

# Preparation and Characterization of Fruit Smoothies Supplemented with Sissoo Spinach

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## Abstract

Obesity is a growing concern in Malaysia, with the 2023 National Health Morbidity Study reporting that 50.1% of Malaysians are overweight or obese. Poor dietary habits, including insufficient consumption of fruits and vegetables, contribute significantly to this issue. To address this, a banana smoothie supplemented with sissoo spinach was developed as a healthy, nutritious, and appealing alternative for individuals seeking a balanced diet. This study also aimed to determine the physicochemical characteristics, nutritional composition, and sensory evaluation of the produced smoothies. The physicochemical analysis involved evaluating parameters such as colour, pH, total soluble solids (TSS), and moisture content. Nutritional composition was determined by analysing protein, dietary fiber, fat, ash, and carbohydrate content. A sensory evaluation was conducted with 50 panellists to assess the acceptability of the smoothies in terms of appearance, taste, aftertaste, aroma, texture, and overall acceptance. The findings revealed that adding sissoo spinach, honey, and lemon influenced the physicochemical properties, resulting in reduced lightness ( $L^*$ ) ( $37.27 \pm 1.33$ ) and pH ( $5.21 \pm 0.01$ ) while maintaining an acceptable TSS level ( $14.98 \pm 0.39$  °Brix). Nutritional analysis showed that Formulation 3 had the highest protein ( $1.59 \pm 0.02\%$ ) and ash content ( $0.96 \pm 0.02\%$ ), indicating improved nutritional value. However, the sensory evaluation demonstrated that Formulation 1 was the most acceptable sample ( $6.18 \pm 1.57$ ), scoring well for appearance ( $6.32 \pm 1.76$ ) and texture ( $6.42 \pm 1.57$ ) while balancing nutritional quality and taste. Finally, the banana smoothie supplemented with sissoo spinach is a promising option for promoting the consumption of dark green vegetables and fruits, particularly among individuals who are reluctant to consume vegetables due to their bitter taste. The incorporation of honey and lemon effectively masked the bitterness, making the smoothie a palatable, convenient, and nutrient-dense choice.

## 1. Introduction

Smoothies are widely recognized as versatile, nutritious beverages that offer various health benefits, depending on their ingredients. They are typically made by blending fruits, vegetables, and liquids, with optional additions such as nuts, seeds, yogurt, or oats, resulting in a creamy, thick texture like milkshakes [1]. Green smoothies, for example, combine leafy greens like spinach with a base liquid, offering a convenient way to meet daily intake requirements for vitamins, minerals, and fiber, while retaining the beneficial fiber from whole foods [2].

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Similarly, fruit smoothies are rich in vitamins, minerals, fiber, and antioxidants contributing to improved health and wellness.

Bananas, a staple ingredient in many smoothies, are a popular fruit base due to their natural sweetness and nutrient content. They are rich in carbohydrates, dietary fiber, and essential vitamins such as C, A, and B-complex, as well as minerals like magnesium and iron [3]. Bananas also provide various health benefits, including improved carbohydrate metabolism, weight management, and a reduced risk of gastrointestinal diseases. In this study, the developed green smoothies feature leafy greens such as Sissoo spinach (*Alternanthera sissoo*) blended with bananas, creating a nutrient-rich base. Besides being readily available, Sissoo spinach was selected for its exceptional nutritional profile and potential health benefits. It is a rich source of antioxidants, vitamins (such as A and C), and minerals, particularly iron and calcium, which are essential for overall health. This leafy green not only significantly enhances the smoothie’s nutritional value but also improves its visual appeal and freshness. Furthermore, Sissoo spinach was chosen because previous studies have primarily focused on other vegetables, such as carrots and baby spinach, in smoothies, resulting in limited research on the incorporation of Sissoo spinach. Honey and lemon are added to enhance the flavour and balance the taste, while a liquid base is used to achieve the desired consistency. This combination offers a convenient and wholesome option for meeting daily nutritional requirements.

The quality of smoothies is influenced by their physicochemical, sensory, and nutritional properties, which are determined by the proportions of ingredients used. Key physicochemical properties include pH, total soluble solids (TSS), moisture, and optical characteristics such as colour, which play a vital role in consumer perception [4]. Sensory analysis, which evaluates appearance, aroma, taste, aftertaste, and texture, is crucial in understanding consumer preferences and acceptance [2]. Additionally, analysing the nutritional content of smoothies is essential to assess their health benefits and align with dietary recommendations. In this study, the nutritional content of the smoothies, including macronutrients like carbohydrates, protein, dietary fiber, and fat, as well as micronutrients such as minerals, was evaluated to ensure the smoothies offer a balanced and beneficial option for consumers.

## 2. Materials and Methods

### 2.1 Materials

The raw materials used to produce smoothies included fresh ripe bananas purchased at a nearby supermarket in Pagoh. Sissoo spinach, utilized as a supplement in the fruit-base smoothie, was obtained from a local cultivation farm. Honey and lemon were obtained from the local supermarket in the Pagoh area as additives in the smoothies. The pure honey used in the smoothie was from the CED brand, while the type of lemon used was Eureka lemon from the species *Citrus limon*.

### 2.2 Preparation of Banana Smoothie Supplemented with Sissoo Spinach

Fresh bananas were selected and properly washed, then peeled with a knife and diced [5]. The stems of the sissoo spinach were removed, leaving only the leaves, which were then washed under running clean water. Lemons were squeezed using a lemon squeezer. The diced bananas and sissoo spinach were weighed using an analytical weighing balance. Honey and lemon were added to the smoothie following the ratios presented in Table 1 following the previous study [5]. The mixture was carefully poured into the electric blender to produce smoothie samples. They were homogenized, bottled, and stored in the refrigerator until analysis was conducted [6].

**Table 1** Formulation of fruit smoothie supplemented with sissoo spinach (g/100g)

	Fruit (Banana) (g)	Dark Green Vegetable (Sissoo Spinach) (g)	Honey (g)	Lemon Juice (g)	Water (g)
Control	50	-	-	-	50
Formulation 1	40	10	2	4	44
Formulation 2	40	20	4	2	34
Formulation 3	40	30	3	3	24

## 2.3 Analysis on Physicochemical Properties

### 2.3.1 pH value

The pH value of the smoothies was assessed using a digital pH meter (Sanxin SX751, China), which underwent calibration using pH 4.0 and 7.0 buffers. 50 ml of the smoothies was transferred into a beaker, and the pH was measured after calibrating the meter [7]. The average value was obtained from the triplicate of each sample.

### 2.3.2 Colour

The colour of the smoothies was measured using an in-house method (STP-SPL-3003) with a colorimeter. Colour was expressed in  $L^* a^* b^*$  system coordinates [5].

### 2.3.3 Total Soluble Solid

The Brix value, or sugar content, was assessed by measuring the refractive index. The refractive index was determined using a digital refractometer (Model: Atago PAL-BX/RI) [8]. Two drops of the smoothies were placed onto the refractometer and the result of the Brix measurement was recorded in triplicate.

### 2.3.4 Moisture content

The moisture content of the sample was analysed using a moisture analyzer (AND MS-70), which combines heat application and weight measurement for precise determination [9]. The device applies heat to the sample, typically using a halogen heating lamp to facilitate drying. A 1g sample was placed on the weighing pan, and its initial weight was recorded. The heating lamp was activated, and the sample was heated until it achieved a constant weight, indicating the complete evaporation of moisture. The final weight was then measured to determine the moisture content.

## 2.4 Nutritional Analysis

### 2.4.1 Protein Content

Protein content was analysed using the AOAC 976.05 standard protocol, commonly called the Kjeldahl method [10]. The procedure began by placing three Kjel-tabs into a 500 mL compatible flask at station 1. Next, 10 mL of 30–35%  $H_2O_2$  and 12–15 mL of 96–98%  $H_2SO_4$  were added, with the volume adjusted based on the sample's fat content. A sample (1.0 g for protein content below 45% or 0.5 g for protein content above 45%) wrapped in paper was introduced, and the flask was sealed. The flask sequentially moved through digestion at stations 2 and 3, cooled at station 4 with the addition of 140 mL of water, and then advanced to station 5, where  $NaOH-Na_2S_2O_3$  was added. The released  $NH_3$  was steam-distilled into a beaker with an indicator and automatically titrated with  $H_2SO_4$  to determine nitrogen or protein content, which was displayed or printed. At position 6, the flask was emptied. The instrument was calibrated daily with standard solutions as per the manual. The protein content was calculated using the equation (1) [10].

$$\% \text{ Protein content} = \% N \times \text{Protein factor} \quad (1)$$

### 2.4.2 Fat Content

The fat content of the smoothies was analysed using the Soxhlet extraction method [11]. Initially, the smoothie sample was frozen at  $-50^\circ C$  and dried using a freeze-dryer. The dried sample, approximately 30 grams, was finely ground using a mortar and pestle. With gloves on, three pre-dried cellulose extraction thimbles were retrieved from a desiccator and accurately weighed using an analytical balance. Each thimble was filled with 2–3 grams of the ground sample, reweighed, and sealed with a small plug of dried glass wool. The thimbles were then placed in a Soxhlet extractor. A flask containing around 350 mL of petroleum ether and glass boiling beads was prepared for the extraction, which was conducted for at least 6 hours. Post-extraction, the thimbles were carefully removed using tongs, air-dried overnight in a fume hood, and further dried in a vacuum oven at  $70^\circ C$  for 24 hours. After drying, the samples were cooled in a desiccator and reweighed, then calculated using equation (2) to determine fat content [11].

$$\% \text{ Fat content} = (\text{Weight of fat}) / (\text{Weight of sample}) \times 100 \quad (2)$$

### 2.4.3 Ash Content

The ash of the smoothies was obtained following the method outlined by the Food Analysis Laboratory Manual [11]. To prepare, the crucible and its lid were placed in a furnace at 550°C overnight to remove any surface impurities. After cooling in a desiccator for 30 minutes, the crucible and lid were weighed. A 5g sample of the smoothie was then added to the crucible, which was partially covered with the lid and heated over a low Bunsen flame until no fumes were emitted. The crucible was subsequently transferred to a furnace and heated at 550°C overnight, with the lid left off during the heating process. Once the heating was complete, the lid was replaced to prevent ash loss, and the crucible was cooled in a desiccator. Finally, the ash, along with the crucible and lid, was weighed. The resulting ash was required to be white or light grey in appearance. Ash content was calculated using equation (3) [11].

$$\% \text{ Ash content} = (\text{weight of crucible with ash} - \text{weight of empty crucible}) / (\text{weight of sample}) \times 100 \quad (3)$$

### 2.4.4 Dietary Fiber Content

The analysis of the dietary fiber content of smoothies was determined according to the method of the Association of Official Analytical Chemists, AOAC 985.29 & 991.4, also known as the Enzymatic-Gravimetric method [12]. The sample is digested with amylase, amyloglucosidase, and protease, and then the residue is isolated and measured gravimetrically. Initially, the sample is treated with  $\alpha$ -amylase at pH 6.0  $\pm$  0.2 and incubated at 95-100°C for 15-30 minutes to break down starches. This is followed by protease treatment at pH 7.5  $\pm$  0.2, 60°C for 30 minutes to degrade proteins. Then, amyloglucosidase is added at pH 4.0-4.6, 60°C for 30 minutes to further degrade any remaining starch. Afterward, 280 mL of 95% ethyl alcohol at 60°C is added to the mixture, and the precipitate is allowed to form at room temperature for 60 minutes. The residues, which include both soluble and insoluble fiber, are collected by filtration into pre-weighed crucibles. A second sample is used to determine protein content, applying a conversion factor of N  $\times$  6.25. To measure ash content, the sample is incinerated at 525°C for 5 hours. This method ensures an accurate determination of dietary fiber by excluding protein and ash, leaving only the fiber content. The dietary fiber content was determined according to the AOAC 985.29 & 991.4 method by using the following equation (4) [12].

$$\text{Total Dietary Fiber (\%)} = ((\text{weight residue} - \text{protein} - \text{ash} - \text{blank})) / (\text{weight of sample}) \times 100 \quad (4)$$

### 2.4.5 Carbohydrate Content

The carbohydrate content of the smoothies was calculated using the difference method, which involves subtracting the percentages of protein, fat, ash, and moisture from 100% as seen in equation (5).

$$\% \text{ Carbohydrate} = 100 \% - (\% \text{ protein} + \% \text{ fat} + \% \text{ ash} + \% \text{ moisture}) \quad (5)$$

## 2.5 Sample preparation for sensory evaluation

Four formulations of banana smoothies supplemented with sissou spinach were prepared and served in plastic cups labelled with randomized three-digit codes at room temperature [13]. Each smoothie portion was approximately 10 mL. The samples were presented to panellists in a completely randomized order for sensory evaluation, with panellists assessing the smoothies one at a time using a provided scorecard.

## 2.6 Selection of Panelist

Fifty panellists from Universiti Tun Hussein Onn Malaysia (UTHM) participated in a sensory evaluation test to assess the smoothies based on various attributes, including taste, appearance, aftertaste, texture, aroma and overall acceptability. The evaluation was using a 9-point hedonic scale, with 9 indicating "like extremely" and 1 representing "dislike extremely" [13].

### 3. Result and Discussion

#### 3.1 Physicochemical Analysis

##### 3.1.1 pH, Total Soluble Solid, Colour, Moisture Content

Table 2 shows results of pH, total soluble solid, colour, and moisture content for the banana smoothies with various proportions of sissoo spinach, honey, and lemon.

**Table 2** pH, total soluble solid, colour, and moisture content of the smoothies

	pH	Total Soluble Solid (°Brix)	Colour			Moisture Content (%)
			L*	A*	B*	
Control	5.77 ± 0.04 <sup>a</sup>	14.92 ± 0.13 <sup>a</sup>	35.64 ± 0.49 <sup>ab</sup>	-0.66 ± 0.07 <sup>a</sup>	-3.75 ± 0.14 <sup>b</sup>	84.81 ± 0.53 <sup>a</sup>
Formulation 1	5.21 ± 0.01 <sup>c</sup>	14.98 ± 0.39 <sup>a</sup>	37.27 ± 1.33 <sup>a</sup>	-2.25 ± 0.05 <sup>b</sup>	-2.25 ± 1.12 <sup>a</sup>	82.92 ± 0.53 <sup>b</sup>
Formulation 2	5.25 ± 0.02 <sup>bc</sup>	15.36 ± 0.46 <sup>a</sup>	34.01 ± 0.09 <sup>bc</sup>	-2.24 ± 0.05 <sup>b</sup>	-4.72 ± 0.08 <sup>b</sup>	80.59 ± 1.05 <sup>c</sup>
Formulation 3	5.30 ± 0.03 <sup>b</sup>	14.65 ± 0.12 <sup>a</sup>	33.59 ± 0.14 <sup>c</sup>	-2.35 ± 0.09 <sup>b</sup>	-4.90 ± 0.17 <sup>b</sup>	81.64 ± 0.21 <sup>bc</sup>

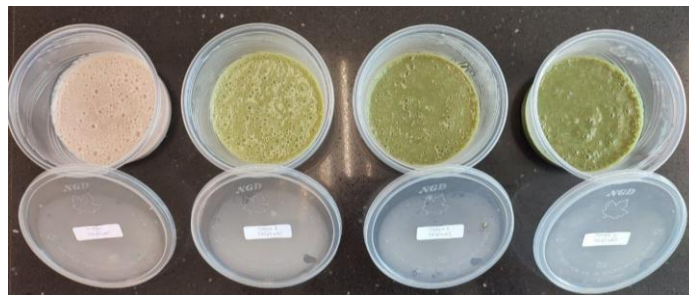
Data are expressed as mean ± S.D., with a significance of  $p < 0.05$  indicated by different letters.

The pH value, as indicated in Table 2, ranges from 5.21 to 5.77. However, the previous study reported that fresh green smoothies typically have a pH of 4.22 [14]. In this study, the pH values of the smoothies are slightly acidic, with the lowest recorded value being 5.21. This acidity is attributed to the presence of organic acids in fruits and vegetables [9]. Additionally, the Control is expressed as  $5.77 \pm 0.04^a$  with a significance of  $p < 0.05$  indicated by different letters between the formulated smoothies. The addition of lemon juice in the formulated smoothies enhanced acidity and flavour while balancing sweetness and bitterness. Lemon, being rich in citric acid, effectively lowers the pH and can replace artificial acidity regulators.

In the context of smoothies, TSS values, which reflect sweetness, range from 14.65% to 15.36% °Brix. The results showed no significant difference ( $p > 0.05$ ) in TSS values among the samples, indicated by the same letters. Formulation 2, with the highest honey content, exhibited the highest TSS value, while Formulation 3 had the lowest due to its lower honey content and a higher proportion of sissoo spinach. The control, consisting only of bananas, showed TSS levels comparable to Formulation 1, as bananas are naturally sweet. The °Brix content obtained in this study was similar to those reported by previous studies [5] for fruit and vegetable smoothies, particularly in samples containing bananas. The °Brix, also referred to as the sugar index, indicates the degree of sweetness and reflects the sugar present in the fruit and honey used in the smoothies.

Colour analysis was conducted using the CIE-defined colour-space system, which includes the parameters  $L^*$ ,  $a^*$ , and  $b^*$ . The  $L^*$  axis represents lightness, ranging vertically from 0 (black) to 100 (white). The  $a^*$  axis measures the red-green spectrum, with positive  $a^*$  values indicating red and negative  $a^*$  values indicating green. Similarly, the  $b^*$  axis represents the yellow-blue spectrum, where positive  $b^*$  values correspond to yellow and negative  $b^*$  values correspond to blue [15]. This study showed  $L^*$  values ranging from 33.59 to 37.27, with the highest lightness observed in Formulation 1 and the darkest sample in Formulation 3. The negative  $a^*$  values indicate the greenish colour. Formulation 3 exhibited the greenest appearance (negative  $a^*$  values), which can be attributed to the chlorophyll present in sissoo spinach leaves [16].

The moisture content ranged from 80.59% to 84.81%. The control exhibited the highest moisture content ( $84.81 \pm 0.53$ ), while Formulation 2 had the lowest moisture content ( $80.59 \pm 1.05$ ). Bananas significantly contribute to the moisture content due to their high-water content, influencing the overall moisture level in the smoothies. Table 2 shows the significance of  $P < 0.05$  in moisture content between the control and the formulated smoothie samples indicated by different letters. These findings differ from those reported by the previous study [6], who observed higher moisture content (ranging from 89.04% to 90.80%) in a similar study. This discrepancy may be due to the different fruits used in their research, such as mango, pineapple, and watermelon, which have higher moisture levels compared to the fruits used in the current study. Therefore, the banana and sissoo spinach used in this study contribute significantly to the overall moisture level [5].



**Fig. 1** Colour of smoothies for Control, Formulation 1, Formulation 2, and Formulation 3

### 3.2 Nutritional Analysis

Five nutritional compositions for smoothie samples including protein, dietary fiber, fat, ash, and carbohydrate were analysed and the results are shown in Table 3.

**Table 3** Nutritional content of the smoothies

	Protein (%)	Dietary Fiber (%)	Fat (%)	Ash (%)	Carbohydrate (%)
Control	1.17 ± 0.07 <sup>b</sup>	0.01 ± 0.01 <sup>b</sup>	0.01 ± 0.01 <sup>c</sup>	0.82 ± 0.06 <sup>b</sup>	13.19 ± 0.41 <sup>c</sup>
Formulation 1	0.88 ± 0.07 <sup>c</sup>	0.10 ± 0.01 <sup>a</sup>	0.15 ± 0.02 <sup>b</sup>	0.86 ± 0.02 <sup>b</sup>	15.20 ± 0.49 <sup>b</sup>
Formulation 2	1.18 ± 0.05 <sup>b</sup>	0.09 ± 0.01 <sup>a</sup>	0.36 ± 0.04 <sup>a</sup>	0.91 ± 0.03 <sup>ab</sup>	16.96 ± 1.05 <sup>a</sup>
Formulation 3	1.59 ± 0.02 <sup>a</sup>	0.09 ± 0.01 <sup>a</sup>	0.34 ± 0.01 <sup>a</sup>	0.96 ± 0.02 <sup>a</sup>	15.47 ± 0.21 <sup>ab</sup>

Data are expressed as mean ± S.D., with a significance of  $p < 0.05$  indicated by different letters.

Fruits and vegetables have been identified as potential protein sources due to their protein content. According to the previous study [17], fruits contain approximately 1% protein, while vegetables contain around 4%. The protein content of the produced smoothies ranged from 0.88% to 1.59%, with Formulation 3 showing the highest value (1.59%) due to its increased sissoo spinach content and the addition of honey and lemon in the smoothie. This value surpasses the highest value reported in the previous study (1.06%) [18], highlighting the role of sissoo spinach in enhancing protein levels. Formulation 1 had the lowest protein content at 0.88%, possibly due to a lower proportion of sissoo spinach in this formulation. However, the overall protein content remained low, making these smoothies more suitable for hydration and micronutrient delivery rather than as significant protein sources.

The dietary fiber content in the produced smoothies, influenced by the sissoo spinach ratios, ranged from 0.01% in the Control to 0.10% in Formulation 1, the highest among the formulations. The data with no significant difference ( $p > 0.05$ ) in dietary fiber content among the formulations is indicated by the same letters. Unexpectedly, Formulations 2 and 3, despite containing more sissoo spinach, showed slightly lower dietary fiber values (0.09%). This may be due to variations in ingredient proportions, processing factors, or fiber solubility during smoothie preparation, which could have affected the overall measurable fiber content. Nevertheless, all formulations demonstrated improved fiber content compared to the Control.

The fat content, as indicated in Table 3, ranged from 0.01% to 0.36%. The Control had the lowest fat content at 0.01%, while Formulation 2 had the highest fat value at 0.36%, which may be due to the honey content in the banana smoothies. The fat content of the smoothies supplemented with sissoo spinach, honey, and lemon varied across the formulations, reflecting the impact of ingredient composition on the nutritional profile. Formulation 3 (0.34%) had marginally lower fat due to ingredient variations. The results show a significant difference ( $P < 0.05$ ) in fat content between the control and the formulations, indicated by different letters. Despite this variation, these smoothies remained low in fat, making them suitable for health-conscious consumers.

Ash content increased with higher sissoo spinach levels, ranging from 0.82% in the Control to 0.96% in Formulation 3, reflecting enhanced mineral content from the sissoo spinach. Similarly, carbohydrates ranged from 13.19% in the Control to 16.96% in Formulation 2, driven by honey and banana. In contrast, Formulation 3 recorded a slightly lower carbohydrate content (15.47%) due to the dilution effect of sissoo spinach, which has fewer carbohydrates compared to honey and banana. The higher carbohydrate content in Formulation 2 can be

attributed to the inclusion of 4 g of honey, a concentrated carbohydrate source, and 40 g of banana, which is naturally rich in carbohydrates. The data show a significant difference ( $p < 0.05$ ) in carbohydrate content between the Control and the formulations, indicated by different letters. These results emphasize the role of bananas and honey in enhancing the energy-providing potential of the smoothies, making them suitable as energy-dense beverages. These smoothies provide balanced nutritional benefits, including improved fiber, mineral, and carbohydrate content.

### 3.3 Sensory Evaluation Test

A sensory test on four (4) samples of banana smoothie supplemented with sissoo spinach was carried out for fifty (50) panellists. The sensory attributes evaluated included appearance, taste, aftertaste, aroma, texture, and overall acceptability. Each attribute was determined using a 9-point hedonic scale. All samples were accepted by panellists with sensory scores of 5-7. The result of each smoothie sample is shown in Table 4.

**Table 4** Sensory results of the smoothies

	Appearance	Taste	Aftertaste	Aroma	Texture	Overall Acceptance
Control	4.76 ± 1.76 <sup>b</sup>	6.50 ± 1.82 <sup>a</sup>	6.30 ± 1.83 <sup>a</sup>	6.60 ± 1.68 <sup>a</sup>	6.60 ± 1.55 <sup>a</sup>	6.48 ± 1.89 <sup>a</sup>
Formulation 1	6.32 ± 1.76 <sup>a</sup>	5.78 ± 1.97 <sup>ab</sup>	5.72 ± 1.95 <sup>ab</sup>	5.36 ± 1.56 <sup>b</sup>	6.42 ± 1.57 <sup>ab</sup>	6.18 ± 1.57 <sup>ab</sup>
Formulation 2	6.22 ± 1.96 <sup>a</sup>	5.88 ± 1.93 <sup>ab</sup>	5.70 ± 1.93 <sup>ab</sup>	5.26 ± 1.83 <sup>b</sup>	6.18 ± 1.70 <sup>ab</sup>	6.00 ± 1.91 <sup>ab</sup>
Formulation 3	6.18 ± 2.07 <sup>a</sup>	5.30 ± 1.80 <sup>b</sup>	5.00 ± 1.88 <sup>b</sup>	5.00 ± 1.81 <sup>b</sup>	5.62 ± 1.84 <sup>b</sup>	5.36 ± 1.52 <sup>b</sup>

Data are expressed as mean ± S.D., with a significance of  $p < 0.05$  indicated by different letters.

In terms of appearance, it was observed that Formulation 1 smoothie recorded the highest taste score (6.32 ± 1.76). The acceptability of the appearance increased when 10g of sissoo spinach was added. The results obtained show a significant difference ( $p < 0.05$ ) in appearance scores between the Control and the formulations, indicated by different letters. The Control exhibited the lowest mean score for appearance (4.76 ± 1.76), likely due to its plain yellow colour. In contrast, the supplemented formulations (Formulation 1, Formulation 2, and Formulation 3) recorded significantly higher appearance scores which are 6.32, 6.22, and 6.18, respectively, which can be attributed to the vibrant green hue imparted by sissoo spinach. This finding aligns with previous studies indicating that visually appealing colours, particularly green tones, are associated with higher consumer perceptions of freshness and nutritional value.

For taste, the Control smoothie achieved the highest score at 6.50, reflecting panellists' preference for the naturally sweet and familiar flavour of banana. Formulation 1 recorded a slightly lower taste score (5.78), followed by Formulation 2 (5.88) and Formulation 3 (5.30). The Control and Formulations 1 and 2 show no significant difference ( $p > 0.05$ ) in taste scores, indicated by the same letters. The decline in taste scores with increasing sissoo spinach content (10 g to 30 g) can be attributed to the mildly bitter notes of sissoo spinach, which are less favourable to some consumers. However, the inclusion of honey and lemon in Formulation 1 and Formulation 2 helped balance the bitterness, as evidenced by their slightly higher taste scores compared to Formulation 3. According to the previous study, the inclusion of honey in mixed beverages is beneficial for enhancing their quality as it maintains their instrumental colour and presents a good sensorial acceptance while lemon imparts a refreshing citrusy flavour [7][19].

The aftertaste scores followed a similar trend, with the Control receiving the highest mean score (6.30), while Formulation 1, Formulation 2, and Formulation 3 recorded 5.72, 5.70, and 5.00, respectively. The results obtained indicate no significant difference in aftertaste scores between the Control and formulations 1 and 2 ( $p > 0.05$ ). The reduced aftertaste scores in the formulations could be linked to slightly bitter and acidic aftertaste from sissoo spinach and lemon, as previously reported in studies involving vegetable-based smoothies.

For aroma, the Control smoothie achieved the highest score (6.60), reflecting the appealing natural aroma of bananas. The supplemented formulations showed a significant reduction in aroma scores, with Formulation 1 (5.36), Formulation 2 (5.26), and Formulation 3 (5.00). The Control and the formulated smoothies show a significant difference ( $p < 0.05$ ), indicated by the different letters. This decline may be due to the addition of sissoo spinach, which can impart a mild grassy or earthy smell that is less preferred by panellists.

The texture scores were relatively high across all formulations, with the Control achieving 6.60. The control and Formulations 1 and 2 showed no significant difference ( $p > 0.05$ ) in texture scores, as indicated by the same letters. Formulation 1 recorded a comparable score of 6.42, indicating that the addition of 10 g of sissoo spinach did not significantly affect the smoothie's smoothness. However, as sissoo spinach content increased to 20 g (Formulation 2) and 30 g (Formulation 3), texture scores decreased to 6.18 and 5.62, respectively, likely due to the fibrous nature of sissoo spinach, which affects the smooth consistency of the smoothie.

The overall acceptance scores further reinforce these findings, with the control smoothie receiving the highest score (6.48), followed closely by Formulation 1 (6.18) and Formulation 2 (6.00). These results indicate that panellists found the formulations with 10 g and 20 g of sissou spinach acceptable. However, Formulation 3, which contained 30 g of sissou spinach, recorded the lowest overall acceptance score (5.36), suggesting that higher sissou spinach content negatively impacted consumer preference due to its pronounced slightly bitter taste and impact on texture. Finally, while the Control smoothie was the most preferred in all sensory attributes, Formulations 1 and 2 demonstrated good overall acceptance, indicating the potential for incorporating moderate levels of sissou spinach (10-20 g) into banana-based smoothies to enhance nutritional value without significantly compromising sensory quality. These findings are consistent with previous studies, which highlight the importance of balancing functional ingredients to maintain product acceptability among consumers [5, 20].

#### 4. Conclusion

Banana smoothies made with sissou spinach, honey, and lemon can be considered valuable products, as they demonstrate significant improvements in nutritional composition and sensory attributes. The findings revealed that Formulation 3 exhibited the highest protein content ( $1.59 \pm 0.02\%$ ) and ash content ( $0.96 \pm 0.02\%$ ). Sensory panellists approved of the formulated smoothies. However, the Control received excellent scores for taste, aroma, and overall acceptance, while Formulation 1 achieved better scores for appearance and texture, providing a balance between nutrition and sensory appeal. The smoothies containing sissou spinach, honey, and lemon had lower pH values, indicating greater acidity compared to the Control, which contained only banana. Variations in pH, colour, TSS, and moisture content highlighted the influence of sissou spinach, honey, and lemon, which enhanced acidity, freshness, sweetness, and nutritional value. Consequently, smoothies made with these ingredients present a convenient and nutritious alternative to traditional banana smoothies. Nevertheless, further research is required to explore the incorporation of other dark green vegetables, optimize ingredient proportions for enhanced antioxidant and micronutrient content, and apply preservation techniques such as pasteurization or high-pressure processing (HPP). These efforts could extend shelf life, maintain quality, and address consumer demand for convenient and nutritious beverages.

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#### Conflict of Interest

Authors declare that there is no conflict of interests regarding the publication of the paper.

#### Author Contribution

The authors confirm contribution to the paper as follows: **study conception and design:** Nurelysha Eriena Md. Nizam, Hatijah Basri; **data collection:** Hatijah Basri; **analysis and interpretation of results:** Nurelysha Eriena Md. Nizam, Hatijah Basri; **draft manuscript preparation:** Nurelysha Eriena Md. Nizam, Hatijah Basri. All authors reviewed the results and approved the final version of the manuscript.

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