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Formulation and evaluation of stevia-sweetened ready-to-eat mixed fruit milkshake powder: proximal analysis, sensory, and microbial assessment

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Abstract

The growing demand for nutritious, sugar-free, and convenient food options has spurred innovation in product development, particularly for health-conscious consumers. Additionally, converting highly perishable fruits into ready-to-eat products offers an effective solution to reduce waste. This study focused on the development of a ready-to-eat (RTE) mixed fruit milkshake powder sweetened with *Stevia rebaudiana*. The product aims to meet the need for sugar-free beverages, catering to health-conscious individuals and those with dietary restrictions. The milkshake powder formulation combined banana, date, dragon fruit, and custard apple powders, with stevia incorporated at levels of 3%, 6%, and 9%, alongside a control sample sweetened with 10% sugar for comparison. Proximate composition, sensory evaluation, and microbial analyses were conducted to assess the nutritional content, consumer acceptability, and safety of the product. The results indicated that the 9% stevia formulation was the most appealing to consumers, offering superior sensory attributes and meeting acceptable microbial standards. Proximate analysis revealed a balanced distribution of macronutrients, with carbohydrates at 69.54%, protein at 7.20%, and fats at 9.10%. This stevia-fortified, ready-to-eat milkshake powder is a novel, enjoyable, and shelf-stable beverage, offering a healthy alternative that could help address malnutrition and health issues related to excessive sugar consumption.

1. Introduction

For generations, beverages have been an important part of our lives since they provide relief from the intense heat, slake our thirst, and satisfy our palates (Muhammad *et al*., 2019). Milkshake is a cold, sweet beverage that is typically made with milk, ice cream, or iced milk and flavorings or sweeteners like chocolate sauce or fruit syrup and ready for consumption at the retail location (Goff, 2022). In recent decades, the manufacturing of different kinds of milk beverages has been growing quickly all over the world, and their popularity is growing on a daily basis (Kayshar *et al*., 2024).

Banana (*Musa acuminate*) fruit is a great source of essential phytonutrients (Zhang *et al*., 2022), date (*Phoenix dactylifera L.*) fruits are mostly composed of carbohydrates, including dietary fiber and soluble sugars, with minimal amounts of proteins and fats (Kayshar *et al.,* 2024) and also custard apples (*Annona squamosa L.*) are rich in carbs and also contain a considerable portion of proteins and minerals, including iron, calcium, and phosphorus (Bala *et al*., 2020). Dragon fruit (*Selenicereus undatus*) is a tropical fruit high in vitamins (e.g., vitamin B, vitamin C), minerals (e.g., calcium and iron), antioxidants, and fiber (Luu *et al*., 2021; Islam *et al.,* 2022). The leaves of the *Stevia rebaudiana* plant are used to make stevia sugar and has no calories and is significantly sweeter than sugar without the energy or carbohydrate content and often used as a sugar substitute in foods, drinks, and baking recipes (Gupta *et al*., 2013).

Fruits have a limited shelf life (Monira *et al.,* 2019; Rana *et al*., 2024; Sarker *et al*., 2024) with compact of nutrition. In addition to reducing fruit waste, using these fruits to make milkshakes would result in dairy products that are more appealing, nutritious, and valuable (Angami *et al*., 2023). Snacking on foods that satisfy

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hunger and provide vital nutrients is becoming more popular among modern consumers, who are showing signs of a change in dietary habits. Scientists in the food industry now have a chance to capitalize on this trend by coming up with new, fruit-based products that are rich in nutrients.

The current study aimed to develop an RTE mixed fruit milkshake drink (powder) with stevioside incorporation, as well as to assess milkshake shelf life and adaptability. This study also evaluated the sensory acceptability of formulated milkshakes incorporating both stevia and white sugar.

2. Materials and methods

2.1 Collection of raw materials

Raw materials, such as sugar, milk, stevia powder, bananas, dates, dragon fruit, and custard apples, were purchased from the local supermarket and food safety information was evaluated. To store the ingredients, high -density polyethylene was used as a packaging material.

2.2 Preparation of mixed fruit powder

The preparation of the mixed fruit powder followed the method described by Dutta *et al*. (2021) with some modifications. The process involved washing, peeling, and slicing various fruits, followed by drying them in a cabinet dryer (AIO-1600G, China) at 60°C for 3 days. For banana slices, an additional step of incorporating rice starch was carried out to prevent enzymatic discoloration. After drying, the fruits were blended into a fine powder using a blender. The formulated powder was then sieved to remove large fragments and stored in an airtight container for further analysis. The ready-to-eat (RTE) mixed fruit milkshake powder was prepared according to the method suggested by Pandey *et al*. (2020). The mixed fruit powder and milk powder were combined with sugar at a concentration of 9% to prepare the control sample (S1). For the experimental samples (S2, S3, and S4), stevia powder was added at concentrations of 3%, 6%, and 9%, respectively, replacing sugar (Table 1). To ensure uniform distribution of particles, the mixture was blended thoroughly using a high-speed blender. The prepared samples were stored in airtight containers for subsequent analysis.

Table 1. Formulation of RTE mixed fruit milkshake powder.

Ingredients	S1 (g)	S2 (g)	S3 (g)	S4 (g)
Banana powder	20	20	20	20
Dragon fruit powder	15	15	15	15
Dates powder	20	20	20	20
Custard apple powder	15	15	15	15
Milk powder	20	20	20	20
Sugar	10	0	0	0
Stevioside	0	3	6	9

2.3 Preparation of ready-to-eat mixed fruit milkshake drink

The RTE mixed fruit milkshake drink was prepared following the method described by Dutta *et al*. (2021). The preparation process involved incorporating distilled water with the pre-formulated RTE mixed fruit milkshake powder to create the beverage. The resulting mixture was homogenized using a magnetic stirrer to ensure uniform consistency.

2.4 Sensory evaluation of ready-to-eat mixed fruit milkshake drink

The sensory evaluation and preference test of the RTE mixed fruit milkshake drink were conducted following the methods described by Aziz *et al*. (2011) and Rafi *et al*. (2020). A panel of 9 trained judges, each with two years of experience in using quantitative response scales for sensory analysis (ISO 4121:2003), assessed the samples. The panelists underwent a 45-minute training session to familiarize themselves with the 9-point hedonic scale, which ranged from 1 (dislike extremely) to 9 (like extremely).

2.5 Proximate analysis

The proximate analysis of S4 was selected as the final product based on sensory evaluation, was conducted following the AOAC INTERNATIONAL (2016) guidelines, using AOAC Official Method 925.10 for moisture, AOAC Official Method 984.13 for crude protein, AOAC Official Method 962.09 for crude fiber, and AOAC Official Method 920.39 for crude fat. The total carbohydrate content in the formulated beverage was estimated using the methods recommended by the Food and Agriculture Organization of the United Nations (FAO, 1998).

2.6 Microbial analysis of ready-to-eat mixed fruit milkshake drink

The microbial analysis was conducted following the method by Awan *et al.* (2019) . In order to maintain their microbial integrity, all milkshake samples were kept at 4°C prior to culturing for the duration of the storage study. Microbial analyses were performed on 0, 3, 6, 9, 12, and 15 days of storage. To create a tenfold (10^{-1}) dilution, 1 mL of milkshake was transferred using a sterile pipette into a sterile test tube that contained 9 mL of sterile normal saline after each sample had been thawed. Subsequently, 1 mL was transferred from one tube to the next, each containing 9 mL of sterile saline, and thoroughly mixed to create serial dilutions up to 10^{-4}. The pour-plate method was used to plate 1 mL aliquots from the 10^{-3} dilution in duplicate onto Nutrient Agar (HiMedia, India). Plates were incubated inverted at

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37°C for 24 to 48 hrs and counted. Colony-forming units per milliliter (CFU/mL) were used to report the results. Plates with colony counts between 30 and 300 were considered valid, and the results for each dilution were recorded. The following formula was used to calculate the total bacterial count.

$$\text{CFU/mL} = \frac{\text{colony number} \times \text{dilution factor}}{\text{volume of culture plate}}$$

2.7 Statistical analysis

The mean values were presented as the results after analyzing all data in triplicate. The mean scores from the sensory evaluation were plotted on a radar chart and subsequently analyzed to assess statistically significant differences among the samples using two-way Analysis of Variance (ANOVA) in Microsoft Office Excel 2013. Additionally, preference test results for color, flavor, texture, and overall acceptability of the final beverage were evaluated using the Least Significant Difference (LSD) test, with significance set at $p < 0.05$.

3. Results and discussion

3.1 Sensory evaluation

Figure 1 shows the sensory differences amongst the samples. S4 (9% stevioside) had the highest sensory scores for color (8.39), flavor (7.80), texture (8.00), and overall acceptability (8.09). The taste and overall sensory experience clearly show the need for increased high-quality stevioside content. S1 (10 g of white sugar) fared reasonably well, especially in terms of color (7.78), texture (8.00), and overall acceptability (7.07). However, it received a lower flavor rating than S4, indicating that customers preferred the stevioside's natural sweetness. In contrast, S2 and S3 (3% and 6 % of stevioside) received significantly lower scores across all sensory attributes, particularly texture and flavor. Reduced stevioside amounts may have affected their taste character and texture, resulting in less pleasant sensory experiences. Kuchekar *et al*. (2019) reported similar findings, with sensory attributes like color (7.95-8.33), flavor (8.03-8.22), texture (7.41-8.21), and overall acceptability (8.06 -8.44) in almond milkshake samples, supporting the results of this experiment. Ubale *et al*. (2014) also reported that sensory attributes of milkshakes developed with 9% sapota pulp varied from 6 to 6.96 for color, 6.55 to 6.90 for flavor, 6.22 to 7.28 for texture, and 6.10 to 7.17 for overall acceptability. These values support the experimental findings, although slight deviations may be due to variations in experimental design and raw ingredient selection.

3.2 Proximate analysis of ready-to-eat mixed fruit milkshake powder

The proximate analysis of the selected RTE mixed fruit milkshake powder (S4) showed satisfactory results.

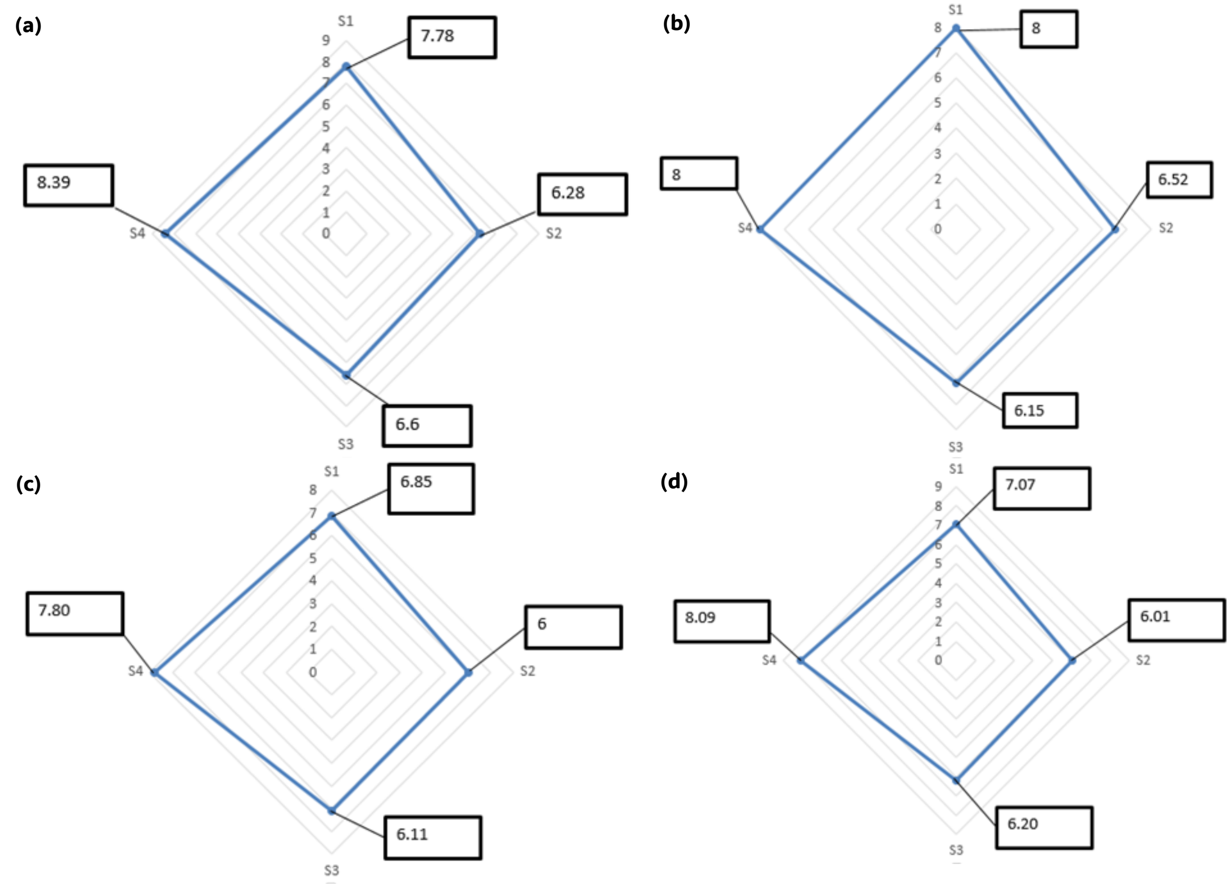

Figure 1. A graphical representation of mean scores for different attributes of the RTE mixed fruits: (a) color, (b) flavor, (c) texture, and (d) overall acceptability.

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According to the data in Table 2, the moisture, protein, fat, ash, carbohydrate, and dietary fiber contents were 9.33%, 7.20%, 9.10%, 3.33%, 69.54%, and 1.50%, respectively. Comparing these results, Pandey *et al*. (2020) reported that phytonutrient-enriched avocado milkshake powder has much lower moisture (2.3%) but higher fat (42.2%) and protein (17.2%) content. Jerlin *et al*. (2022) found that milkshakes with *Cucurbita maxima* had higher fat (49.8%) but lower carbohydrate (23.65%) content. The mixed fruit milkshake powder's nutritional profile appears consistent with other studies, underscoring the influence of fruit type on its nutritional makeup.

Table 2. The proximate nutritional composition of RTE mixed fruit milkshake powder.

Ingredients	Composition (%)
Moisture	9.33
Protein	7.20
Fat	9.10
Ash	3.33
Carbohydrate	69.54
Dietary fiber	1.50
Total	100

3.3 Microbial analysis

Table 3 tabulates the microbial analysis of S4. Even after 15 days of storage, the microbial count in S4 remained within the acceptable limit of 5.0×10^4 CFU/mL set by the Gulf Standard for fruit juices and beverages. While our starting count on Day 0 was 1.0×10^4 CFU/mL and it gradually increased to 4.1×10^4 CFU/mL by Day 15, which is well within the safe range. Although the final count was slightly higher than the typical average for fresh juice and dairy products (around 2.4×10^4 CFU/mL as per Ahmad and Haq, 2014), it still aligns with regional standards and international findings for safe consumption. We believe the low moisture content of the dried powder used in the milkshake played a key role in maintaining this microbial stability. While some studies have shown much higher contamination in milk drinks, like one that found counts up to 7.5×10^7 CFU/mL, S4 clearly demonstrates its safety for consumption.

Table 3. Microbial study of RTE mixed fruit milkshake drink sample (S4).

Day	CFU/mL
0	1.00×10^4
3	1.33×10^4
6	1.76×10^4
9	2.33×10^4
12	3.09×10^4
15	4.10×10^4

4. Conclusion

This study successfully developed and tested RTE mixed fruit milkshake powder with *Stevia rebaudiana* in its composition. These findings come in the context of the increasing demand for healthy and sugar-free drinks. Results showed that the formulation with 9% stevia (S4) was the most acceptable because it had the best sensory qualities as well as a safe microbial profile and contained a reasonably well-balanced nutritional content of carbohydrates (69.54%), protein (7.20%), and fats (9.10%). These findings reinforce the idea that stevia-fortified milkshake powder could provide a healthy and storage-friendly substitute to sugar-based drinks, especially for diet-sensitive groups such as diabetic patients.

Conflict of interest

All authors clarified that they have no conflict of interest to declare that are relevant to the content of this article, and they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

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