ratio exhibited at Table 1. Later, the highest level of performance was recorded.

2.3 Preparation of Jelly

Jelly was prepared according to Srivastava and Singh⁽²⁷⁾ with necessary modifications. Oranges that were just picked were cleaned, peeled, and their seeds were taken out. After that, it was filtered and mixed. The ground *S. chirata* was put in a different pan, mixed with hot water (1:20), and then it was filtered. Then, the two mixes were mixed together in the way that was described before. The comparison sample was made up of nothing but orange juice (T0). It was heated up until it boiled. After that, agar gel, sugar (60–65%), and lemon juice (0.1%) were added. While it was being cooked and stirred all the time, the mixture turned thick. A refractor was used to make sure the end point was correct. Then put in a glass jar while still hot.

Table 1. Formulations of jelly

| Treatments | Orange (%) | <i>S. Chirata</i> (%) |
|------------|------------|-----------------------|
| T0 | 100 | 0 |
| T1 | 95 | 05 |
| T2 | 90 | 10 |
| T3 | 85 | 15 |

2.4 Quantification of Antioxidant Activity by DPPH Assay

The DPPH (2, 2-diphenyl-1-picrylhydrazyl) test was used to measure antioxidant activity with a few minor modifications, as described by Almey et al.⁽²⁸⁾ using the following formula:

$$\% \text{ Inhibition} = \frac{1 - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

2.5 Quantification of Total Phenolic Content (TPC)

The Folin-Ciocalteu method was used to measure the TPC of the jellies, but with minor modifications.⁽²⁹⁾ The findings were given in milligrams of gallic acid equivalents (GAE) (mg GAE/g) for each gram of extracts.

2.6 Quantification of Total Flavonoid Content (TFC)

Aluminum chloride colorimetric method with slight modifications were used to measure flavonoids content.⁽³⁰⁾ TFC content was calculated as milligrams of quercetin equivalents (QE) per gram of extract (mg QE/g) by comparing the absorbance of the sample extracts with a quercetin standard curve.

2.7 The Chemical Analysis of Prepared Jelly

Moisture content, titratable acidity and pH

The Latimer et al.⁽³¹⁾ and Einhardt et al.⁽³²⁾ methods were followed for the analysis of the moisture, acidity, and pH. Three separate analyses were carried out.

TSS (Total soluble solids)

In order to determine the total soluble solids of jelly using a refractometer, a tiny quantity of jelly was mixed with hot water to make a solution. A drop of this diluted jelly solution was then placed onto the glass surface of the refractometer. Finally, gaze was directed towards the point of intersection between the blue and white regions using the eyepiece of the refractometer in order to determine the concentration of total soluble solids, typically expressed in °Brix.

Vitamin C analysis

A 25 mL aliquot was titrated with 0.025% DCFI (2,6-dichlorophenolindophenol) solution after the samples had been diluted in 100 mL of 2% oxalic acid solution until a pink hue was achieved. L-ascorbic acid solution was used to standardize the solution earlier. The findings are given in mg of ascorbic acid per 100 g of flour.⁽³³⁾ In this instance, the color pigment helped the vitamin C oxidize into dehydroascorbic acid.

2.8 Microbiological Analysis

The quantity of moulds and yeasts that were found by plating the homogenate on PDA (potato dextrose agar), which had been acidified with 10% tartaric acid, was used to assess the microbiological quality of the jelly. The outcomes were compared to the standard criteria and represented as colony forming units per gram of jelly (CFU/g).⁽³⁴⁾

2.9 Sensory Evaluation

A panel conducted sensory evaluation. The 9 point Hedonic scale is the most often employed method for sensory evaluation. The prepared jelly was assessed for its hue, visual presentation, consistency, taste, and overall level of satisfaction. The panel was chosen through a random selection process, taking into account both gender and age. They were given a brief explanation of the sensory attributes that they were required to evaluate. Where 1 represents extreme dislike and 9 represents great liking. A mean score of 7 or above often indicates a sensory quality that is considered highly acceptable. Therefore, a product that attains this

score can be utilized with confidence as a reliable representation of the quality expected from "Target".

2.10 Statistical Analysis

A Microsoft Excel 2019 spreadsheet was used to sort, code, and record the data. Subsequently, one-way ANOVA (Analysis of Variance) procedures were used to do statistical analysis (Tukey's pairwise comparison analysis) using SPSS (Statistical Package for the Social Sciences) software (version 19.0) in order to determine the significant degree of variance at a 95% confidence interval. The statistical analysis was performed at a significance level of 5% (≤ 0.05).

3. RESULTS AND DISCUSSIONS

3.1 Phytochemicals in the Formulated Jelly

Table 2 summarizes the bioactive and antioxidant capacity of the formulated jellies.

Total phenolic content

Phenolic substances are abundant antioxidants found in the human diet and are commonly present in fruits and vegetables. These chemicals are highly

significant due to their exceptional antioxidant properties.⁽³⁵⁾ The Total Polyphenolic Content (TPC) of citrus jellies was examined and the findings are presented in Table 2. The values ranged between 76 and 168.3 mg GAE/g. The TPC of the control jelly, produced without the inclusion of *S. chirata*, was found to be lower than the value reported in reference⁽³⁶⁾ for orange jelly. However, the TPC of the jelly samples T1, T2, and T3 was found to be higher. The phenolic content of jelly increases when various fruits and herbs are fortified throughout the jelly-making process, as a result of the bioactive components, specifically phenolics, present in the fruits. The bioactive substances found in fruits, such as phenolics, flavonoids, carotenoids, and vitamins, are sensitive to heat and can become more concentrated when fruits are processed into jelly.⁽³⁷⁾ Our result aligns with this discovery. The phenolic content is categorized into three levels: low (<100 mg GAE/100 g), medium (100–500 mg GAE/100 g), and high (>500 mg GAE/100 g).⁽³⁸⁾ According to Table 2, the control orange jelly had a low total phenolic content (TPC), whereas the fortified ones had a medium TPC. It can be inferred that adding *S. chirata* increased the total phenolic content (TPC) of orange jelly.

Table 2. Phytochemical Content of formulated jelly

| Sample | Antioxidant activity DPPH (trolox equ.) mg/100 g | Total phenolic content (TPC) (mg GAE/g) | Total flavonoid content (TFC) (mg QE/g extract) |
|--------|--|---|---|
| T0 | 110.5±0.408 ^a | 76±0.816 ^b | 42.5±0.408 ^b |
| T1 | 130.46±0.410 ^b | 161.5±0.408 ^c | 45.53±0.368 ^c |
| T2 | 133.53±0.205 ^d | 164.4±0.309 ^d | 46.8±0.244 ^a |
| T3 | 135.63±0.124 ^c | 168.3±0.216 ^a | 49.1±0.081 ^d |

Table indicates the mean ± S.D. and superscript shows non-significant difference between the treatments

Total flavonoids content

Citrus flavonoids are regarded as potential cancer preventive agents and encompass a wide array of distinct compounds that can lead to various biological activities.⁽³⁵⁾ The bioactive secondary metabolites are found in nearly all plant species, and their effectiveness is determined by the quantity and arrangement of hydroxyl groups inside the molecules. The most prevalent family members consist of flavonol, flavanol, flavones, flavanes, flavonols, and catechins.⁽³⁹⁾ Table 2 exhibits that, TFC ranged from 42.5 to 49.1 mg QE/g. Nevertheless, the existing literature lacks sufficient references for the determination of flavonoid content in products produced from citrus. Significant disparities

among jelly samples were detected based on the acquired results. There is a continuous rise in TFC level observed from T1 to T3. One possible explanation for this phenomenon could be the significant increase in the concentration of *S. chirata* from sample T1 to T3. *S. chirata* is recognised to have the highest concentration of total phenolics (32.77 mg GAE/g) and total flavonoids (27.01 mg RE/g) among all species.⁽⁴⁰⁾

Antioxidant Activity

The amount of phenolic compounds in fruits and fruit jellies is proportional to their antioxidant potential. Citrus jellies' antioxidant activity, as measured by the DPPH technique, varied from 110.5 to 135.63 mg/100g. The findings of our study exhibit a significant increase

compared to the results reported in a prior investigation.⁽³⁶⁾ The latter group determined that the antioxidant activity in orange jelly was 0.549 $\mu\text{mol TE}/100\text{ g}$ using the DPPH technique. The inclusion of *S. chirata* significantly enhanced the antioxidant activity of the prepared jelly. The species *S. chirata* exhibits a DPPH activity of $91.85 \pm 0.12\%$.⁽⁴⁰⁾

3.2 Quality Parameters of Jellies

Table 3 provides the physical and chemical characteristics of the formulated citrus jellies. The composition of plants and fruits is influenced by several elements, mostly include variety, maturation stage, growth, climatic circumstances, and soil type. The composition of citrus fruit directly influences the qualities of jellies.⁽³⁵⁾ The moisture content of the jellies ranged from 34.40 to 31.80%. The finding of this study conform to the 32 to 35% moisture levels reported for jellies.⁽⁴¹⁾ In this study, moisture content was observed to decrease with increased *S. chirata* proportion. Which is because fruits are high moisture foods than plant or medicinal sources.⁽⁴²⁾

Table 3. Quality parameters of formulated jelly

| Sample | Moisture Content (%) | Titratable Acidity | pH | T.S.S (°Brix) | Vitamin C Content (mg/100gm) |
|--------|--------------------------------|--------------------------------|-------------------------------|--------------------------------|--------------------------------|
| T0 | 34.40 \pm 0.294 ^a | 0.068 \pm 0.002 ^b | 2.90 \pm 0.081 ^a | 66.50 \pm 0.408 ^b | 201.5 \pm 0.285 ^b |
| T1 | 33.60 \pm 0.081 ^c | 0.067 \pm 0.008 ^b | 3.03 \pm 0.047 ^b | 66.67 \pm 0.471 ^a | 152.6 \pm 0.216 ^c |
| T2 | 32.13 \pm 0.124 ^b | 0.066 \pm 0.001 ^a | 3.07 \pm 0.047 ^c | 67.30 \pm 0.471 ^b | 150.3 \pm 0.163 ^a |
| T3 | 31.80 \pm 0.216 ^d | 0.062 \pm 0.001 ^c | 3.13 \pm 0.047 ^c | 68.30 \pm 0.471 ^c | 147.3 \pm 0.235 ^d |

Table indicates the mean \pm S.D. and superscript shows non-significant difference between the treatments ($P \geq 0.05$)

The total soluble solids (TSS) content of the jellies in the study ranged between 66.50 to 68.30° Brix. The jelly's total soluble solids (TSS) content conforms to the CODEX STAN standard, which stipulates a range of 60 to 65%.⁽⁴⁶⁾ The Total Soluble Solids (TSS) is a quantitative assessment of the quantity of substances that can dissolve in water, represented as a percentage. A product that contains 100% soluble solids is completely devoid of water, while a product with 0% soluble solids consists entirely of water. Accurate sugar content is essential for the development of gel and the preservation of jelly. If the final Total Suspended Solids (TSS) concentration is below 60-65%, the shelf life of the product will be decreased. The product will have a fluid consistency, which will facilitate the growth of bacteria and moulds within it. If the Total Soluble Solids (TSS) exceeds 65%, the jelly will exhibit a high level of rigidity,

The acidity level of jelly items directly impacts their inherent features, including taste, texture, color, and flavor. Citrus juices with low pH values result in increased jelly stability. The pH values varied between 2.90 to 3.13. Moreover, the pH values of all the products were below 3.5, ensuring the preservation of microbiological stability, as observed in similar fruit-based products made with peach, strawberry, apricot, plum, and lemon.⁽⁴³⁾ On the other hand, low pH is necessary for the setting of jams or jellies.⁽⁴⁴⁾

The jelly samples were analyzed for acidity, and values ranged between 0.062 and 0.068. The level of acidity in jams and jellies are inversely related to the pH as seen from Table 3. An elevated level of acidity is necessary for the preservation, enhancement of color and flavor, and solidification of the jelly. The high acidity of the jelly inhibits the availability of water and prevents the growth of microorganisms. An optimal quantity of acidity is crucial, since excessive acidity might result in the jelly releasing liquid, while insufficient acidity may prevent the product from solidifying.⁽⁴⁵⁾

and there is a possibility of sugar crystallization occurring within the product.⁽⁴¹⁾

The vitamin C content of the prepared jelly ranged between 147.3 to 201.5 mg/100g. Fruits and fruit products are categorized as low (<30 mg/100 g), medium (30-50 mg/100 g), or high (> 50 mg/100 g) depending on the amount of vitamin C they contain.⁽⁴⁷⁾ Therefore, despite the reduction of vitamin C content during processing, *S. chirata* blended orange jellies can be categorized as having a high amount of vitamin C. Consuming 100 g of jelly can provide a sufficient intake of vitamin C, aligning with the recommended daily intake for adults (90 mg/day for men and 75 mg/day for women).⁽⁴⁸⁾

3.3 Microbiological analysis of jelly

Table 4 analyzes the microbiological load of the formulated products to assess the presence and absence of pathogenic bacteria and fungus. There were moulds and yeasts growth in the jellies. Although there was growth of moulds and yeasts, the values were within the permissible range, with a maximum of 10^4 CFU/g.⁽³⁴⁾ The growth of moulds and yeasts during storage can be influenced by various circumstances, including acidic pH, high moisture levels, elevated storage temperatures,

the presence of oxygen, the chemical makeup of the food, and the addition of sugars, which serve as an energy source for microbial growth.⁽⁴⁹⁾ The significant observation is that the growth of yeast and mould is visibly suppressed in the *S. chirata* blended jelly compared to the control sample. The lowest growth value was seen in the sample with the highest *chirata* ratio. One possible cause is the antifungal characteristic of *chirata*,⁽⁵⁰⁾ which ultimately aided in mitigating the proliferation of yeast and mould.

Table 4. Microbiological analysis of formulated jelly

| Sample | Mould and Yeast (cfu/ml) | | | <i>E. coli</i> ($\times 10^1$ cfu/ml) | | | <i>Salmonella</i> ($\times 10^1$ cfu/ml) | | |
|--------|--------------------------|--------------------|--------------------|--|--------|--------|---|--------|--------|
| | Day 1 | Day 30 | Day 60 | Day 1 | Day 30 | Day 60 | Day 1 | Day 30 | Day 60 |
| To | 2.3×10^1 | 5.6×10^2 | 7.8×10^4 | Nil | Nil | Nil | Nil | Nil | Nil |
| T1 | 3.67×10^1 | 4.43×10^2 | 3.35×10^3 | Nil | Nil | Nil | Nil | Nil | Nil |
| T2 | 2.00×10^1 | 2.10×10^2 | 3.00×10^3 | Nil | Nil | Nil | Nil | Nil | Nil |
| T3 | 1.67×10^1 | 1.33×10^2 | 2.32×10^2 | Nil | Nil | Nil | Nil | Nil | Nil |

Testing for the presence of *Salmonella* and *E. coli* is essential for guaranteeing food safety because they are both known to cause food illness when consumed. The presence of *E. coli* and *Salmonella* in food can lead to food poisoning, which can have serious health implications. Therefore, testing for these bacteria is a preventive measure to ensure food safety.⁽⁵¹⁾ All these samples showed absence for salmonella and *E. coli* upto 2 months. The absence of growth for *Salmonella* and *E. coli* in a jelly sample indicates that the sample is free from

these specific bacteria, ensuring its safety for consumption.

3.4 Sensory evaluation

Figure 1 symbolizes the output of sensory evaluation of the 4 formulated jellies. Organoleptic assessment is a critical aspect in both the creation of new products and the monitoring of food quality. The quality of food is assessed by an intricate perception that arises from the interplay of various senses, such as smell, taste, and touch.⁽³⁵⁾

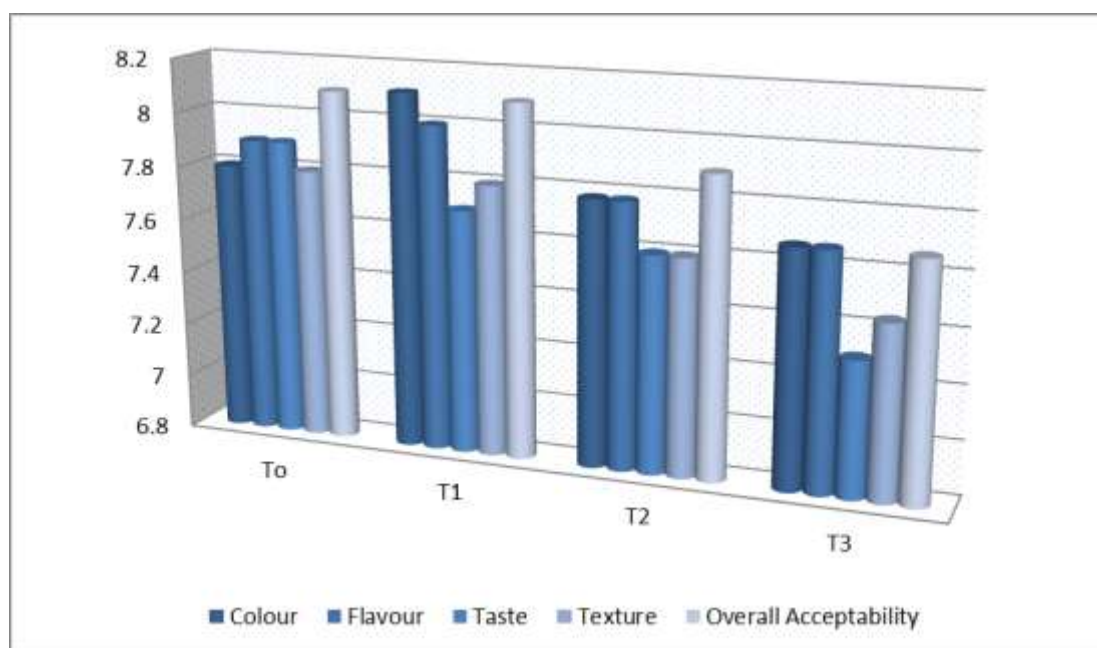


Figure 1. Sensory attributes of formulated jellies

Figure 1 displays the mean scores for each assessed attribute using a radial chart. Average scores of overall acceptability ranged from 7.67 to 8.11. Notably, both the control and sample T1 demonstrated the highest level of overall acceptability. what this means is that people might have the same preference for the orange jelly sample as they do for the T1 sample. Which implies that, to some extent, the aim or purpose of this entire investigation. For future reference, one could study the changes in the physico-chemical attributes of the formulated jellies over a period of time using a commercially available orange jelly.

4. CONCLUSION

Through a comprehensive comparative analysis involving the examination of bioactive compounds, microbiological load, and sensory evaluation, it has been demonstrated that *S. chirata* blended orange jelly exhibits superior characteristics in contrast to conventional orange jelly. The enhanced accessibility of bioactive compounds not only enhances the health advantages but also enhances the whole sensory encounter. Moreover, the advantageous microbiological composition ensures the product's safety and extends its storage lifespan. These findings not only contribute to expanding the range of jelly products, but also provide opportunities for developing functional foods with improved nutritional benefits. This study offers potential for further exploration and application of *S. chirata* in the development of food items, catering to the needs of customers who need healthier and more appealing options in the food industry. A major drawback of the study may lie in the poor comprehension of the possible synergistic interactions between the bioactive chemicals found in the orange and *S. chirata*. Although specific compounds have been found and examined, the intricate interactions among these compounds and their collective impact on health, such as bioaccessibility results, are still not well understood.

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No funds received for this study.

Conflict of Interest

The authors declare no conflict of interest.

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