Comparative analysis of phytochemical properties in preserved and non-preserved butterfly pea flower beverages during storage

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Abstract. The COVID-19 pandemic has increased public consciousness of the significance of ensuring food safety and promoting good health. The butterfly pea flower (Clitoria ternatea) is recognized for its therapeutic properties, as it harbors valuable components that promote health, notably a rich concentration of antioxidants. This study aims to analyze the changes in phytochemical content in butterfly pea flower beverages with and without preservatives during storage at different temperatures. The method involved preparing butterfly pea flower beverages with the addition of sodium benzoate at concentrations of 0 mg/L, 200 mg/L, 400 mg/L, and 600 mg/L, which were then stored at temperatures of 4°C, 27°C, and 45°C for five weeks. Weekly analyses were performed to quantify the levels of vitamin C, anthocyanins, and free radical inhibition activity. The sensory properties were also conducted to compare hedonic levels between preserved and non-preserved beverages. The findings indicated that the pH of the beverages tended to maintain stability during the storage period. However, there was a notable reduction in the vitamin C concentration. The level of anthocyanin is reduced, particularly when stored at higher temperatures. The inhibition of free radicals showed substantial variance among the different treatments. Sensory properties, including color and aroma, remained generally preferred by the panelists for both preserved and non-preserved beverages. Overall, the addition of sodium benzoate preservatives was effective in maintaining the phytochemical stability and sensory properties of butterfly pea flower beverages during storage.

1 INTRODUCTION

The COVID-19 pandemic has raised public awareness about the value of safe and nutritious food. Functional foods and beverages are more popular due to their health benefits [1]—natural sources of functional ingredients, particularly those containing less commonly found bioactive compounds. The butterfly pea flower/BF (Clitoria ternatea) is widely acknowledged for its medicinal properties due to the presence of beneficial chemicals. [2]. The flowers possess an appealing hue, unique fragrance, and a significant amount of

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antioxidants. Currently, BFs are extensively utilized as a primary ingredient in the production of food and beverages. Popular herbal teas in Southeast Asia are made from dried BFs [3].

The development of herbal sources into functional drinks involves an understanding of the content of active components. This method is necessary to ensure that the taste and health benefits are both acceptable. A challenge in the development of functional beverages, which provide bioactive compounds to consumers in a convenient format, is the poor stability of the compounds [4]. The chemicals are heat-labile and susceptible to autooxidation. In a multi-component system, interactions amongst polyphenols may have varying effects on compound stability. Furthermore, their stability is impacted by common beverage additives such as ascorbic acid and citric acid [5,6].

The growing popularity of veganism has led to an increased demand for non-dairy beverages, which are also advantageous for individuals with lactose intolerance [7]. Functional beverages made from flowers and other plant components are mostly consumed fresh or in the form of dry powder. Recent years have seen a significant increase in the popularity of ready-to-drink products. Consumer preference for ready-to-drink beverages is attributed not only to their advantageous health properties but also to their nutritional composition and convenient liquid form. Stability throughout its store life is essential information for the development of functional beverages. As the shelf-life is determined by the length of time the functional components are retained, the retention of the bioactive compounds can be regarded as a crucial determinant of quality. The degree of consumer acceptability is also influenced by the flavor and appearance of a product, which are additional factors to consider when estimating its shelf life [8,9].

A prior investigation concentrated on the development of butterfly flower beverages that demonstrated admirable antioxidant properties and were well received by consumers [10]. The research findings indicated that the beverage formulated from 4.5 g/L of telang flower and 5 ml/L of lime juice contained the lowest levels of sugar and calories compared to all other treatment combinations. The current investigation continued in examining the stability of these bioactive compounds in a ready-to-drink BF product, both with and without preservation, across various temperature conditions. The objective of this study was to evaluate the impact of varying storage temperatures on the levels of vitamin C, anthocyanin, free radical scavenging activity, and sensory properties of BF beverages.

2 Materials and Method

2.1 Materials

Fresh BFs were collected from the plant collection of the Teaching Factory of Politeknik Pembangunan Pertanian Yogyakarta Magelang. Fress lemongrass was supplied from Taruna Agro Nusantara Farmers, Bantul Regency, Yogyakarta Special Region. BFs were subsequently dehydrated using a solar dryer equipped with a black fabric covering for three days, resulting in a moisture content of less than 15% db. Lime, sugar, and sodium benzoate were purchased from the local market. 2,2-diphenyl-1-picrylhydrazyl (DPPH), methanol, ethanol, buffer solution, chloric acid, ascorbic acid, and acetic acid were utilized as chemical reagents. Except otherwise mentioned, all reagents were analytical grade.

2.2 BF Beverages Preparation

The preparation of beverages was followed by previous methods with slight modifications [10]. A mixture of 1 liter of water, 30 g of lemongrass, and 120 g of sugar was brought to a boiling point. Subsequently, 4.5 g of the flower was added and the burner was deactivated.

The lime juice was applied to the flower while it was being soaked for 30 minutes. Subsequently, the solution underwent filtration and was then readied for packaging. Sodium benzoate was introduced into the mixture while it was being heated, with concentrations of 0 mg/L, 200 mg/L, 400 mg/L, and 600 mg/L. The packaged product was thereafter stored at temperatures of 4, 27, and 45 ^{0}C for 5 weeks. A weekly inquiry was done to study the changes in phytochemicals.

2.3 Anthocyanin analysis

The measurement of anthocyanin content was conducted using the pH differential method [11]. BFs extract is dissolved in two separate buffer solutions, each containing a specific volume. The initial solution was dissolved in a potassium chloride buffer with a concentration of 0.025 M and a pH of 1.0. In comparison, the remaining solutions were dissolved in a sodium acetate buffer with a concentration of 0.4 M and a pH of 4.5. The number of samples used was determined, resulting in the absorbance value at the maximum visible wavelength, which falls within the linear range of the spectrophotometer. In addition, wavelengths ranging from 200 nm to 750 nm were employed to measure the anthocyanin concentration and viscosity of the sample solutions in both KCl and Sodium Acetate buffers. The absorbance of each solution was measured, and the result was computed using the following equation.

$A = (A540 - A700)1 - (A540 - A700)4.5$

Total monomeric anthocyanin from extract BFs was estimated as cyaniding-3- glucoside based on the following equation.

$MAP\ (\quad) = [(AxMWxDFx\ \ \varepsilon\ \ 10)\ x\ \ 1]$

description:
A: Absorbance of the solution
MW: Molecular weight
DF: Dilution factor
ε: Molar absorptivity of cyaniding-3-glucoside
b: the thickness of cuvette = 1
MAP: Monomeric Anthocyanin Pigment

2.4 Free radical inhibition activity analysis

The activity of inhibiting free radicals was assessed using the DPPH methods [12]. 1 mL of the sample solution was introduced into the test tube, followed by the addition of 4 mL of a 50 □L DPPH solution. The alteration in color was then examined. The mixture was homogenized and incubated for 30 minutes in a light-restricted environment, after which it was analyzed using a UV – Vis spectrophotometer at a wavelength of 517 nm.

2.5 Vitamin C analysis

The spectrophotometric method is employed to analyze Vitamin C, with ascorbic acid provided as the standard [13].

2.6 Sensory evaluation

The analysis involved 50 untrained panelists. They were presented with two samples of selected treatment beverages and evaluated them based on specific criteria, such as colour, taste, and flavour. The hedonic assessment ratings range from strongly like (5), like (4), moderate (3), dislike (2), and strongly dislike (1).

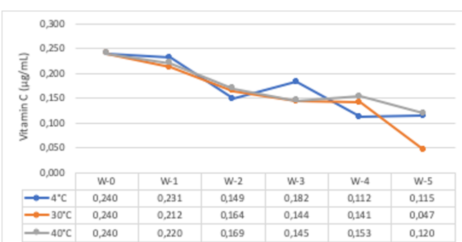
2.7 Statistical analysis

SPSS IBM 25 was used to do the one-way ANOVA and Duncan Multiple Range Test (DMRT) on the data. Before the one-way ANOVA, the hedonic scores were turned into interval data using the Methods of Successive Interval (MSI). A 95% confidence range showed that there was a significant difference.

3 RESULTS AND DISCUSSION

3.1 The change of vitamin C and pH during storage

The alterations in the levels of vitamin C and pH of the BF beverages are displayed in Table 1 and Fig 1, respectively. Observations indicated that the pH of BF beverages remains consistent while stored at temperatures of 4, 30, and 40°C, regardless of whether they were preserved or not. Conversely, a notable reduction in vitamin C was observed in all treatments. The beverages analyzed in this study have a pH ranging from 3.5 to 4.3, indicating that they possess acidic qualities. The inclusion of lime in the drink was the cause of this. Lime is a naturally occurring substance that contains citric acid and has a pH level ranging from 2 to 3 [14].



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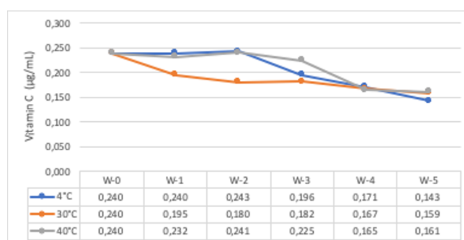

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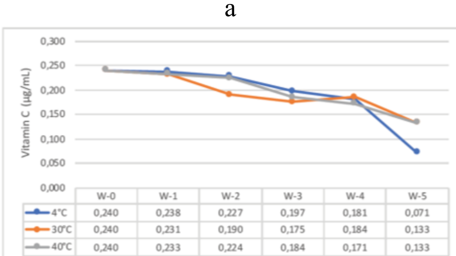

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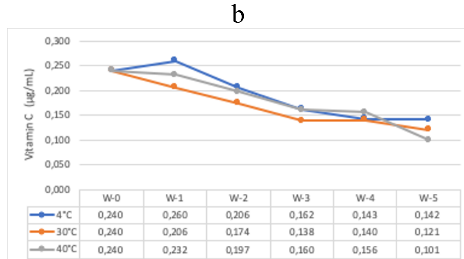

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Fig. 1. Changes in vitamin C during storage.

a) P1: non-preserved beverages b) P2: beverages with sodium benzoate 200 mg/L c) P3: beverages with sodium benzoate 400 mg/L; d) P4: beverages with sodium benzoate 600 mg/L.

The stability of vitamin C is affected by various parameters, including storage temperature, pH, oxygen levels, mineral concentration, salt and sugar content, and the presence or absence of enzymes or light [14]. The previous study revealed numerous chemical processes that lead to the degradation of vitamin C and the occurrence of biochemical reactions during storage, together with microbial activity, which collectively contribute to variations in pH in all fruit beverages. The degradation of vitamin C over time may not be consistent with other fruits stored under similar conditions. Furthermore, boiling samples significantly reduced the amount of vitamin C in the fruits. The nutritional value of vitamin C declined when exposed to volatile environmental conditions. Consequently, a decrease in the vitamin C level was seen over the storage period of our experiment. According to another study, storage temperature had a significant impact on the rate of vitamin degradation in citrus juice; at 7 °C, the rate of degradation is lower than at 28 °C [15]. The storage of orange juice in the refrigerator can maintain vitamin C levels and its acceptability [16].

Table 1. pH of BF beverages during storage

Week	pH											
	P1			P2			P3			P4		
	4°C	30°C	40°C	4°C	30°C	40°C	4°C	30°C	40°C	4°C	30°C	40°C
0	3.9	3.90	3.90	3.97	3.97	3.97	4.10	4.10	4.1	4.20	4.20	4.20
1	3.97	3.90	3.90	4.00	4.07	4.00	4.10	4.27	4.1	4.23	4.33	4.20
2	3.93	3.90	3.90	4.00	4.10	4.00	4.20	4.10	4.1	4.27	4.20	4.20
3	3.93	3.27	3.87	4.07	3.50	4.07	4.13	4.40	4.1	4.33	4.20	4.20
4	3.93	3.50	3.93	4.00	3.60	4.00	4.10	4.10	4.1	4.23	4.30	4.17
5	3.9	4.13	3.83	4.00	4.10	3.90	4.20	4.17	4.1	4.20	4.20	4.10

3.2 The Change of Antocyanin and Free Radical Inhibition Activity during Storage

Table 2 provides an overview of the anthocyanin content of BFs. A statistically significant difference ($p<0.05$) in the anthocyanin content of BFs during storage has been observed, according to the data collected. Significant variations in statistical analysis were observed for each storage temperature starting in the initial week of storage ($p<0.05$). The product can be preserved at low temperatures, which can reduce anthocyanin degradation. The treatment containing a preservative concentration of 200 mg/L (P2) and subjecting it to a temperature of 400C for five weeks exhibited the greatest anthocyanin degradation, at 91.89%. The treatment contained a 400 mg/L preservative (P4) and storing it at 40°C for five weeks exhibited the highest level of anthocyanin degradation (21.62%).

Anthocyanins are generally more stable in acidic solutions than neutral or alkaline solutions. Anthocyanins have different chemical structures depending on the pH of the solution. The stability of anthocyanins is also affected by temperature. The rate of anthocyanin breakdown (degradation) tends to increase during the storage process, accompanied by an increase in temperature [17]. Thermal degradation leads to loss of color in anthocyanins, which eventually results in browning. Anthocyanin degradation in this study can also be seen visually (Fig. 2). In the color change of BFs, which is fading in storage treatment at 40°C of BFs beverage without and with preservatives (P1, P2, P3, P4).

The stability of antioxidants was examined in several fruit juices. The findings revealed that the majority of the samples experienced significant deterioration in both bioactive components and antioxidant activity within 7 days. The degradation of the antioxidant activity of fruit juice during storage was shown in a previous study, which claimed that the decrease in ascorbic acid and total phenolic compounds was the possible reason for the reduction of antioxidant activity [18]. Another study demonstrated that watermelon juice was affected by storage temperatures and conditions, resulting in significant changes in its physical and chemical properties. This led to a drop in the overall amount of phytochemicals, which in turn reduced the antioxidant activities of the juice during 9 days of storage [19].

Table 2. Anthocyanin content of flower drink during storage.

Week	**4°C**				**30°C**				**40°C**			
	P1	**P2**	**P3**	**P4**	**P1**	**P2**	**P3**	**P4**	**P1**	**P2**	**P3**	**P4**
0	3.33^{d}	3.33^{d}	3.33^{d}	3.33^{d}	3.33^{d}	3.33^{d}	3.33^{e}	3.33^{c}	3.33^{d}	3.33^{d}	3.33^{e}	3.33^{c}
1	2.68^{cA}	3.30^{bB}	3.24^{dB}	3.10^{bcAB}	2.73^{dA}	2.52^{cB}	2.80^{dB}	1.95^{bAB}	2.09^{cB}	1.64^{cAB}	1.49^{dA}	1.23^{bA}
2	2.4^{A}	3.56^{Bb}	2.95^{cAB}	2.95^{abcAB}	1.98^{c}	2.40^{c}	2.04^{c}	1.72^{ab}	1.41^{b}	1.22^{bc}	1.19^{cd}	0.97^{b}
3	2.24^{abA}	3.52^{bC}	2.81^{bcB}	2.41^{aA}	1.32^{bcA}	2.07^{bC}	1.56^{bB}	1.37^{aAB}	1.10^{bcB}	0.81^{bAB}	1.03^{bcB}	0.45^{aA}
4	2.03^{aA}	3.44^{bB}	2.59^{bA}	2.51^{abA}	1.65^{bcB}	1.96^{bC}	1.55^{bAB}	1.29^{aA}	0.95^{abD}	0.27^{aA}	0.70^{abC}	0.45^{aB}
5	2.50^{bcB}	2.01^{aA}	1.89^{aA}	2.61^{abB}	1.20^{aAB}	1.64^{aC}	1.00^{aA}	1.38^{aB}	0.86^{aC}	0.27^{aAB}	0.38^{aB}	0.12^{aA}

The data represents the mean value (n = 3). The mean values with lowercase superscripts in different rows indicate significant differences ($p<0.05$). The mean values with capital letter superscripts in different columns at the same temperature also indicate significant differences ($p<0.05$). P1 refers to non-preserved beverages, P2 refers to beverages with sodium benzoate 200 mg/L, P3 refers to beverages with sodium benzoate 400 mg/L, and P4 refers to beverages with sodium benzoate 600 mg/L.

The study found that the storage of BF beverages had a noticeable impact on the ability to suppress the formation of free radicals, with a statistically significant difference ($p < 0.05$). The treatment had free radical inhibition activity ranging from 37.10% to 61.54%. According to the obtained results, it was evident that the treatment of BF beverages with a high anthocyanin content does not always result in stronger free radical inhibitory activity. This was due to the fact that other antioxidant compounds of BF may significantly impact free radical inhibition. The predominant components included in BF include flavonoids, anthocyanins, alkaloids, saponins, tannins, taraxerol, and taraxerone. Previous research has found 14 kinds of flavonol glycosides in flowers using spectrophotometry [2]. Free radical inhibition is shown in Table 3.

Table 3. Inhibition of free radicals of BFs beverages during storage.

Week	4°C				30°C				40°C			
	P1	P2	P3	P4	P1	P2	P3	P4	P1	P2	P3	P4
0	47.92^{bA}	47.57^{bcA}	44.43^{aA}	61.54^{eB}	47.92^{bB}	47.57^{bB}	44.43^{cA}	61.54^{cC}	47.92^{bcB}	47.57^{bB}	44.43^{bA}	61.54^{dC}
1	43.32^{aA}	48.41^{cB}	47.43^{bB}	57.14^{dC}	45.27^{aB}	48.41^{bC}	37.10^{aA}	50.65^{bD}	48.55^{cB}	40.94^{aA}	49.39^{cB}	56.58^{cC}
2	44.99^{aA}	46.46^{bA}	48.97^{bcB}	51.55^{cC}	43.18^{aB}	44.50^{abB}	37.52^{aA}	45.62^{aB}	44.15^{aB}	40.87^{aA}	40.87^{aA}	56.23^{cC}
3	45.20^{aA}	48.41^{cB}	50.23^{cC}	57.56^{dD}	46.04^{aB}	46.46^{bB}	37.38^{aA}	45.69^{aB}	46.25^{abc}	44.22^{ab}	45.34^{b}	45.20^{a}
4	44.78^{aB}	42.69^{aA}	48.76^{bcC}	48.06^{bC}	44.99^{aB}	40.73^{aA}	40.87^{aA}	45.20^{aB}	45.20^{ab}	45.48^{ab}	45.27^{b}	45.55^{a}
5	44.85^{aA}	48.41^{cB}	45.62^{aA}	45.62^{aA}	47.99^{bB}	41.43^{aB}	45.62^{cAB}	45.55^{aAB}	44.29^{aA}	44.85^{abA}	45.62^{bAB}	48.62^{bB}

The data represents the mean value (n = 3). The mean values with lowercase superscripts in different rows indicate significant differences ($p<0.05$). The mean values with capital letter superscripts in different columns at the same temperature also indicate significant differences ($p<0.05$). P1 refers to non-preserved beverages, P2 refers to beverages with sodium benzoate 200 mg/L, P3 refers to beverages with sodium benzoate 400 mg/L, and P4 refers to beverages with sodium benzoate 600 mg/L.

3.3 BF beverage's sensory quality

The sensory test for the BF beverages was conducted by making visual observations and surveying panelists to determine their level of satisfaction. The visual state of the BF beverages during storage at 4, 30, and 40 0C included color. Fig 2 illustrates the appearance of the color of the beverages. Throughout the five weeks of storage, the color of the beverages at P4 remained relatively constant. The addition of up to 600 mg/L of sodium benzoate had a significant impact on the color stability of the product. Previous research has shown that using sodium benzoate as a preservative can extend the shelf life of nutmeg jam to 60 days while reducing the number of microbe colonies at 750 mg of sodium benzoate. Sodium benzoate also influences the vitamin C concentration, total acid content, and pH value of nutmeg jam [20]. In Fig 2 it can be seen that in the storage of the BF beverages at a temperature of 400C there is a change in the purple color of the product, the change is visually visible starting at week 3 at P1, P2, and P3.

In this study, the level of preference for BF beverages was carried out by BF beverages with the best shelf life, namely BF beverages with preservatives 600 mg/L (P4) (data not shown) compared to controls (P1). Statistical investigation revealed that there was no significant difference in products with and without preservatives. Both treatments were categorized as "like". This demonstrated that the panelists found it acceptable to add the preservative sodium benzoate to the flower drink at a concentration of 600 mg/L.

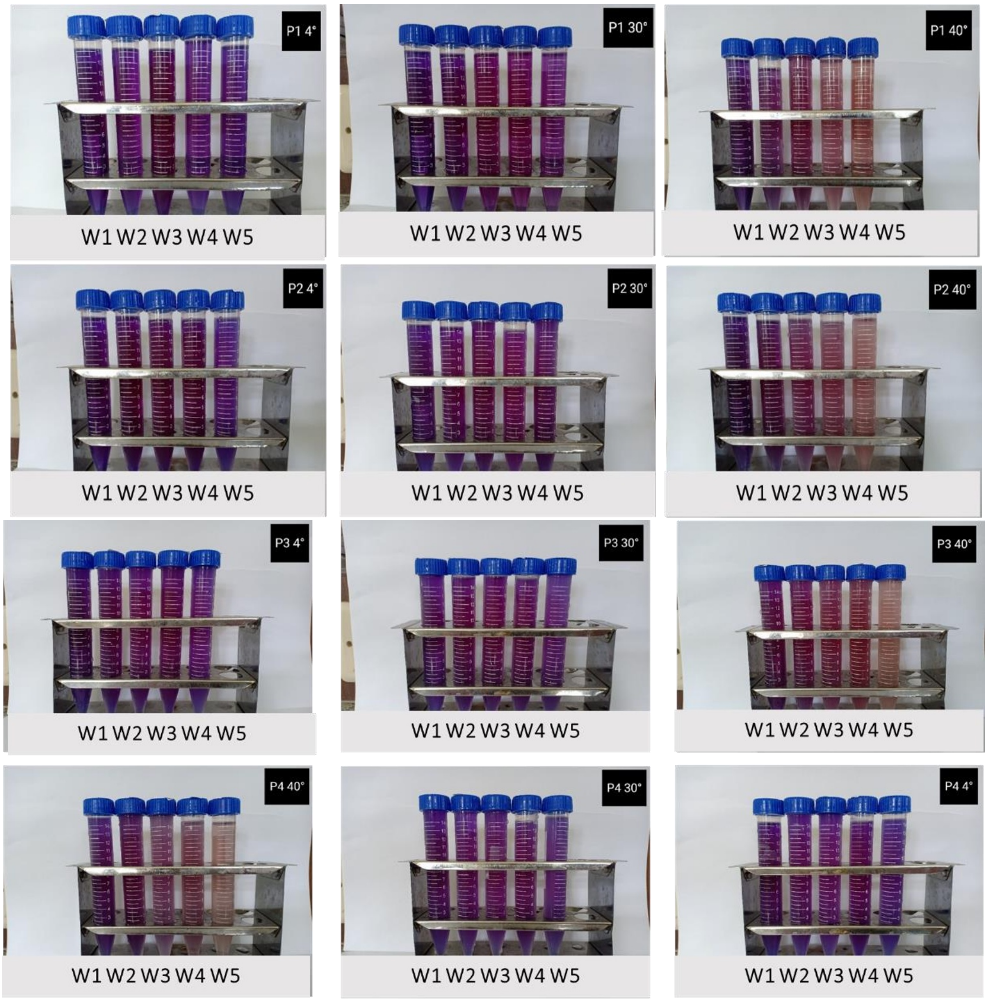

Fig. 1. Visual appearance of the flower drink during storage

Table 4. Hedonic level of BF beverages

Treatment	Colour	Taste	Flavour	Average	Criteria
P1	3.80	3.78	4.03a	3.87	Like
P4	3.52	3.05	3.80b	3.46	Like

3.4 BF Nutrition Value

Table 5 shows the nutritional value of BFs beverages. According to the findings, there are no significant differences in sugar, fat, or protein composition between BFs beverages with and without preservatives. Some of the nutritional value of beverages was unaffected by the presence of sodium benzoate. The vitamin C component showed that the content of vitamin C grows directly proportional to the concentration of sodium benzoate employed in the beverage manufacturing process. The levels of iron, calcium, and potassium in telang flower drinks follow the same trend of increase. The reverse tendency was observed in cholesterol concentrations, which tend to fall after adding sodium benzoate to the beverage.In previous investigations, the addition of sodium benzoate altered the total microorganisms in pineapple juice. The addition of sodium benzoate reduces the total microbial concentration and increases the inhibitory power of microbial activity. Adding additional sodium benzoate to pineapple juice inhibits microbial development more effectively (Nurman, Muhajir, & Muhardina, 2018). Microbial development will degrade the quality of beverage, which waslikely to reduce their nutritional value. Table 6 shows that striped floral beverages with the preservative sodium benzoate maintain a consistent nutritional content during storage.

Table 5. Nutritional Content of BF Beverages.

Component	P1			P2			P3			P4		
Total sugar (%)	1.59	±	0.00	1.55	±	0.00	1.65	±	0.00	1.64	±	0.00
Fat (%)	0.03	±	0.00	0.03	±	0.00	0.03	±	0.00	0.02	±	0.00
Protein (%)	0.11	±	0.00	0.10	±	0.00	0.08	±	0.00	0.13	±	0.00
Total Cholesterol (mg/100g)	5.30	±	0.27	2.42	±	0.13	2.81	±	0.13	2.07	±	0.13
Energy (kal/g)	471.34	±	1.37	478.24	±	8.30	919.10	±	15.07	498.86	±	1.93
Fe (mg/100g)	6.61	±	0.04	8.41	±	0.12	7.55	±	0.04	8.51	±	0.04
Ca (mg/100g)	10.89	±	0.34	13.35	±	0.12	12.73	±	0.13	14.46	±	0.07
K (mg/100g)	16.38	±	0.04	18.20	±	0.04	17.18	±	0.02	18.39	±	0.02

Table 6. Comparison Nutritional Content of BF Beverages after 30 days storage.

Component	P1						P4					
	Week 0			Week 5			Week 0			Week 5		
Total sugar (%)	1.59	±	0.00	1.59	±	0.00	1.64	±	0.00	1.69	±	0.00
Fat (%)	0.03	±	0.00	0.02	±	0.00	0.02	±	0.00	0.02	±	0.00
Protein (%)	0.11	±	0.00	0.10	±	0.00	0.13	±	0.00	0.10	±	0.00
Total Cholesterol (mg/100g)	5.30	±	0.27	2.08[a]	±	0.13	2.07	±	0.13	2.05	±	0.13
Energy (kal/g)	471.34	±	1.37	469.12	±	4.25	498.86	±	1.93	480.33	±	9.84
Fe (mg/100g)	6.61	±	0.04	6.79	±	0.02	8.51	±	0.04	9.30	±	0.02
Ca (mg/100g)	10.89	±	0.34	15.89	±	0.07	14.46	±	0.07	16.49	±	0.05
K (mg/100g)	16.38	±	0.04	18.14	±	0.00	18.39	±	0.02	19.33	±	0.00

4 Conclusion

Vitamin C flower drink on storage for 5 years degraded in BF beverages without and using preservatives. Storage at cold temperatures can minimize the degradation of vitamin C. The anthocyanin content of beverages had decreased significantly during storage, especially in drinks stored at temperatures of 270C and 400C. There was no significant difference in the taste and color attributes of BF beverages without and using preservatives, while in the aroma attributes, there were significant differences.

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