Characteristics and stability of benzoic acid-enriched aloe-buni drinks: potential as functional drink

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Abstract. Aloe-buni is a popular functional beverage due to its flavonoids and anthocyanins that function as antioxidants and antidiabetics. However, it has a short shelf life and is unstable because flavonoids easily form precipitates. To maintain quality and extend shelf life, the addition of food additives such as sorbic acid, benzoic acid, and sodium benzoate is required. The purpose of this study was to determine the type of preservative, concentration, and preservation temperature that can maintain the quality of Aloe-buni drinks. This study used a two-factor complete randomized design (CRD), namely the type of preservative (sorbic acid, benzoic acid, and sodium benzoate) and concentration (0.01%; 0.02%; 0.03%), Observation variables include vitamin C content, Antioxidant Activity, Viscosity, Degree of acidity (pH), total microbes and determination of shelf life by the Extended Storage Studies (ESS) method. The characteristics of this aloe-buni drink with benzoic acid added with a concentration of 0.02% are pH 3.35, viscosity 2.38 m.Pa.s, inhibiting antioxidant activity 7.40%, and vitamin C 10.57 µg/g, and total microbes <2.5 x 102 cfu/g. The quality of Aloe-buni drinks lasted until the 14th day at cold temperature storage. The addition of 0.02% benzoic acid to Aloe-buni drinks produced the best characteristics. The addition of benzoic acid can maintain the quality of the drink until the 14th day at cold temperature storage.

1. INTRODUCTION

The need for foods and beverages that can improve health is currently driving the development of functional food innovations [1]. Aloe-buni drink is one of the functional drinks. Its functional properties are evidenced by the presence of antioxidant and antidiabetic bioactive compounds such as flavonoids and anthocyanins. Flavonoids can regulate glucose metabolism, liver enzyme activity, and lipid metabolism, which can effectively reduce the incidence and development of diabetes [2]. Anthocyanins can reduce blood glucose levels by protecting β-cells, improving insulin resistance, increasing insulin secretion, and improving liver and lipid metabolism [3]. This antioxidant and antidiabetic compound is obtained from the buni fruit. Besides buni fruit, this drink is made from aloe vera gel. Aloe vera gel has antidiabetic properties. Research conducted by [4], found that panellists consumed 10-20 ml

of aloe vera gel per day and obtained results of cholesterol decreased by 15%, triglycerides by 30%, and hyperlipidemia in patients with low-density lipoprotein cholesterol decreased by 18%. In 2023, clinical trials were conducted on Aloe-buni drinks using experimental rats. The test found that the aloe vera drink could lower the blood sugar levels of mice suffering from diabetes. Aloe vera and buni have many benefits, but in Indonesia and the Philippines have not been maximally utilised for food. Aloe vera is usually used in beauty products, and buni fruit is eaten fresh.

Aloe-buni in liquid form has the disadvantage of a very short shelf life. It only lasts for 6 days if stored at room temperature (28±2°C) and can last for 15 days if stored at refrigerated temperature (10±2°C) [5]. The consumption time of an aloe vera drink is very short because it contains flavonoids. Beverages containing flavonoids in large quantities will precipitate. Precipitation is one of the characteristics of drinks that have low stability, so the shelf life is short. The addition of additives in the form of food preservatives is needed to help extend the shelf life of liquid Aloe-buni drinks [6].

Additives commonly used in fruit juice drinks are sorbic acid, benzoic acid and sodium benzoate. The use of these additives is regulated by the Food and Drug Administration. The maximum limit of sorbic acid use is 0-25 mg/kg body weight [7]. The maximum use of benzoic acid in soft drinks is 600 mg/kg, and sodium benzoate is 5 mg/kg body weight. The advantage of sorbic acid as a preservative is its ability to suppress the growth of mould and yeast, making it effective in extending the shelf life of foods such as margarine, fruit juice, cheese, and wine [8]. In addition, sorbic acid is also an antimicrobial agent commonly used as a preservative in food products, and is generally considered safe for consumption in reasonable amounts. The advantage of benzoic acid is that it can limit the growth of various microorganisms that can cause spoilage in beverages, making it effective as a preservative [9]. The advantage of sodium benzoate is that it actively inhibits the growth of bacteria and yeast, making it effective in maintaining the quality of beverages [10].

Based on the type of additives and their concentrations, researchers want to conduct research on the provision of various types of food preservatives in Aloe-buni drinks with different concentrations. With this research, it is expected to know the type of preservative and its concentration that is most effective for maintaining the quality and extending the shelf life of Aloe-buni drinks.

2. METHODS

This research has been conducted for 10 months, from February to November 2024, in Denpasar, Bali, Indonesia. Buni fruit and aloe vera were used as the main ingredients in this study. Buni was obtained in Dauh Pala Village, Tabanan, Bali, while aloe vera was obtained in Taro Village, Gianyar, Bali. The data obtained were analysed by ANOVA variety. The results of single treatment fingerprints that showed a real effect ($P<0.05$) became very real ($P<0.01$), followed by 5% BNT Examination, and if the results of various treatment fingerprints showed an interaction, followed by Duncan's Multiple Range Test. This study used a completely randomised design (CRD). Analysis was carried out in this study:

2.1 Vitamin C

The reagent solution was prepared by mixing 500 ml of 0.6 M sulfuric acid with 5.322 g sodium phosphate and 2.471 g ammonium molybdate. The reagent (3 ml) was mixed with 0.3 ml of sample and incubated at 95°C for 90 min in a water bath. After incubation, it was cooled in water for 5 min before absorbance was measured at 695 nm. Results are expressed as ascorbic acid equivalents in mg/g.

2.2 Antioxidant Activity

The radical inhibitory activity of the samples was based on their inhibition of 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals. Sample solutions were prepared at various concentrations (0.9µL to 100µL/ml) in methanol. Put each sample solution as much up 2 ml into a test tube. After that, 2 ml of 0.002% DPPH solution (in methanol) was added. Incubated in a dark room for 30 minutes, the absorbance of the sample was measured with a UV-Vis spectrophotometer. The amount of antioxidant activity was measured with the per cent inhibition parameter.

2.3 Viscosity

Viscosity will be measured with a viscosity measuring instrument also known as a viscometer. To be able to measure viscosity, the sample in the material viscometer must be in the container while the shaft moves while immersed in the liquid. The shaft or router must not touch the bottom of the container during the analysis process. The router used in analysing the viscosity of the Aloe-buni drink is router 2 with a speed of 60 rpm.

2.4 pH

The pH value of Aloe-buni health drinks is measured with a ph meter. Before using the pH meter, standardise the pH meter first. Turn on the pH meter, then rinse the electrode with distilled water, and then dry it using tissue paper. Drying the pH meter electrode is enough to attach tissue paper to the edges and ends of the electrode, scratched electrodes, and validation of its accuracy can be changed, for it needs to be recalibrated.

2.5 Total Plate Count

A sample preparation was prepared, 10 grams of sample was dissolved in an Erlenmeyer each has been filled with 90 ml of 0.86% NaCl. 1 ml of sample was pipetted and put into 9 ml of 0.86% NaCl solution (10-2 dilution). Further dilutions were made up to dilution 10^{-6} and then plated on dilutions 10^{-1} to 10^{-6}. Take 1 ml of each sample from each dilution and plant in PCA media that has been sterilised, incubate for 24 hours at 37 ± 5°C in an inverted position and the number of colonies that grow is observed and counted.

2.6 Determination of Shelf Life (The Extended Storage Studies Method)

The Extended Storage Studies (ESS) method is the determination of the expiration date by storing a series of products under normal conditions. This method is accurate and precise, but the use of this method requires a long time and analysis of relative quality parameters, as well as, expensive and used for products that have an expiration date of less than 3 months. Each treatment on the sample has been coded. The questionnaire will be distributed to consumers, to give a score to the sample to be studied as for the measurement scale of the favorability test or ranking test, which is as follows: 1. Very dislike, 2. Dislike, 3. Somewhat dislike, 4. Ordinary, 5 Somewhat Like, 6. Like, and 7. Very Like. The results of the analysis with this method will be inscribed best before on the product packaging.

3. RESULTS

3.1 Characteristics of Aloe-buni Drink

Aloe-buni drink is one of the products that has functional properties. The fiber obtained from aloe vera gel and the bioactive components derived from buni fruit, such as vitamin C, flavonoids, and anthocyanins, categorize Aloe-buni as a functional beverage. Aloe vera gel is combined with buni fruit to eliminate the bitter taste of aloe vera gel. The characteristics of aloe-buni drink can be seen in Table 1.

Table 1. Characteristics of Aloe-buni Drink

No	Component	Amount
1	pH	3,49
2	Water Content	68,69%
3	Carbohydrate	20,12%
4	Ash Content	1,33%
5	Protein Content	0,20%
6	Fat Content	9,84%
7	Vitamin C	3,55%
8	Total Anthocyanin	0,94 mg/100ml
9	Total Flavonoid	0,13 mg/gr

Fresh Aloe-Buni has a drawback, which is low stability, causing changes in the characteristics of the drink as well as antioxidant activity within 3 days at room temperature.

3.2 Vitamin C

The Aloe-buni beverage with different preservatives and concentrations did not have a significant effect on the vitamin C content of the Aloe-buni drink. Aloe-buni drinks with preservatives Benzoic Acid concentration 0.02% have the lowest vitamin C, 10.57 µg/g, and aloe-buni drinks with preservatives Sodium Benzoate concentration 0.03% have the highest vitamin C, which is 14.46 µg/g. Vitamin C functional aloe-buni drinks can be seen in Table 2.

Table 2. Results of Vitamin C Analysis of Aloe-Buni Beverages with Different Types and Concentrations of Preservatives

Treatment	Concentration			Average	
	0,01%	0,02%	0,03%		
Day 0					
Sorbic Acid	9,28	9,94	9,37	9,53	a
Sodium benzoate	9,21	9,72	9,22	9,38	a
Benzoic Acid	11,68	8,08	9,83	9,86	a
Average	10,06 a	9,25 a	9,47 a		
Day 7					
Sorbic Acid	11,64	10,61	12,74	11,66	a
Sodium benzoate	10,63	11,71	14,46	12,27	a
Benzoic Acid	11,09	10,57	11,81	12,27	a
Average	11,12 a	10,96 a	13,01 a		

Description: Mean values in one row followed by the same letter mean not significantly different in the 5% BNT test.

3.3 Antioxidant Activity

Aloe-buni with preservatives and different concentrations significantly affects the viscosity of Aloe-buni drinks. Aloe-buni drinks with Benzoic Acid preservative concentration of 0.01% had the lowest antioxidant activity of 11.33% inhibition, and aloe-buni drinks with Benzoic Acid preservative concentration of 0.03% had the highest antioxidant activity of 17.98% inhibition. The antioxidant activity of aloe-buni functional drinks can be seen in Table 3.

Table 3. Results of Antioxidant Activity Analysis of Aloe-Buni Drinks with Different Types and Concentrations of Preservatives

Treatment	Average	
	Day 0	Day 7
Sorbic Acid 0,01%	5,52 bc	16,26 abc
Sodium Benzoate 0,01%	8,33 ab	12,11 bc
Benzoic Acid 0,01%	9,27 a	11,33 c
Sorbic Acid 0,02%	8,33 ab	17,56 a
Sodium benzoate 0,02%	6,18 bc	15,74 abc
Benzoic Acid 0,02%	7,40 abc	17,34 ab
Sorbic Acid 0,03%	6,37 abc	12,11 bc
Sodium Benzoate 0,03%	5,43 bc	15,74 abc
Benzoic Acid 0,03%	4,49 c	17,98 a

Description: Mean values followed by the same letter are not significantly different at 5% Duncan test.

3.4 Viscosity

Aloe-buni drink with preservatives and different concentrations does not have a significant effect on the viscosity of the Aloe-buni drink. Aloe-buni drinks with Benzoic Acid preservatives at a concentration of 0.03% have the lowest viscosity of 1.5 Pa.s and aloe-buni drinks with Benzoic Acid preservatives at a concentration of 0.01% have the highest viscosity of 3.88 m.Pa.s. Viscosity of aloe-buni functional beverages can be seen in Table 4.

Table 4. Viscosity Analysis Results of Aloe-Buni Beverages with Different Types and Concentrations of Preservatives

Treatment	Concentration			Average	
	0,01%	0,02%	0,03%		
Day 0					
Sorbic Acid	2,25	2,00	2,63	2,29	a
Sodium benzoate	2,50	2,75	1,75	2,33	a
Benzoic Acid	2,75	2,38	2,88	2,67	a
Average	2,50 a	2,38 a	2,42 a		
Day 7					
Sorbic Acid	3,00	3,00	2,75	2,92	a
Sodium benzoate	2,38	2,13	2,25	2,25	a
Benzoic Acid	3,88	2,38	1,50	2,58	a
Average	3,08 a	2,50 a	2,17 a		

Description: Mean values in one row followed by the same letter mean not significantly different in the 5% BNT test.

3.5 pH

Aloe-buni drinks with preservatives and different concentrations, they have no significant effect on the pH of Aloe-buni drinks when stored at cold temperatures. Aloe-buni drinks with 0.01% concentration of Benzoic Acid preservatives have the lowest pH of 3.32, and aloe-buni drinks with 0.03% concentration of Sodium Benzoate preservatives have the highest pH of 3.43. The degree of acidity of aloe-buni functional drinks can be seen in Table 5.

Table 5. Results of pH Analysis of Aloe-Buni Beverages with Different Types and Concentrations of Preservatives

Treatment	Concentration			Average
	0,01%	0,02%	0,03%	
Day 0				
Sorbic Acid	3,33	3,34	3,3	3,33 a
Sodium benzoate	3,34	3,34	3,4	3,35 a
Benzoic Acid	3,29	3,32	3,33	3,31 a
Average	3,32 a	3,33 a	3,34 a	
Day 7				
Sorbic Acid	3,35	3,37	3,39	3,37 a
Sodium benzoate	3,39	3,38	3,43	3,40 a
Benzoic Acid	3,32	3,35	3,40	3,35 a
Average	3,35 a	3,36 a	3,41 a	

Description: Mean values in one row followed by the same letter mean not significantly different in the 5% BNT test.

3.6 Total Plate Count

Aloe-buni drink with preservatives and different concentrations does not have a significant effect on the total microbes in the Aloe-buni drink. Aloe-buni drinks with preservatives Benzoic Acid concentration 0.02% has the lowest total microbes of 0.00 cfu/g. Aloe-buni drinks with preservatives, Sodium Benzoate concentration 0.03%, has the highest total microbes of 60.00 cfu/g. The total number of microbes in aloe- buni functional drinks can be seen in Table 6.

Table 6. Results of Total Microbial Analysis of Aloe-Buni Beverages with Different Types and Concentrations of Preservatives

Treatment	Concentration			Average
	0,01%	0,02%	0,03%	
Day 0				
Sorbic Acid	20,00	10,00	20,00	16,67 a
Sodium benzoate	5,00	5,00	15,00	8,33 a
Benzoic Acid	45,00	45,00	55,00	48,33 a
Average	23,33 a	20,00 a	30,00 a	
Day 7				
Sorbic Acid	15,00	25,00	15,00	18,33 a
Sodium benzoate	30,00	10,00	60,00	33,33 a
Benzoic Acid	30,00	0,00	10,00	13,33 a
Average	25,00 a	11,67 a	28,33 a	

Description: Mean values in one row followed by the same letter mean not significantly different in the 5% BNT test.

3.7 Determination of Shelf Life with the Extended Storage Studies Method

Storage temperature affects the quality of Aloe-buni beverages. Storage at 13±1°C kept the beverage quality high until day 14 but started to decline on day 16. In contrast, at 29±1°C, the quality of the beverage decreased dramatically from day 2. This shows that storage at low temperatures is more effective in maintaining product quality.

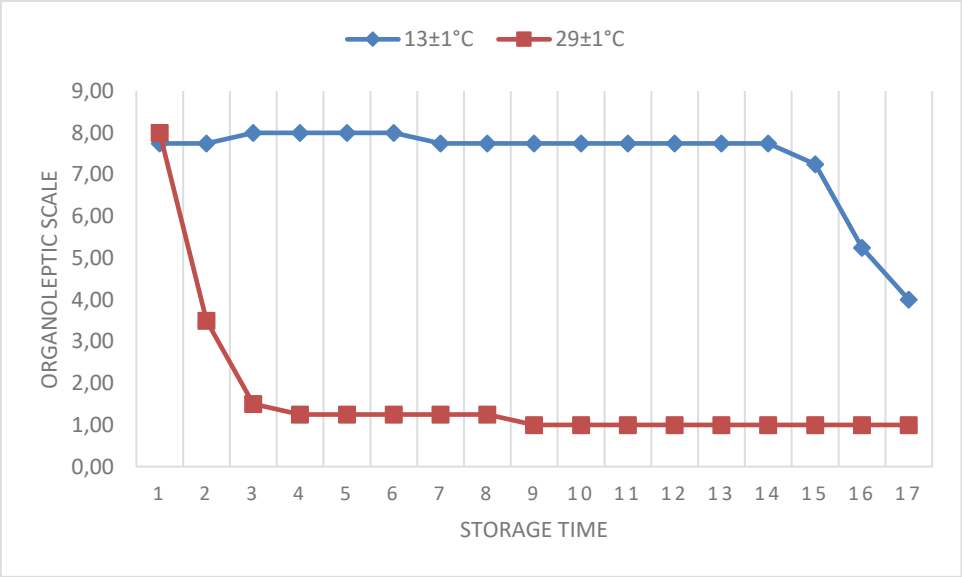

Fig. 1. Chart of Panellist Acceptance of Aloe-Buni Quality

Based on the organoleptic evaluation of Aloe Buni beverage aroma over a 17-day storage period at two different temperatures (13±1°C and 29±1°C), it was found that storage temperature significantly influenced the stability of the product's aroma (Figure 2.). At the lower temperature (13±1°C), the aroma scores remained relatively stable for the first 15 days, ranging between 7 and 8, indicating that the aroma quality was still acceptable to the panelists. However, a sharp decline in aroma score was observed after day 15, suggesting the onset of sensory degradation likely due to the breakdown of volatile aromatic compounds. In contrast, storage at the higher temperature (29±1°C) led to a rapid deterioration in aroma. The aroma score dropped dramatically from approximately 7 on day one to below 2 by the second day and remained consistently low for the remainder of the storage period. This indicates that elevated temperatures accelerate both chemical and microbiological reactions responsible for aroma degradation, thereby significantly reducing the sensory shelf life of the product.

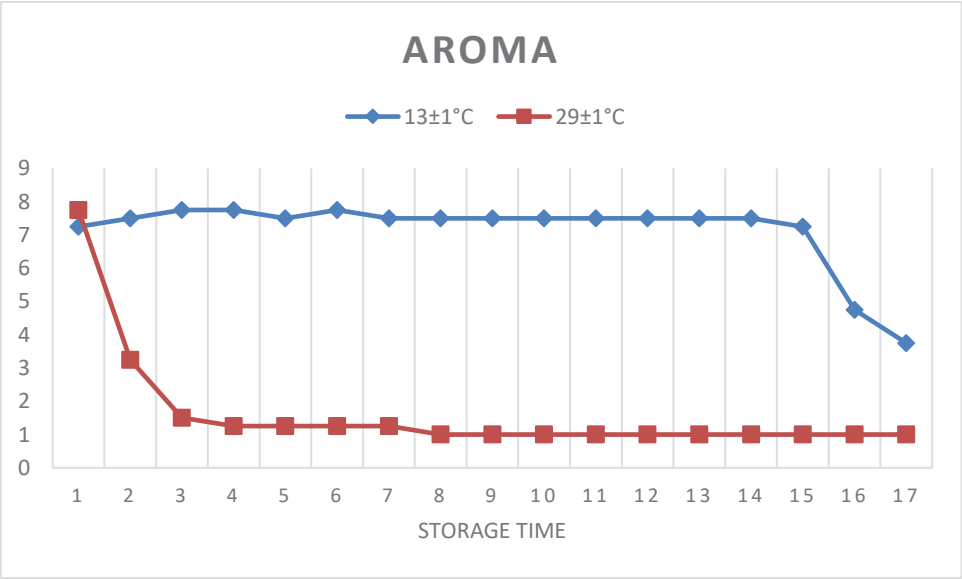

Fig. 2. Chart of Aroma Aloe-Buni

Based on the color evaluation of Aloe Buni beverage during 17 days of storage at two different temperatures (13±1°C and 29±1°C), it was observed that storage temperature had a notable effect on the color stability of the product (Figure 3.). At a higher temperature (29±1°C), the color score started at approximately 16 and gradually decreased over time, reaching around 13 by day 17. Although the decrease was relatively moderate, it indicates a gradual loss of visual quality, which could be attributed to pigment degradation or non-enzymatic browning reactions enhanced by higher temperature conditions. In contrast, the beverage stored at a lower temperature (13±1°C) maintained a relatively stable color score around 9 for the majority of the storage period, with only a slight decline observed toward the end of the storage duration. This suggests that lower temperatures are more effective in preserving the visual appearance of the beverage, likely by slowing down chemical reactions and pigment oxidation that affect color.

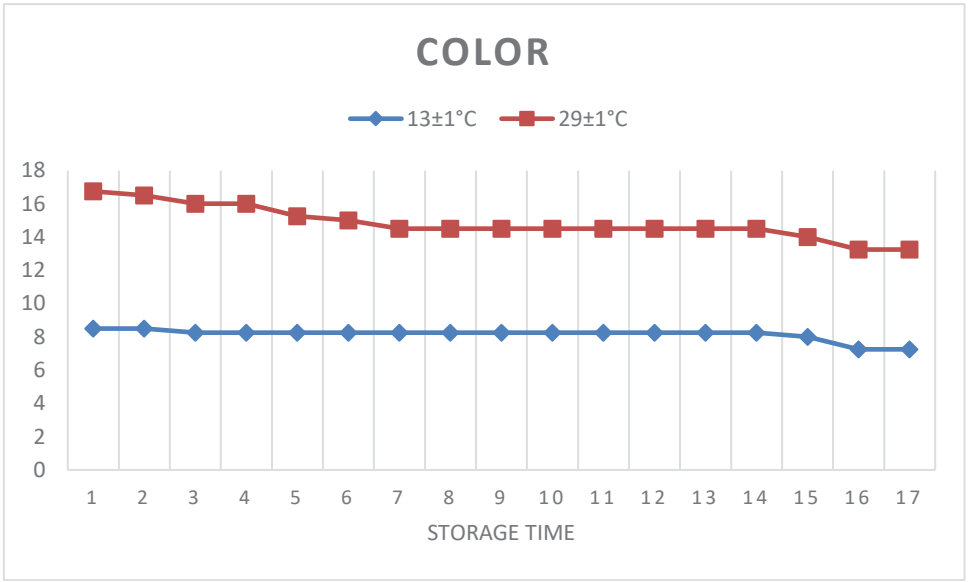

Fig. 3. Chart of Color Aloe-Buni

The flavor evaluation of Aloe Buni beverage over a 17-day storage period at two different temperatures (13±1°C and 29±1°C) reveals a significant impact of storage temperature on flavor stability (Figure 4.). At the lower temperature of 13±1°C, the flavor score remained consistently high, around 8, for the first 14 days, indicating that the product retained its desirable taste characteristics. However, a noticeable decline was observed from day 15 onward, with scores dropping to approximately 4 by day 17. This suggests that flavor degradation began to accelerate after two weeks, even under refrigerated conditions. In contrast, samples stored at 29±1°C showed a rapid decline in flavor quality. A sharp decrease was recorded as early as day 2, with scores plummeting from around 8 to below 3. The flavor score continued to decline gradually until it stabilized at approximately 1 by day 6, remaining at that level for the rest of the storage period. This indicates that high storage temperatures greatly accelerate the deterioration of flavor, likely due to microbial activity and chemical changes such as oxidation or Maillard reactions.

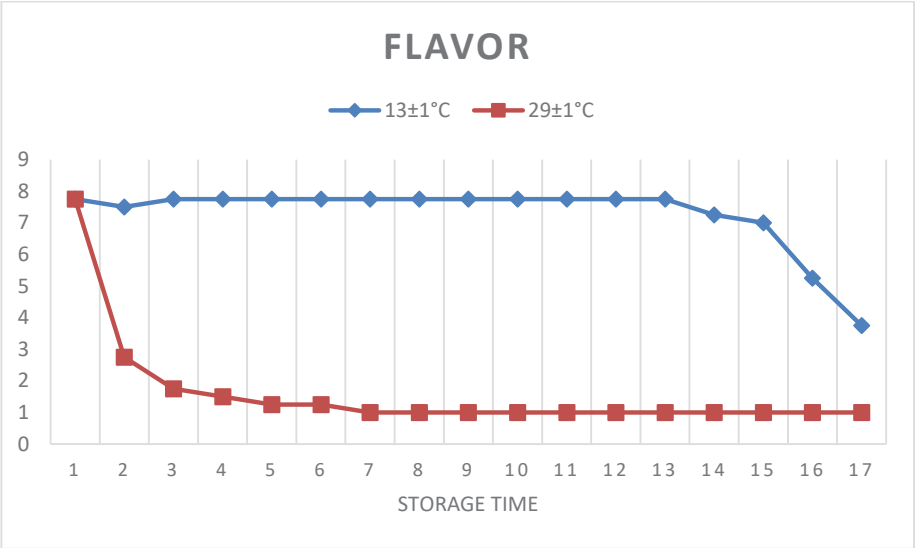

Fig. 4. Chart of Flavor Aloe-Buni

4. DISCUSSION

The disadvantage of fruit juice drinks is that there is sediment in the drink, which is one of the causes of the pectin contained in the fruit [11]. Therefore, to reduce sediment in beverages, the addition of additives is necessary. Food additives are substances that are intentionally added during the production, processing, packaging, transportation, or storage of commercial food products. The additives added to this beverage are sorbic acid, sodium benzoate and benzoic acid, which act as stabilisers and preservatives for the Aloe-Buni functional beverage [9]. Different types and concentrations of additives affect the characteristics of Aloe-buni beverages.

Based on the analysis results, there is a difference in pH. This pH difference occurs due to the chemical properties of the two preservatives. Benzoic acid is a weak acid that, in solution, will release hydrogen ions (H^+) and produce a more acidic atmosphere, thus lowering the pH of the drink [8]. Based on the analysis results, there is a difference in pH. This pH difference occurs due to the chemical properties of the two preservatives. Benzoic acid is a weak acid that, in solution, will release hydrogen ions (H^+) and produce a more acidic atmosphere, thus lowering the pH of the drink. Sodium Benzoate tends to be more stable in the acidic pH environment of fruit drinks, such as Aloe-buni, so it is able to retain vitamin C better [9]. At a concentration of 0.02%, Benzoic Acid is not enough to provide optimal protection to the vitamin C component, leading to lower vitamin C levels compared to Sodium Benzoate.

At a concentration of 0.02%, the acid type and concentration of preservatives play an important role in determining the stability of antioxidant activity in Aloe-Buni drinks during storage. Sorbic acid and sodium benzoate have different mechanisms of action in inhibiting the growth of microorganisms and slowing down oxidative reactions that can damage antioxidant compounds. Sorbic acid tends to be more effective at the beginning of storage due to its ability to inhibit the growth of mould and yeast, and stabilise bioactive components, while Benzoate is not enough to provide optimal protection to vitamin C components, which causes lower vitamin C levels compared to Sodium Benzoate [11]. However, their stability may decrease over time due to degradation of the active compounds by the effects of temperature, pH, and exposure to oxygen. [12]. In contrast, sodium benzoate showed higher effectiveness in maintaining or even increasing antioxidant activity up to the 7th day of

storage, especially at 0.03% concentration. This is due to its ability to be more stable against storage conditions, as well as its ability to inhibit antioxidant-damaging enzymes such as polyphenol oxidase [13]. In addition, higher concentrations of preservatives allow for the creation of a more protective environment against the degradation of phenolic compounds and vitamins that play a role in antioxidant activity.

The addition of additives also affects the viscosity of the drink. This difference in viscosity can be caused by the interaction that occurs between the preservative components and the components in the Aloe-buni drink, including the fibre content of aloe vera or buni, which is affected by the concentration of preservatives. The lower concentration of Benzoic Acid does not change the structure of the fibre components much, so that the viscosity remains high [12]. The addition of additives also affects the viscosity of the drink. This difference in viscosity can be caused by the interaction that occurs between the preservative components and the components in the Aloe-buni drink, including the fibre content of aloe vera or buni, which is affected by the concentration of preservatives. The lower concentration of Benzoic Acid does not change the structure of the fibre components much, so that the viscosity remains high. In addition, the addition of preservatives also affects the number of microbes. This difference is caused by the difference in the effectiveness of the two preservatives under certain conditions. Benzoic acid is known to have better preservation ability in acidic environments, such as in Aloe-buni drinks, especially at the right concentration. At a concentration of 0.02%, Benzoic Acid can create unfavourable conditions for the growth of microorganisms, so that the total number of microbes can be suppressed to the maximum [14]. In addition, Benzoic Acid's stronger antimicrobial properties in acidic environments make it more effective in maintaining the microbiological cleanliness of beverages. Many factors affect the stability of the drink, one of which is the presence of microorganisms. Microbial activity in beverages can result in a decrease in the stability of food products, such as food products becoming acidic or tasting unpleasant due to fermentation, the formation of taste and smell and soap due to bacterial growth. The microbes that grow will cause the drink to become stale, damaging the taste, aroma and colour [15]. Microbial activity in beverages can result in a decrease in the stability of food products, such as food products becoming acidic or tasting unpleasant due to fermentation, the formation of taste and smell and soap due to bacterial growth [29].

The shelf life of the drink using the ESS method showed that Aloe-buni drinks stored at cold temperatures were able to maintain their quality of Aloe-buni drinks until the 14th day. Storage temperature has an influence on the organoleptic quality of the product during the storage period. At low temperatures (13±1°C), the product can maintain a high organoleptic value, with scores ranging from 8 on the first day and remaining stable until about day 14. The decline only occurred after the 14th day, and on the 17th day, the organoleptic score was around 5, which is still quite acceptable. On the other hand, at room temperature (29±1°C), there was a drastic decrease in organoleptic quality. Although on the first day the score was still high (around 8), on the second day it had dropped sharply and continued to decline until it was below a score of 2, which shows that the product is no longer suitable for organoleptic consumption. These results show that storage at lower temperatures is much more effective in maintaining the organoleptic quality of the product, while high temperatures accelerate the deterioration of product quality. Therefore, low storage temperatures are highly recommended to maintain quality and extend the shelf life of the product.

5. CONCLUSION

This study showed that the addition of preservatives such as sorbic acid, sodium benzoic, and benzoic acid in Aloe-buni functional drinks affects the quality and stability during storage. Among the various treatments, benzoic acid with a concentration of 0.02% provides the best results in maintaining the physicochemical and microbiological characteristics of the drink, including pH, antioxidant activity, viscosity, vitamin C levels, and total microbes. Storage at a low temperature (13±1°C) has also been shown to be more effective in maintaining the

organoleptic quality of the beverage until day 14, compared to room temperature storage, which accelerates quality degradation. Thus, the combination of using 0.02% benzoic acid and cold temperature storage is recommended to extend the shelf life and maintain the quality of Aloe-buni drink as a functional product.

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