January – March, 2025
Original Article
DOI: http://dx.doi.org/10.30867/action.v10i1.1888

| Pages: 37 – 45 |

AcTion:
Aceh Nutrition Journal
p-issn 2527-3310; e-issn 2548-5741

Boba kefir pineapple beverage innovation: Probiotic and antioxidant refreshment

Inovasi minuman nanas boba kefir: Minuman penyegar probiotik dan antioksidan

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Article History:
Received: June 16, 2024; Revised: September 01, 2024; Accepted: November 19, 2024; Published: March 06, 2023.

Publisher:

Politeknik Kesehatan Aceh
Kementerian Kesehatan RI

Abstract

Nutritional intake plays an important role in boosting the immunity. This study aimed to modify the boba drink into a probiotic- and antioxidant-rich drink. Kefir is a source of probiotics. Pineapples, which are rich in vitamin C, have antioxidant properties. The experimental study used a completely randomized design (CRD) with three formulations: F1 (75% kefir: 25% pineapple), F2 (50% kefir: 50% pineapple), and F3 (25% kefir: 75% pineapple). Product manufacturing and organoleptic tests were conducted at the Food Laboratory of Poltekkes Kemenkes Bandung. Laboratory testing was conducted at Saraswanti Indo Genetech and Sibaweh Lab. This research was conducted from December 2022 to January 2023. Organoleptic testing was performed by 30 trained panelists. Analysis using the Kruskal–Wallis test. Results: There were differences in the organoleptic aspects (p= 0,012) of each formulation. Thus, F3 was found to be the best formulation. The number of lactic acid bacteria was 6,7 x 106, with antioxidant activity of 1238,02 ppm or 0,1238%, and vitamin C content of 19,88 grams. In conclusion, the high antioxidant activity of this drink suggests that it has the potential to increase body immunity. Further testing is needed to determine the type and number of other probiotic bacteria present in the product.

Keywords: Antioxidant, kefir, pineapple, probiotic, vitamin C

Abstrak

Asupan gizi memainkan peran penting dalam meningkatkan kekebalan tubuh. Penelitian ini bertujuan untuk memodifikasi minuman boba menjadi minuman kaya probiotik dan antioksidan. Kefir merupakan sumber probiotik. Nanas, kaya akan vitamin C, memiliki sifat antioksidan. Penelitian eksperimental menggunakan desain acak lengkap (RAL) dengan tiga formulasi: F1 (75% kefir: 25% nanas), F2 (50% kefir: 50% nanas), dan F3 (25% kefir: 75% nanas). Pembuatan produk dan uji organoleptik dilakukan di laboratorium pangan, Poltekkes Kemenkes Bandung. Pengujian laboratorium dilakukan di Saraswanti Indo Genetech dan Sibaweh Lab. Penelitian dilakukan pada Desember 2022 hingga Januari 2023. Pengujian organoleptik dilakukan oleh 30 panelis terlatih. Analisis menggunakan uji Kruskall-Wallis. Hasil, terdapat perbedaan pada aspek organoleptik (p= 0,012) pada masing-masing formulasi. F3 merupakan formulasi terbaik. Jumlah bakteri asam laktat adalah 6,7 x 106, dengan aktivitas antioksidan sebesar 1238,02 ppm atau 0,1238%, dan kadar vitamin C sebesar 19,88 gram. Kesimpulan, aktivitas antioksidan yang tinggi, membuat minuman ini berpotensi meningkatkan imunitas tubuh. Diperlukan pengujian lanjutan terhadap jenis dan jumlah bakteri probiotik lain yang terkandung dalam produk.

Kata Kunci: Antioksidan, kefir, nanas, probiotik, vitamin C

Introduction

Nutritional consumption plays an important role in boosting the immune response. This can be achieved through the intake of foods and beverages that function as immune boosters, helping the body combat infectious diseases, including COVID-19 (Zhang & Liu, 2020). Sugar-sweetened beverages, commonly known as sweetened drinks, are currently popular among children (Al Rahmad et al., 2020). In a study in the United States, 64,5% of boys and 61,3% of girls aged 12-19 years consumed sweet drinks once a day. Consumption of sweetened drinks increases with age, with the largest consumers being aged 12-19 years (Rosinger et al., 2017). Boba drinks are a type of contemporary drink that is much in demand in Indonesia. This drink is based on tea, milk and "boba". Boba are balls with a chewy texture made from tapioca flour with brown sugar. However, these modern drinks are high in sugar and calories (Ramadhaningtyas et al., 2021).

Based on the 2022 Socio-Economic Survey (SUSENAS) in the last two decades, consumption of packaged sweetened drinks (MBDK) in Indonesia increased 15 times from 5 million liters in 1996 to 405 million liters in 2014 (CISDI, 2022). The trend of increasing consumption of sweet drinks in Indonesia is due to the increasingly widespread availability of sweet drinks and the ease of access to get them (CISDI, 2022). In Indonesia, boba drinks are consumed more frequently by women aged 17 – 22 years (84,6 %) (Tinambunan et al., 2020). Based on a study by Hanifah et al. (2023), as many as 95% of teenagers aged 15 – 18 years in Surabaya prefer sweet drinks (Hanifah et al., 2023). The most commonly consumed sweet drinks are chocolate and boba milk tea. High intake of added sugars in beverages such as tea, coffee and cereals is positively correlated with glycemia and the inflammatory profile (O'Connor et al., 2018). High sugar intake can impair the function of the immune system and trigger pathological conditions (Shomali et al., 2021).

Consuming drinks high in sugar during the pandemic risks reducing the body's immunity; therefore, modifications to boba drink products are needed. Boba drinks can be modified to increase immunity by combining kefir, pineapple, pakchoy, and honey as drinks and cilembu sweet potato as a boba. Immunity plays an important role in protecting individuals from various diseases, especially during pandemics or when the risk of infection increases. A strong immune system can recognize and fight pathogens such as viruses and bacteria and prevent serious infections. In the context of a global pandemic, increasing immunity through a healthy diet and lifestyle has become increasingly crucial. Each ingredient contained the best nutrients for use as an immune booster.

Kefir is a fermented milk. Based on the literature review carried out Valentino et al. (2024) consumption of fermented products is positively correlated with a healthier metabolic profile through reduced inflammation and serum lipid peroxidation. The quantity of live microbes in food is negatively correlated with blood pressure, inflammation, body mass index (BMI), insulin levels, and triglycerides. Kefir has bioactive compound components, such as retinol, folic acid, serine, methionine, tryptophan, iron, and zinc, as well as unique peptides. These components play a role in regulating the balance of intestinal microbiota, reducing inflammation, and improving body health (Apalowo et al., 2024; O'Connor et al., 2018).

Pineapples have the ability to increase immunity because there are a number of derivatives, namely phenolic compounds, such as phenolic acids, flavonoids, tannins, lignin, and non-phenolics, such as carotenoids and vitamin C, which have antioxidant and anticarcinogenic abilities (Mappa et al., 2021). Pineapple has been proven to have health benefits as an anti-inflammatory, high in antioxidants, plays a role in nervous system function, and improves bowel movements (Mohd Ali et al., 2020).

One solution that can be offered is the combination of kefir and pineapple in boba drinks. Kefir is a source of probiotics that supports the balance of intestinal microflora and improves the immune response of the body. Pineapple is rich in vitamin C, which is a powerful antioxidant, as well as the enzyme bromelain, which supports digestion and has anti-inflammatory properties. The combination of the two not only provides benefits for digestive health and the immune system but also creates an attractive and delicious drink for modern consumers. Boba kefir and pineapple drink products have the potential to improve the immune system through several biomolecular mechanisms: Kefir as an immunomodulator, is a fermented milk product that is rich in probiotic microorganisms, such as lactic acid bacteria (LAB) and yeast (Wisudanti, 2017).

Kefir can reduce oxidative stress conditions, improve the function of biological proteins, and increase the production of amino acids such as glutamine, arginine and nucleotides through enzyme and bacterial hydrolysis (Judiono et al., 2014). The benefits of kefir for health are as an antimicrobial, slows cancer growth, accelerates the wound healing process, immunomodulation, anti-inflammatory, anti-obesity, lowers cholesterol, source of antioxidants, increases lactose tolerance, reduces liver fat, and improves intestinal flora (Bengoa et al., 2019).

Based on research Ellatif et al. (2022), kefir acts as an immunomodulatory. Kefir significantly suppressed the increase in TNF-α and increased the secretion of IL-10 and INF by 1,38- and 1,4-fold, respectively, compared to untreated HCV-infected patients. Kefir has the potential to act as an immunomodulator by suppressing the cytokines TNF-α and IL-6 and increasing CD8+. Immunoglobulin (Ig) G+ cells increase IL-2 and IFN-C production. Probiotic microorganisms replenish natural microbes in the gut This causes positive immunomodulation and balance of pro-inflammatory and anti-inflammatory cytokine production (González-Orozco et al., 2022).

Antioxidants in pineapple contain several phenolic and non-phenolic compounds, including phenolic acids, flavonoids, tannins, lignin, carotenoids, and vitamin C. These compounds have strong antioxidant properties, which can protect cells from damage caused by free radicals and reduce inflammation. Thus, regular consumption of pineapple can help improve the immune system by reducing oxidative stress and reducing inflammation (Mappa et al., 2021).

By combining kefir and pineapple in boba drinks, consumers can obtain synergistic benefits of these two ingredients in improving the immune system. The probiotics in kefir help improve the immune response, whereas the antioxidants in pineapple protect the body's cells from damage and inflammation. This study aimed to determine the quality and acceptability of kefir and pineapple boba drinks.

Methods

The research was an experimental study with a two-factor completely randomized design (CRD) for the liking test. The independent variable was the treatment of kefir and pineapple boba drinks, which influenced the dependent variable, namely organoleptic properties (color, aroma, taste, consistency, and overall). This study included three types of treatments: F1 (75% kefir: 25% pineapple), F2 (50% kefir: 50% pineapple), and F3 (25% kefir: 75% pineapple).

This research was conducted from August 2022 to February 2023. The product manufacturing process was conducted in the Food Technology Laboratory. Department of Nutrition, Data Organoleptic testing was carried out by 30 moderately trained panelists who met the inclusion criteria in the taste testing laboratory of the Department of Nutrition.

The research collected in the research data included the number of lactic acid bacteria, antioxidant activity, and vitamin C levels carried out for the best formula based on organoleptic test results. Tests were carried out at the Diponegoro University Laboratory, Semarang and Sibaweh Laboratories Bandung to obtain data on antioxidant activity, and at the Saraswanti Indo Genetech Bogor Laboratory for the number of lactic acid bacteria and vitamin C levels. Data analysis of nutritional value was performed using the 2017 Indonesian Food Composition Table (IFCT).

Tools and Materials

The raw materials used in making kefir and pineapple boba drinks are kefir, pineapple, bok choy, honey, cilembu sweet potato, tapioca flour, gelatin and water. The tools used to make the product include product manufacturing tools, organoleptic testing tools, antioxidant activity testing tools, the number of lactic acid bacteria, and vitamin C levels.

Product Processing Methods

Product manufacturing process: preparing the materials to be used according to the balance. The drink making process was divided into four stages. The first stage involves making boba dough from Cilembu sweet potatoes, tapioca flour, gelatin, and water. The mixture was then stirred until it became smooth and shaped into a ball, and then boiled until cooking. The second stage involves making kefir and pineapple juices by blending the two ingredients and then adding honey. The third stage involved blending kefir with bok choy. The final stage is pouring the boba into glass, pineapple kefir, and bok choy kefir.

Data Analysis

Data analysis was carried out in stages, starting from univariate analysis including testing the assumption of normality of the data, and the results showed that the data were not normally distributed. Bivariate analysis was performed using the Kruskal-Wallis and Mann-Whitney tests, using a significance level of 95%.

Research Ethics

This study was approved by the Bandung Ministry of Health Polytechnic Health Research Ethics Committee (approval number 32/KEPK/EC/II/2023). This approval indicates that all procedures used in this study met internationally recognized ethical standards for human health research.

Result and Discussion

Organoleptic Test Results

Organoleptic properties were tested on 30 panelists who had a somewhat trained level of training using the hedonic test method on a seven-point scale. The obtained data were then analyzed descriptively and presented in the form of a frequency distribution table. The results of the panelists' assessment of organoleptic parameters (color, aroma, taste, consistency, and overall) are presented in Table 1, and the results of statistical tests related to the product's organoleptic properties are presented in Table 2.

Table 1. Percentage distribution of panelists' preference levels for color, aroma, taste, consistency, overall

Organoleptic	Formulas	Very Dislike	Do not like	Kinda Dislike	Neutral	Kinda Like	Like	Very Like
Color	F1 (75:25)	3	0	17	17	47	10	7
	F2 (50:50)	0	0	7	23	23	40	7
	F3 (25:75)	0	3	3	20	7	47	20
Aroma	F1 (75:25)	0	3	10	27	27	30	3
	F2 (50:50)	0	0	17	13	20	43	7
	F3 (25:75)	0	0	0	13	27	43	17
Flavor	F1 (75:25)	3	37	27	20	13	0	0
	F2 (50:50)	0	7	27	10	23	30	3
	F3 (25:75)	0	0	0	17	20	27	37
Consistency	F1 (75:25)	0	10	13	33	27	17	0
	F2 (50:50)	0	0	10	27	20	37	7
	F3 (25:75)	0	0	3	10	20	33	33
Overall	F1 (75:25)	0	7	33	27	23	10	0
	F2 (50:50)	0	3	10	23	27	27	10
	F3 (25:75)	0	0	0	13	23	40	23

Table 2. Statistical test results

	Color	Aroma	Flavor	Consistency	Overall
F1, F2, F3	0,012*	0,027*	0,000*	0,000*	0,000*
F1 dan F2	0,070	0,297	0,000**	0,023**	0,004**
F1 dan F3	0,005**	0,007**	0,000**	0,000**	0,000**
F2 dan F3	0,157	0,125	0,001**	0,009**	0,014**

* Significantly different based on the Kruskall Wallis test (p<0,05)
**Significantly different based on Mann-Whitney follow-up test (p<0,05)

The results of statistical analysis showed that there was a significant influence of differences in kefir and pineapple formulations on organoleptic characteristics, including color, aroma, taste, consistency, and overall assessment. Formulations 1, 2, and 3 received varying ratings from panelists, indicating significant differences in the acceptability of each formula. Based on the preference assessment by panelists, only the most preferred formulations were tested in the laboratory for further analysis.

Color

The results showed differences in color among the three formulations. The comparison between Formula 1 (F1) and Formula 2 (F2) did not show a significant difference, while the comparison between F1 and Formula 3 (F3) showed a significant difference.

However, no significant difference was observed between F2 and F3. The significant difference between F1 and F3 can be attributed to the higher proportion of pineapple in F3, which resulted in increased brightness of the yellow color with the green nuances of the pakchoy. Pineapples, which are rich in bioactive compounds such as flavonoids and carotenoids, contribute to the yellow pigment (Bait et al., 2022). Meanwhile, the green in the drink comes from chlorophyll, the dominant pigment in pakchoy (Istri & Dharmadewi, 2016).

Aroma

The aroma parameters showed no significant differences between Formula 1 (F1) and Formula 2 (F2). A comparison between F1 and Formula 3 (F3) showed significant differences, and there was no significant difference between F2 and F3. The significant difference between F1 and F3 could be explained by the kefir and pineapple formulations used. In F3, the use of more pineapple resulted in a decrease in the sour aroma of kefir, resulting in an aroma dominated by pineapple.

This is in accordance with the preferences of panelists, who tend to prefer a stronger dominant pineapple aroma. The characteristic sour aroma of fermented products such as kefir or yoghurt comes from the transformation of lactose into lactic acid by lactic acid bacteria during the fermentation process (Yunus et al., 2017). Volatile compound components greatly contribute to the quality of the taste and aroma. Most of the volatiles in pineapple have a strong aroma and come from aglycone molecules free from esters, alcohols, aldehydes, terpenes and lactones (Asikin et al., 2022).

Flavor

Taste parameters showed significant differences among all formulations: F1 with F2, F1 with F3, and F2 with F3. In F1, a higher proportion of kefir produced a sour taste. In contrast, F2, with a balance of kefir and pineapple, displays a predominantly sour taste, but with a touch of sweetness. In F3, the use of more pineapple resulted in a predominantly sweet flavor profile, with lighter sour overtones, making it more favorable in terms of taste. The increased use of pineapple consistently increases the sweetness of the such product.

Honey, which is used as a sweetener, contributes its distinctive sweet taste due to its high fructose content (Evahelda et al., 2018). Meanwhile, the sour taste of kefir comes from the metabolic activity of lactic acid bacteria during fermentation, which produces chemical compounds such as lactic acid, acetaldehyde, acetic acid, diacetyl, and 2-3 pentanediones which provide a distinctive flavor (Yunus et al., 2017). The characteristic sweet taste of pineapple comes from the breakdown of carbohydrate polymers during the ripening process, which increases the sugar content and causes a more intense sweet taste (Sujatna & Wisaniyasa, 2017).

Consistency

The consistency parameters showed significant differences among all formulations: F1 and F2, F1 and F3, and F2 and F3. Formula F1, which has a higher proportion of kefir, produces a more liquid consistency compared to F2 and F3. In contrast, F2, which balances kefir with a moderate amount of pineapple, showed thicker consistency than F1. Meanwhile, F3, with the largest use of pineapple, produced the thickest consistency among the three cultivars.

The viscosity of the product was affected by the amount of pineapple added. Pineapples are rich in pectin, which ranges from 1,0 to 1,2% per 100 g. Pectin has the property of forming a gel, where a higher amount of pectin will produce a thicker gel (Saputro et al., 2018).

Overall

Overall, this research shows that there are significant organoleptic differences between all formulations: Formula 1 (F1) and Formula 2 (F2), F1 and Formula 3 (F3), and F2 and F3. This analysis showed that the increase in pineapple use is directly proportional to the increase in the level of panelists' preference for the overall aspect. This increase can be attributed to differences in kefir and pineapple formulations, which significantly affect product quality. The higher quality in every organoleptic aspect, including color, aroma, taste, and consistency, supports this improvement.

Based on the organoleptic evaluation, Formula 3 (F3) was identified as the most preferred formulation by the panelists and was superior in all organoleptic aspects tested. F3 stands out with a brighter color, a sour aroma that is not too strong and is dominated by pineapple, a predominantly sweet taste, and the thickest consistency compared to the other formulations.

Test Results for the Number of Lactic Acid Bacteria (LAB)

The test result for the number of lactic acid bacteria (LAB) was 6,7 x 106 colonies/mL. The number of lactic acid bacteria in kefir and pineapple products was determined using the Total Plate Count (TPC) method. This method is based on the principle of cultivating microorganisms that are still active on agar media, where they multiply and form visible colonies. The colonies were counted directly, without the need for a microscope, using a colony counter. The number of colonies counted is then applied in the formula to calculate the total plate count (Purukan et al., 2020).

In accordance with the Indonesian National Standard (INS) 7552:2009, which sets the standard for kefir as a fermented drink, the minimum number of starter cultures, which in this case are lactic acid bacteria, must reach 1 × 106 colonies/ml. Test results for boba kefir and pineapple drink products showed that the number of lactic acid bacteria was 6,7 x 106 colonies/mL. Based on these results, the product meets the requirements set by INS for fermented drinks.

Antioxidant Activity Test Results

The results of the antioxidant activity test were, namely 1238,02 ppm. Antioxidant activity in boba kefir and pineapple drinks was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method to determine IC50, with UV-Vis spectrophotometry at a wavelength of 517 nm.

The DPPH assay involves the elimination of DPPH compounds to stabilize free radicals. Free radicals from DPPH interact with odd valence electrons (Baliyan et al., 2022). The IC50 value determines the ability of a compound to ward off free radicals (Widyasanti et al., 2016). The kefir and pineapple boba drink showed an IC50 value of 1238,02 ppm, which is equivalent to 0,1238%. These results indicate the high antioxidant activity of the product (Suzery, 2023).

In addition, the antioxidant stability of this product is influenced by environmental factors such as pH, oxygen, light, and temperature. Exposure to air, light, or high temperatures can damage antioxidants. Product processing, storage, and shipping processes involving high temperatures can contribute to the degradation of antioxidant compounds. Antioxidant stability is also greatly influenced by pH, where acidic conditions tend to support antioxidant stability, whereas at neutral or alkaline pH, antioxidants show instability (Khotimah et al., 2018).

Vitamin C Level Test Results

The test results for the vitamin C levels were 9,94 mg/100 g. The vitamin C content in the boba kefir and pineapple drinks was analyzed using high-performance liquid chromatography (HPLC). This process produces a chromatogram which is then interpreted to determine the level of vitamin C. This determination is done by comparing the concentration of vitamin C in the standard solution and the sample solution (Ditri, 2020). The products with significant vitamin C content include pineapple and pakchoy.

Based on the results of the analysis, it is known that one serving (200 ml) of this drink contains 19,88 mg vitamin C. According to TKPI 2017, the product formulation with a ratio of 25% kefir to 75% pineapple has a vitamin C content of 16,95 mg, which meets 26,51% of the daily vitamin C requirement for young women aged 16-18 years. Factors such as fruit variety, growing conditions, and level of maturity at harvest and post-harvest influence vitamin C levels in fresh fruits. During postharvest storage, the ongoing respiration process in the fruit can increase the metabolic rate, resulting in vitamin oxidation and a decrease in vitamin C levels. (Sujatna & Wisaniyasa, 2017).

Results of Macro Nutrient Analysis

The results of the macronutrient analysis are shown in Table 3.

Table 3. Macronutrient analysis

Nutrients*	F1	F2	F3
Energy (Kcal)	270,1	264,1	258,1
Carbohydrate (g)	53,9	56,8	59,8
Proteins (g)	6,3	5,0	3,6
Fat (g)	4,1	2,9	1,9

*Based on the 2017 Indonesian Food Composition Table or TKPI.

Analysis of the macronutrient content in Cilembu sweet potato boba drink products was carried out based on the 2017 Indonesian Food Composition Table (TKPI), considering the type and weight of ingredients used in making the product. The results of the analysis showed that one portion of the product can meet approximately 12% of the daily energy needs of adolescent girls aged 16-18 years. The carbohydrate content in this product contributes approximately 19,9%, protein 5,5%, and fat 2,7% to the total daily requirement for macronutrients.

Based on these data, it can be concluded that kefir and pineapple boba drinks with similar compositions have the potential to meet daily macronutrient needs as snacks for young women. This study underlines the importance of considering nutritional composition in the development of food products aimed at supporting balanced nutritional intake in the adolescent population.

Conclusion

The organoleptic analysis revealed Formula 3 (F3) as the most favorable, with a higher pineapple content affecting color, aroma, taste, and consistency. Boba kefir and pineapple products meet INS standards, with high antioxidant activity and vitamin C levels. They can fulfill 12% of young women's daily energy needs, with carbohydrates, proteins, and fat compositions accounting for 19,9%, 5,5%, and 2,7% respectively. The product has potential as a nutritious snack, and modifications have been made to make it a pineapple kefir drink, containing probiotics and antioxidants.

Further testing of the types and amounts of other probiotic bacteria contained in the product is needed to broaden the understanding of its microbiological profile. In addition, further research on antioxidant activity during storage is needed to ensure the stability of organoleptic properties, especially taste. Research on product shelf life also needs to be conducted to ensure long-term quality.

Acknowledgements

We also express our sincere thanks to Prof. Dr. Dra. Meiny Suzery, MS as Head of the Integrated Laboratory at Diponegoro University Semarang, and Head of the Sibaweh Laboratories Bandung Laboratory, who have provided substantial support in testing antioxidant activity. The Saraswanti Indo Genetech Laboratory team, Bogor, as well as the Director and Head of the Nutrition Department, Poltekkes Kemenkes Bandung, for their guidance and support during the preparation of this scientific work. Furthermore, we thank all the lecturers and the academic community of the Department of Nutrition, Poltekkes Kemenkes Bandung, for their cooperation and support.

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