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Frozen Non–Dairy Kefir With Snake Fruit Puree: Impact of Fermentation Time On Quality And Consumers' Acceptability

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ABSTRACT

Kefir, a fermented milk beverage, is a nutrient-rich dairy product loaded with probiotics. Meanwhile, snake fruit, an exotic fruit rich in vitamins, minerals, and dietary fibre, has shown its nutritional value. Studies reveal that approximately 70% of adults worldwide have struggle with digesting dietary lactose. The study additionally aims to address the increasing digestive issues, the underutilization of snake fruit, and the lack of study on fermenting coconut milk kefir, a non-dairy option. Therefore, this research study explores the impact of different fermentation times on microbial levels, consumer acceptance, physicochemical properties, and scavenging activity of frozen non- dairy kefir with snake fruit puree, offering a lactose-free alternative alongside native snake fruit products. Coconut milk kefir has undergone fermentation for 8, 16, and 24 hours at 32°C in an incubator. The kefir is infused with pasteurized snake fruit puree in a 60:40 ratio. This study encompasses lactic acid bacteria, yeast and mold, alcohol content, pH, colour, viscosity, scavenging activity, and hedonic test. A comparative analysis is conducted against cow dairy milk kefir, specifically focusing on a fermentation time of 16 hours. Findings indicate that consumers prefer the 16-hour fermented coconut milk kefir infused with snake fruit puree. The fermentation time of 16 hours showcases balanced and favourable microbial levels especially probiotics, physicochemical attributes, scavenging activity and consumer preferences. This comparative analysis with dairy milk kefir serves as a benchmark for assessing the viability and desirability of non-dairy alternatives, offering a comprehensive perspective for both scientific and consumer-oriented considerations. Simultaneously, it highlights that this product holds substantial potential for commercialization and marketing.

1.0 Introduction

The market trend for fermented foods and beverages is expected to demonstrate a Compound Annual Growth Rate (CAGR) of 6.70% in the next five years. The growing prevalence of health problems within the expanding population is anticipated to play a crucial role in boosting the demand for fermented foods (Mordor, 2024). Kefir is a fermented milk beverage made from kefir grains that has a creamy consistency and an acidic flavor. The Turkish word kef, which meaning pleasant taste, is derived from the phrase kefir (Mohamed *et al.*, 2020). Kefir and kefir-like products offer a huge potential for commercialization because of their promising advantages.

Among its numerous therapeutic advantages, regular kefir consumption has been shown to improve digestive health, regulate blood sugar and cholesterol and more (Braley *et al*., 2023). In fact, kefir is widely acknowledged as a prominent product among probiotic beverages. According to Market research (2024) A Compound Annual Growth Rate (CAGR) of 6.36% is predicted for the Kefir Market, which was valued at USD 4.08 billion in 2023 and is projected to grow to USD 4.33 billion in 2024, reaching USD 6.28 billion by 2030.

On the other hand, development of new non-dairy probiotic food is vital since consuming dairy products has adverse impacts on lactose intolerance and allergenic milk proteins. Coconut milk is used as a non-dairy as well as lactose free alternative of kefir. Coconut milk is widely used in Southeast Asian cuisines. In addition of being consumed as a beverage, it is an ingredient of various savoury and sweet cuisine. Consuming coconut milk is rarely linked to allergic reactions. Coconut milk contains lauric acid, a saturated fat found in mother's milk that has been linked to promoting brain development (Swathi *et al*., 2016). This is supported by Samuel *et al*., (2022), lauric acid helps to keep blood vessels elasticity and strengthen the immune system. It has anti-viral, anti- microbial, anti-carcinogenic, and fitness benefits associated with its consumption.

Studies reveal that 70% of people worldwide have lactose intolerance, and this varies based on factors like ethnicity and age (Alili *et al*., 2023). Lactose intolerance is a digestive problem characterized as difficulties digesting lactose. Bloating, diarrhoea and stomach pain are some of the symptoms. Lactase non-persistence, for example, is a condition that affects more than half of the population in South America, Africa, and Asia. Lactose is found solely in mammalian milk and is hydrolysed in the small intestine by lactase (Samuel *et al*., 2022).

Snake fruit (Salacca spp.) is categorized as one of the underutilized tropical fruits, despite its valuable nutritious and environmental conservation content (Riry *et al*., 2023). Lack usage of snake fruit is one of the problems that is taken concern to resolve through this research conducted. As stated by Food and Agriculture Organization of the United Nations, (2023), the unclear state of the snake fruit can be linked to various factors, like a lack of focus on modern marketing, the fading of traditional knowledge, and limited awareness of the health benefits of these underused fruits.

Furthermore, Fundo *et al*., (2015) also mentioned in their previous study, tropical fruits like snake fruit and jackfruit are highly perishable and prone to deterioration. It is due to their high respiration rate and water activity. Additionally, this is supported by Albertus *et al*., (2019), that stated they are prone to enzymatic browning reactions, which can compromise their nutritional quality, taste, aroma, and visual appeal, thereby potentially reducing consumer acceptance. Storage time induces the flesh fruit colour to progressively change to creamy-white, then beige, brownish cream, and brown, indicating deterioration (Widayanti *et al*., 2021).

Acknowledging the nutritional benefits of coconut milk, snake fruit, and the associated challenges, the proposed innovative approach integrates snake fruit into coconut milk to create lactose-free kefir that aligns with market demands. However, the innovative non-dairy kefir integrating snake fruit poses a challenge regarding the fermentation duration of kefir. This is because there is a lack of comprehensive understanding and research on how these specific ingredients and their combination affect microbial levels, consumer acceptance, physicochemical properties, and scavenging activity in the non-dairy kefir combined with snake fruit puree. This underscores the importance of further research and exploration in this field to address the knowledge gap. Conducting thorough studies tailored to these products is essential to fill this gap in understanding. Therefore, the research aims to determine the presence of lactic acid bacteria, yeast, and mold, assess consumer acceptability and investigate changes in physicochemical and scavenging activity influenced by varied fermentation time of the frozen non-dairy kefir with snake fruit puree.

2.0 Literature review

In the development of probiotic and health-promoting foods, consideration is given to kefir and its probiotic bacterial species as potential substitutes (Handray *et al*., 2024). Kefir is distinct from other fermented products because it is made from kefir grains, which contain a unique and complex blend of lactic acid- and acetic acid-producing bacteria, as well as lactose-fermenting and non-fermenting yeast that coexist in a symbiotic relationship (Damiana *et al*., 2017).

Many studies have shown evidence to support the use of probiotic foods, such as kefir, in the treatment of gastrointestinal disorders. Diarrhoea is one example, which can be caused by number of disorders. Probiotics provide a promising therapeutic solution for a range of ailments, including gastrointestinal disorders including Crohn's disease, ulcerative colitis, and diarrhoea (Fujie *et al*., 2022). A significant rise in the incidence of viral diseases, particularly those stemming from newly emerging viruses such as Chikungunya, Dengue, Ebola, Zika, and SARS-CoV-2, has caused widespread disruption to global public health. Several studies have investigated whether probiotic products might be utilized as treatments with antiviral agents in the search for antiviral drugs with minimal adverse effects. Several concepts regarding the potential effectiveness of kefir against viruses like SARS-CoV-2 have been formulated, aiming to aid researchers in evaluating the antiviral properties of this natural product (Reham *et al*., 2021).

Coconut milk is one of the sustainable alternatives for lactose free milk. The primary objective of this milk is to treat the issues related with allergies, lactose intolerance, and hypercholesterolemia (Samuel et al., 2022). Lauric acid (48.2%) and myristic acid (16.6%) dominate the fat and oil composition of coconut milk. Unsaturated fatty acids, such as linoleic acid is limited (Feti *et al*., 2017). Medium Chain Triglyceride, a coconut fraction, had been recognized as an essential and therapeutically beneficial food. The fatty acid component of coconut is lauric acid, which is found solely in mother's milk and is believed to offer significant health advantages (Lakshmi & Mary, 2018). The body produces monolaurin, a disease-fighting monoglyceride fatty acid derivative, from lauric acid. Lauric acid is also beneficial in enhancing the immune system and preserving blood vessel flexibility. According to some research, lauric acid helps prevent cardiovascular illnesses, reduce cholesterol, and decrease body weight (Sandhya *et al*., 2016).

Snake fruit, also known as Salacca zalacca (Gaert.) Voss is one of the nutrient dense exotic fruits. Compared to other exotic fruits, the pulp of snake fruit has been observed to have a higher capacity for antioxidants (Mohammed *et al*., 2018). Snake fruit can prevent atherosclerosis in vivo and has massively greater antioxidant value than mangosteen (Ioana *et al*., 2016). In comparison to kiwi fruit, snake fruit contains higher amounts of the basic nutritional elements (fibre, crude protein, and crude lipids), and a high mineral content has also been proven in snake fruits (Petra *et al*., 2021). According to Melissa & Andre (2017) combined consumption of yoghurt and fruit may offer a synergistic combination of beneficial fatty acids, probiotics, prebiotics, high-quality protein, and other vitamins and minerals.

The nutritional composition of kefir varies depending on several factors, including the milk's composition, the type and composition of the grains employed, fermentation duration and temperature, as well as storage conditions (Damiana *et al*., 2017). Throughout the incubation phase, the microbial community within kefir grains undergoes dynamic growth and dissemination, resulting in the introduction or elimination of bacteria, yeasts, and their genetic material (Reham *et al*., 2021). According to a variety of studies, the viability of probiotics in coconut milk during fermentation and storage is also influenced by several key factors. These factors include the fermentation process itself, the duration of fermentation, the temperature at which fermentation occurs, and the specific type of probiotics used (Amal & Ashwag, 2022). Furthermore, fermentation time plays a significant role in determining final acidity levels, acid production rate, and fermentation rate. Both temperature and fermentation duration are recognized as pivotal factors influencing the microbiota of kefir grains and the resulting kefir (Hecer & Kaynarca, 2019).

Therefore, it is essential to identify the optimal fermentation time when fermenting coconut milk using kefir grains to ensure the desired microbial activity and quality of the final product. According to Muhamad (2022), Thus, enhancing the perspectives of young Muslim and non-Muslim about halal food items promotes a rise in their behavioral intentions toward halal food items.

3.0 Methodology

The research methodology focuses on investigating the effects of different fermentation times on microbial loads, consumer acceptability, physicochemical properties, and scavenging activity of frozen non-dairy kefir with snake fruit puree. Figure 1.1 outlines the detailed steps of the methodology for this research study. The study aims to develop frozen coconut milk kefir with snake fruit puree and analyze microbial counts, including yeast, mold, and lactic acid bacteria (LAB). Additionally, attention is given to analyzing physicochemical properties such as pH, colour, and viscosity. Consumer acceptability is assessed through hedonic testing, and scavenging activity analysis is also conducted.

3.1 Sample preparations

3.1.1 Sample preparation of control (cow's milk kefir)

15 g of active kefir grains were inoculated into sterilized glass jars filled with 250 ml of cow's milk. The sample is incubated for 16 hours at 32°C. The incubation was performed in a thermostatically controlled incubator (Biobase). After incubation, the grains were retrieved from cow's milk kefir by filtering grains using a plastic siever. Honey rock sugar and pectin are weighed. The mixture of honey rock sugar and pectin are cooked until dissolve and added into cow's milk kefir. The cream mixture was mixed until no lumps remains. The cow's milk kefir is homogenized for 20 minutes at 6oC. Sample is labelled and then stored in freezer for 24 hours at -18°C before testing.

3.1.2 Sample preparation of snake fruit puree

The snake fruits were cleaned and cut into small pieces. The snake fruits were then blanched for 5 minutes at 55°C and pureed using a food processer. Snake fruit puree are preheated to 65°C and pasteurized at 85oC for 30 seconds to prolong shelf life. Pasteurized snake fruit are stored at refrigerator (5°C).

3.1.3 Sample preparation of frozen non-dairy kefir with snake fruit puree

15 g of active kefir grains were inoculated into sterilized glass jar filled with 250 ml of UHT coconut milk for sample preparation of non-dairy kefir. Three fermentation time has been applied which are 8 hours, 16 hours and 24 hours. Each sample were incubated at 32°C. All the incubation was performed in a thermostatically controlled incubator (Biobase). After incubation, the grains were retrieved from coconut milk kefir and control kefir by filtering grains using a plastic siever. Followed by addition of pasteurized snake fruit puree, honey rock sugar and pectin. The mixture was then cooked until sugar and pectin dissolved. The formulation that has been used for the study is 60 % of coconut milk kefir and 40% of snake fruit puree with addition of honey rock sugar and pectin. The cream mixture was homogenized for 20 minutes at 6°C. The samples are made separately for different fermentation time and stored in freezer for 24 hours at -18°C before further testing.

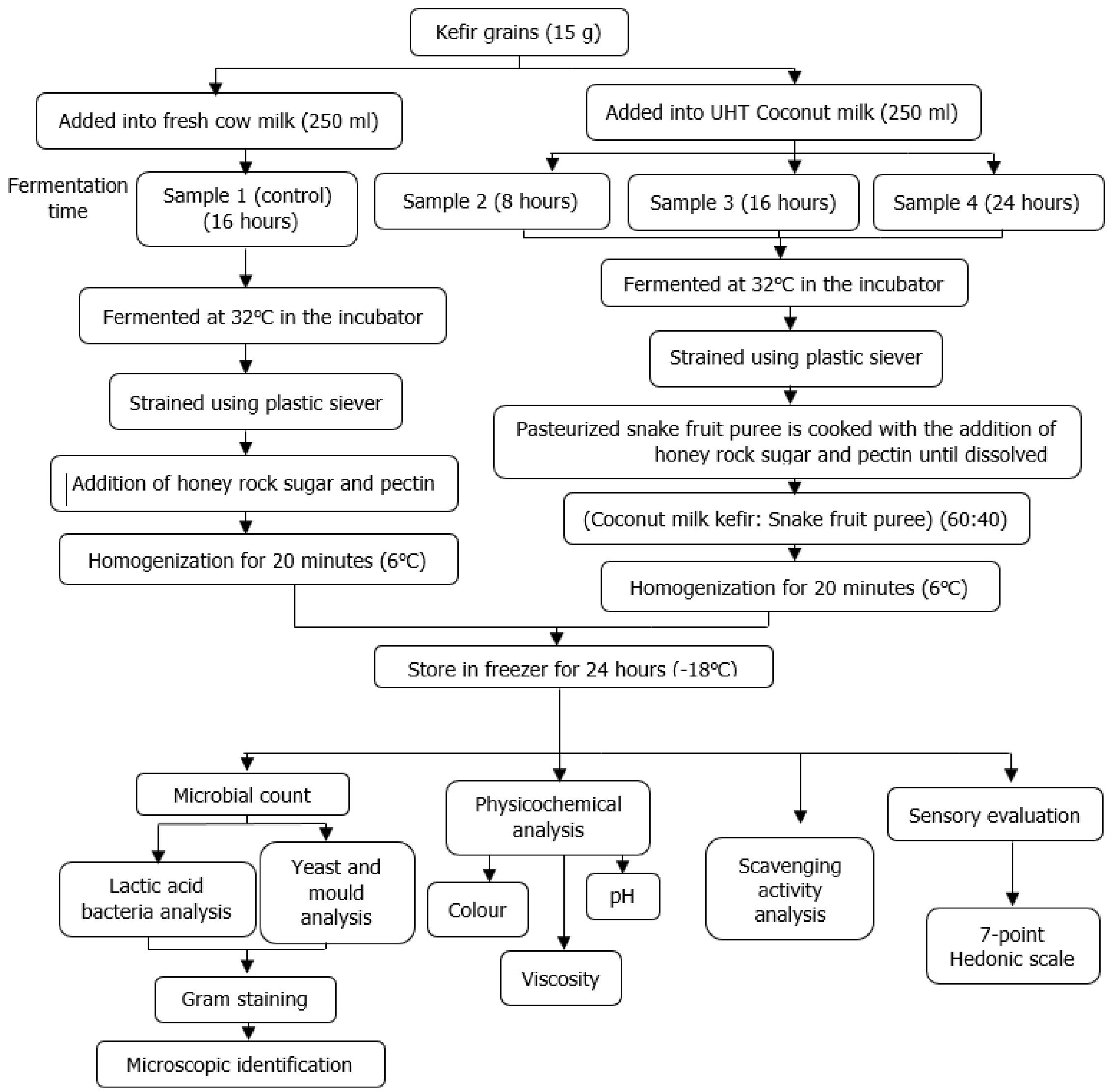

Figure 3.0 Research framework

Table 3.1: Formulation of kefir

Formulations	Frozen non-dairy kefir with snake fruit puree	Cow's milk kefir (Control)
Coconut milk kefir	56.58%	0%
Cow's milk kefir	0%	95%
Snake fruit puree	38.42%	0%
Honey rock sugar	4.5%	4.5%
Pectin	0.5%	0.5%

3.2 Microbial loads

Spread plate method was performed using De Mann Rogosa Sharpe Agar medium for the detection of Lactic acid bacteria (LAB). Whereas, spread plate method was performed using Potato Dextrose agar (PDA) medium for yeast and mold. 10 g of frozen samples are weighed. Stomacher machine was used to extract sample into peptone water. Detection of Lactic acid bacteria (LAB) the petri dish was inserted into anaerobic jar and was incubated at 37ºC for 48 hours. For the yeast count, petri dish was incubated at 37ºC for 48 hours and mold growth was observed on the 5th day. All the colonies were counted using colony counter (Rocker) to determine the number of colony-forming units (CFU) present in a unit volume or weight of the sample. After performing gram staining, all the four samples are analysed under microscope with 10x low power objective lens magnification.

3.3 Physicochemical properties analysis

3.3.1 pH analysis

The pH values of samples were determined using a pH meter (Jenway) and the pH meter calibration was performed with deionised water followed by pH buffer 4 and 7 solutions. Frozen samples temperature was allowed drop within ± 5ºC by placing test sample beaker in controlled water bath 37oC. Probe was inserted into beaker glass containing 50 ml of sample.

3.3.2 Viscosity analysis

Sample's viscosity was tested using a viscometer (Brookfield). Sample's viscosity was measured using a spindle lv-3 at 100 rpm. The spindle was lowered and centred into beaker filled with 50 ml of sample. To achieve accurate viscosity measurements, samples temperature was controlled within ± 5ºc by placing test sample beaker in controlled water bath 37ºC. The viscometer is operated for 2 minutes for each sample to reach stable condition.

3.3.3 Colour analysis

Colorimeter (PCE-CSM 1) was used to analyse colour value differences among the frozen samples of non-dairy kefir with snake fruit puree at different fermentation time. The default setting for this measuring mode is L*a*b*C*H. Testing button are pressed and hold. Next, 4 light cones appear helps to aim at the measuring point. The device was moved as close to the measuring point as possible. The testing button were release and colorimeter takes a measurement. After the measuring process has finished, the results will be displayed.

3.4 DPPH–free radical scavenging analysis

Scavenging analysis was performed to identify the DPPH radical scavenging effect of the samples affected by different fermentation time. Begin the process with sample extraction where 5 g of frozen samples are weighed and extracted using 50 ml of methanol in a conical flask. The flask was placed in a water bath at 25oC for one hour. After 1 hour, the sample extract was filtered using funnel covered with filter paper. The filtered samples extract was then placed in water again for 30 minutes. The DPPH solution was prepared by adding 7.4 mg of 1,1-diphenyl-2-picrylhydrazyl (DPPH) powder with 100 ml of methanol. 400 ul of sample + 2000 ul DPPH solution was measured using pipette and transferred into a small tube. Each tube was wrapped using aluminium foil and left in room temperature (27oC) for one hour. The samples absorbance was analysed using Ultraviolet visible (UV-VIS) spectrophotometer.

3. 5 Sensory evaluations

Hedonic test was performed to determine the degree of acceptability of frozen non-dairy kefir with snake fruit puree at different fermentation time by 50 untrained panellists. Approximately, 25 g of frozen samples were presented on a plate coded with 3-digit random numbers in discrete cubicles with adequate lighting. A cup of water was placed on the tray for each panellist to neutralize their taste buds between samples. The panellists were given briefing and required to indicate their acceptance for each sample by marking (√) at the point that most indicates their level of acceptance in the 7-point Hedonic scale questionnaire (1 =dislike very much, 2 = dislike moderately, 3 = dislike slightly, 4 = neither like nor dislike, 5 = like slightly, 6 = like moderately, 7 = like very much).

3.6 Statistical data analysis method

The One-Way ANOVA was used to analyse the data for each analysis performed except for the yeast and mold analysis as well as the lactic acid bacteria (LAB) analysis. All the data subjected to One- Way Anova Analysis of Variance (ANOVA) to determine the significant difference ($p< 0.05$) among the samples. Data were analysed by using IBM Statistical Package for the Social Sciences (SPSS) Statistics 26 application.

4.0 Discussion of analysis and findings

The microbial loads in non-dairy kefir with snake fruit puree and the control are depicted in Figure 1.2. According to the figure, the total lactic acid bacteria count in the control (cow's milk) was 1.9 x 10^8. In comparison, the total lactic acid bacteria count in coconut milk kefir with snake fruit puree for 8 hours was 4.4 x 10^6, for 16 hours was 3.91 x 10^7, and for 24 hours was 1.1 x 10^9. Notably, the control sample exhibited a higher count of lactic acid bacteria compared to coconut milk kefir with snake fruit puree at the 16-hour fermentation time. This difference may be attributed to the composition of the kefir, as the control sample comprised 100% pure cow's milk kefir, while the coconut milk kefir with snake fruit puree had a ratio of 60% coconut milk kefir and 40% snake fruit puree. According to Shori *et al.*, (2018), it is emphasized that probiotic counts should meet minimum level requirements ranging between 10^6 and 10^7 CFU/ml until the expiry date.

Subsequently, the total yeast count in the control (cow's milk) was 1.0 x 10^7. In coconut milk kefir with snake fruit puree, the total yeast count for 8 hours was 3.2 x 10^7, for 16 hours was 3.8 x 10^7, and for 24 hours was 7.8 x 10^7. This suggests that fermentation time influenced the viability of yeast in the samples, with counts increasing over each 8-hour interval. Raul *et al.*, (2020) reported that cow's milk kefir typically contains yeast counts ranging from 10^6 to 10^7 CFU/mL, and the control of yeast count may vary depending on several factors beyond nutrient availability, such as grain origin.

Furthermore, gram staining analysis revealed the presence of lactic acid bacteria (LAB) in all four samples. LAB, which are gram-positive bacteria used in fermented foods production, appears purple in colour under the microscope, indicative of their gram-positive nature. Morphologically, these microorganisms appeared as cocci or rods and did not form spores. Additionally, yeast and mold analysis were conducted to identify and quantify the presence of yeast and observe mold growth in coconut milk kefir with snake fruit puree across different fermentation times. Potato Dextrose Agar (PDA) was utilized to encourage yeast growth, and spread plate techniques were employed, followed by incubation for 48 hours at 37°C.

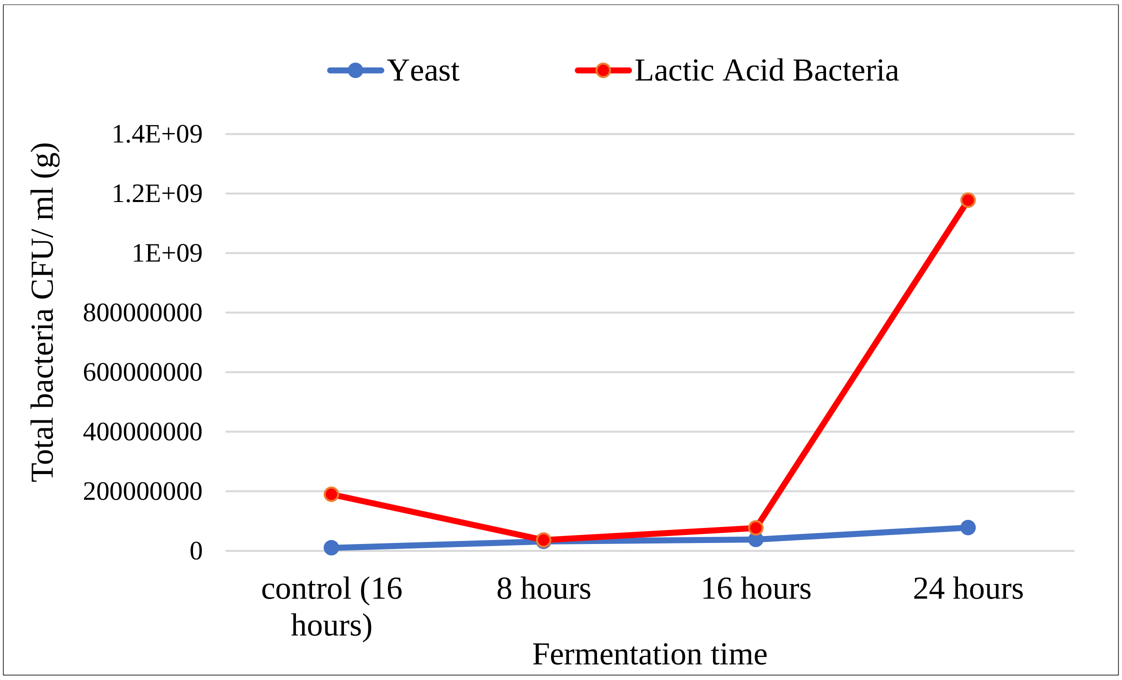

Figure 4.1: Microbial loads in frozen non-dairy kefir with snake puree and control

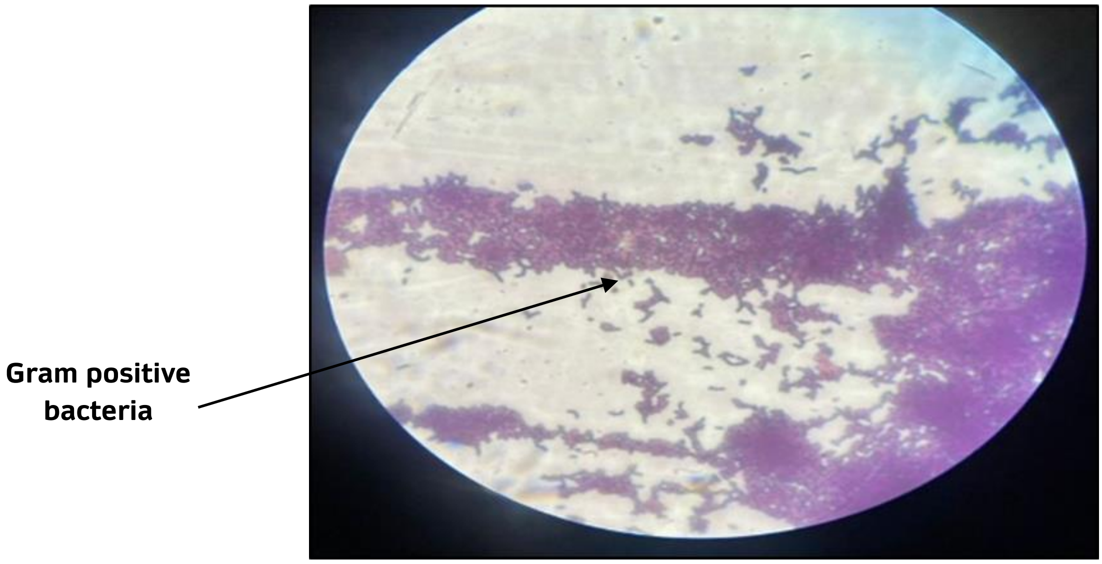

Figure 4.2: Microscopic identification (Lactic Acid Bacteria) of 16 hours fermentation time on frozen non-dairy snake fruit puree (Light microscope) (10x low power objective lens magnification)

The hedonic test was performed to measure consumer overall preferences and satisfaction achieved through the product produced. The statistical analysis exposed that there are significant differences ($p<0.05$) among the samples for the overall acceptance of the non-dairy kefir with snake fruit puree. Overall acceptability rating measures the consumer's degree of likings and preferences in relation to the control sample. The highest overall acceptability means a score of 5.76 was recorded for the coconut milk kefir fermented for 16 hours, which was closely followed by control sample fermented for 16 hours (5.70), 8 hours fermentation (4.56) and 24 hours fermentation (4.34).

It therefore appears that the panellists preferred coconut milk kefir with addition of snake fruit puree at the fermentation time of 16 hours. 16 hours fermentation time of coconut milk kefir yields the most preferred kefir in terms of sensory attributes and overall acceptance. This might be due to the milder flavour and lower acidity compared to longer fermentation time along with addition of snake fruit puree which enhances flavour as well as sweetness. This may appeal to consumers who prefer more subtle taste and lower tartness. Kefir fermented for 24 hours can develop stronger flavours and increased acidity as it has the lowest acceptability among the panellists. As supported by Rusdhi *et al.*, (2020), the quality of sensory tests was influenced by the type of milk and the different fermentation time. According to Davide *et al.*, (2022) when it related to identifying the barriers of customer acceptance for specific plant-based products and the characteristics of those products that make them appeal to consumers, sensory and consumer science can be extremely beneficial.

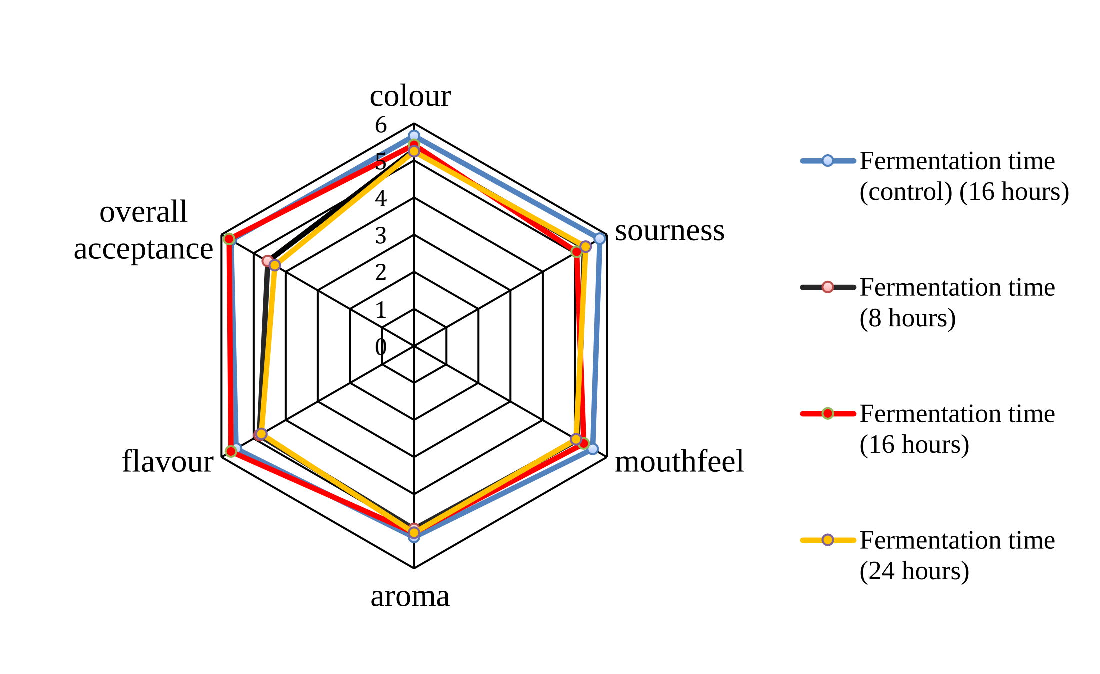

Figure 4.3: Hedonic test of consumer acceptability on different fermentation time

Significant differences ($p<0.05$) were found in the analysis by evaluating all the samples. Alkaline pH values are greater than 7, while acid pH values are less than 7. The pH values for the control, coconut milk kefir with snake fruit puree fermented for 8, 16, and 24 hours were 4.9, 4.8, 4.5, and 4.2, respectively. The pH decreased with longer fermentation times, consistent with previous research indicating that increased incubation time leads to higher viscosity and acidity, accompanied by a lower pH level (Shinta *et al.*, 2020).

According to Wafa *et al.*, (2023) depending on the duration of fermentation, the type of milk, the container that was used, and their interaction term demonstrated that every variable term had significant effects on variations in pH and acidity. Overall, the combination of the initial pH of coconut milk and the acidification process during fermentation might lead to a lower pH in coconut milk kefir with snake fruit puree compared to cow's milk kefir. Exact pH values can vary depending on the specific ingredients, fermentation conditions and the duration of fermentation. As stated by Yeshambel *et al.*, (2021) Lactobacillus spp. can survive in extremely acidic conditions with a pH value of 4 to 5 or even lower. By comparing to the result obtained for the samples, it can be also decided that low pH value obtained can allow the viability of lactic acid bacteria. Low pH levels not only promote the growth of yeasts and probiotic lactic acid bacteria but also inhibit the growth of most of the waste and pathogenic organisms (Anton *et al.*, 2016).

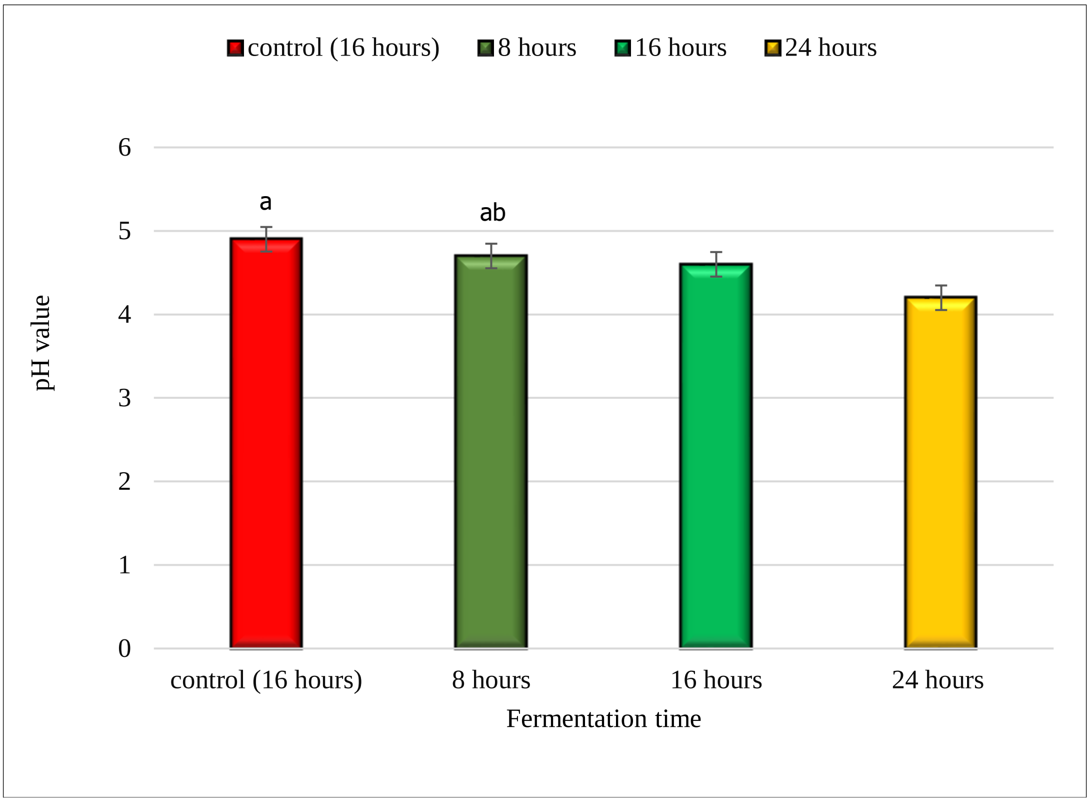

Figure 4.4: pH value of frozen non-dairy kefir with snake fruit puree as affected by different fermentation time

There are significant differences identified between all the four samples analysed. The control (cow's milk kefir) has the least mean score. Generally, coconut milk is more viscous compared to cow's milk. Viscosity can particularly influence by the type of milk used. The viscosity of coconut milk kefir with snake fruit puree at the fermentation time of 8,16 and 24 hours is significantly higher compared with cow's milk kefir as there is no addition of snake fruit puree in the control sample. Addition of snake fruit puree has also contributed to the thickness of the coconut milk kefir with different fermentation time. This is supported by Fitrianingsih et al., (2021) the lactic acid formed in fermentation not only contributes to the taste but also disrupts proteins, resulting in the coagulation of milk proteins.

According to Triana et al., (2020) has stated that matrix formed by a lower pH during the fermentation of milk's solid matter could increase the viscosity of the fermentation product. Through the study conducted, it can also be determined that lower pH resulted from increasing fermentation time might influence the viscosity of the coconut milk kefir with snake fruit puree.

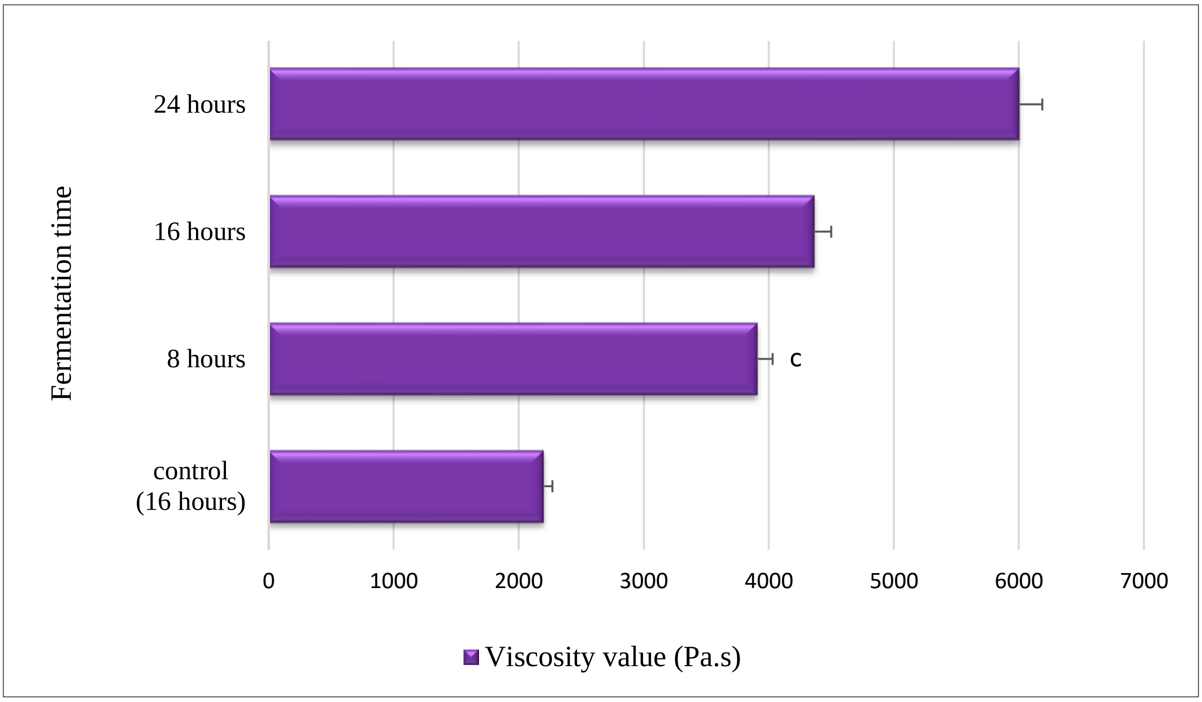

Figure 4.5: Viscosity value of control (cow's milk) and coconut milk kefir with snake fruit puree from different kefir fermentation time

Colour is the most crucial factor in determining the degree to which a food product is deemed acceptable by customers (Kakoli & Rosanin, 2023). Colorimetric analysis can be used to determine customer acceptability. The highest mean value of lightness (24.550 ± 0.07550) was showed in the control (cow's milk kefir), while the lowest mean value (22.8233 ± 0.15011) was in coconut milk kefir with snake fruit puree in the fermentation time of 24 hours. Incorporation of snake fruit puree as an ingredient significantly affected ($p<0.05$) lightness (L*) in kefir produced. It can be observed that as the fermentation time increases, the lightness of the coconut milk kefir with addition of snake fruit puree decreases. Accordingly, cow's milk kefir has higher lightness than coconut milk kefir with snake fruit puree on different fermentation time.

The a* and b* indicate colour directions: a* denotes the red direction, -a* the green direction, +b* the yellow direction, and -b* the blue direction. Parameter a* value was identified where coconut milk kefir with snake fruit puree at the fermentation time of 16 hours shows negative value indicating as it falls under the green colouration. Control sample along with coconut milk kefir with snake fruit at the fermentation time of 8 and 24 hours of a* parameter shows positive value indicating as it falls under red colouration. The b* value was determined for all the samples including control where it shows negative value indicating blue colouration. According to James (2015) centre is achromatic, colour saturation rises as the a* and b* values rise and the point advances further from the centre. By observing the values of the both a* and b* of the samples indicates that the final product colour saturation is around the centre of the chromaticity diagram.

Eun et al., (2020) has stated that during the kimchi fermentation stage, a gradual change in the total colour difference (E) was reported. The greatest E values were observed at 4 (34.87) °C and 10 °C (37.99), after 9 weeks. By the research conducted by Dian *et al.*, (2017), it has been revealed that as fermentation time increased, both the lightness of the soybeans and the mycelia of the tempeh has reduced. From the above statements, it can be concluded that fermentation time can impact the colour of the final product.

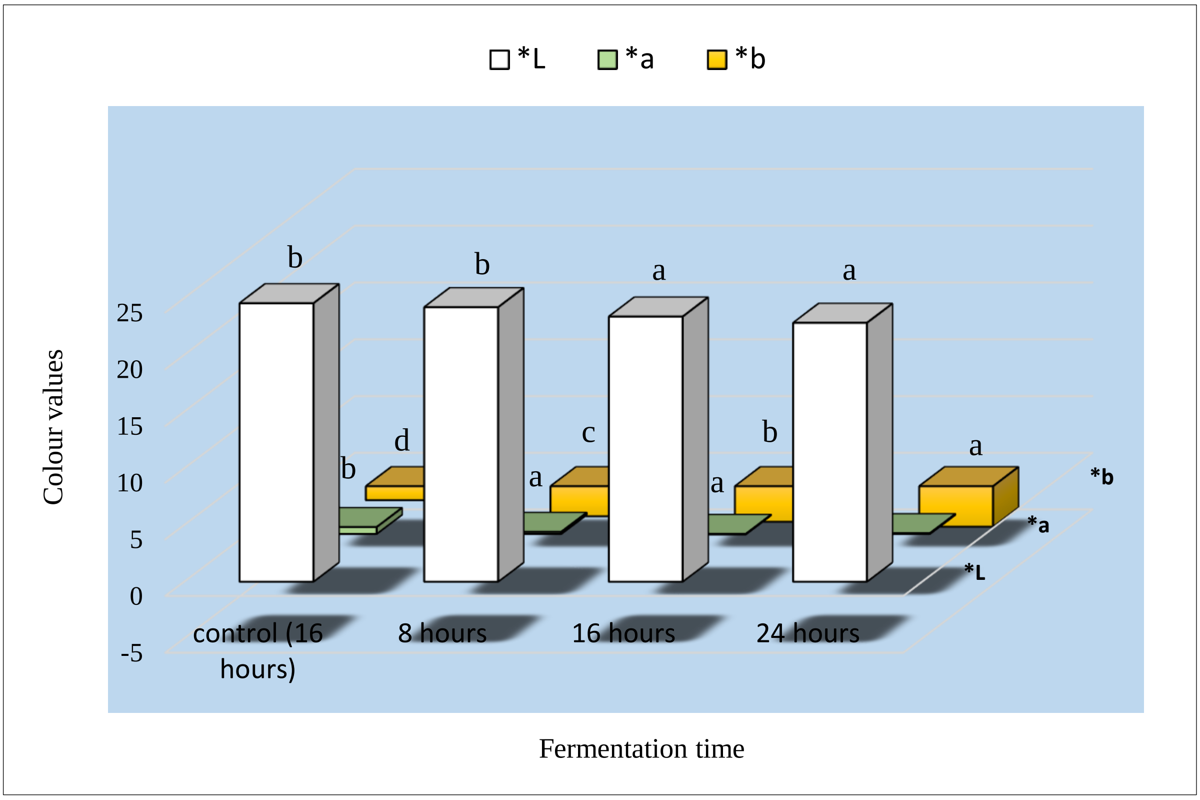

Figure 4.6: Colour value of control (cow's milk) and coconut milk kefir with snake fruit puree from different kefir fermentation time

DPPH radical scavenging effect on coconut milk kefir with snake fruit puree at different fermentation time has been measured. The DPPH assay was included because it is a relatively simple test method that provides an initial indication of a test compound's capacity to scavenge free radicals. It can determine that there are significant differences among the samples. The scavenging activity of control showed 80%. Scavenging activity of coconut milk kefir with snake fruit puree for 8 hours is 79%, for 16 hours is 82% and finally for 24 hours is 85% accordingly.

It can be assumed that the fermentation time of kefir can influence the production as well as the concentration of these antioxidant compounds. As the fermentation time increases, the microbial activity increasing leading to the synthesis of more scavenging activity compared to kefir fermented for a shorter time. The enhancement of scavenging activity is greatly aided by bacterial fermentation (Yan *et al.*, 2021). As stated by Hur *et al.*, (2014) has stated that a rise in phenolic compounds and flavonoids during fermentation, considered as an outcome of a microbial hydrolysis reaction, which is mainly linked to fermentation's ability to boost scavenging activity of the fermented food. Maintaining the optimum temperature as 32°C during fermentation might influence improved microbial growth and enzymatic activity, and consequently the benefits of fermentation are improved.

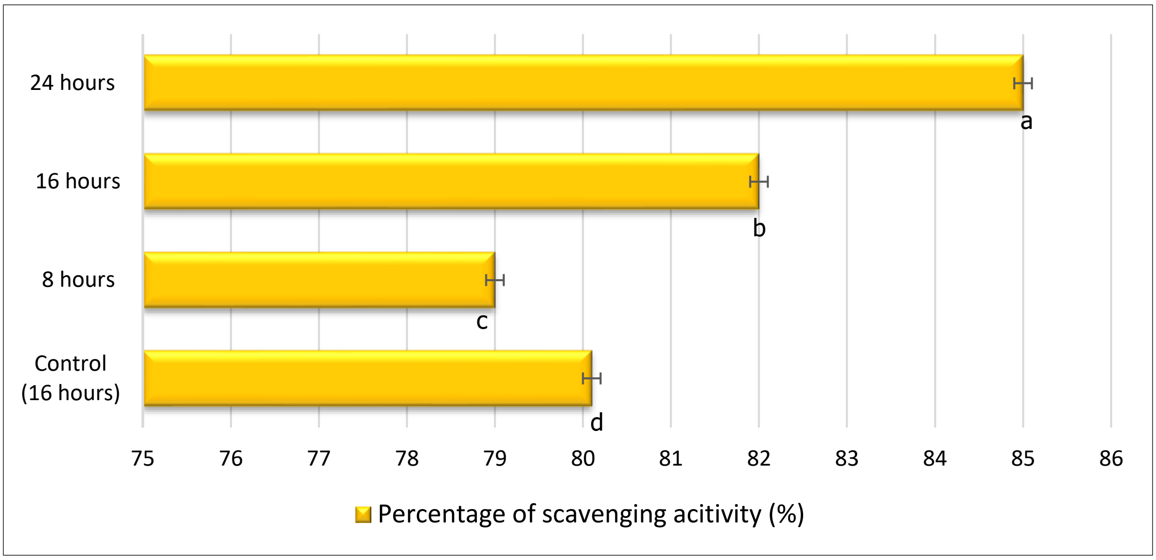

Figure 4.7: Scavenging activity of control (cow's milk kefir) and coconut milk kefir with snake fruit puree from different fermentation time

5.0 Conclusion and future research

In conclusion, the outcomes of this study align with expectations, and the research goal has been successfully achieved. The results indicate that the fermentation time has a significant impact on frozen non-dairy kefir with snake fruit puree, surpassing that of cow's milk kefir. The recommended optimal fermentation time for frozen non-dairy kefir with snake fruit puree is found to be 16 hours at a fermentation temperature of 32°C. Under these optimal fermentation conditions, the sensory evaluation yielded the highest score of 5.76 compared to other samples. Additionally, the pH value was measured at 4.56 and scavenging value at 82%. The viable cell counts of lactic acid bacteria and yeast surviving in the sample were determined to be 3.91 x 10^7 and 3.8 x 10^7 cfu/ml respectively.

Hence, this research project demonstrates the considerable potential of frozen non-dairy kefir enriched with snake fruit puree as a promising alternative among fermented foods for individuals with lactose intolerant, as well as those grappling with digestive health issues within the community. Research on the fermentation of non-dairy milk substitutes has led to evidence-based assessment, findings indicate that plant-based milk function as a viable probiotic carrier. Additionally, the study's findings convincingly demonstrate the commercial viability and market impact of this product, as evidenced by the positive outcomes of the consumer acceptability study.

For future studies, researchers can consider exploring the application of kefir fermentation to various other plant-based dairy alternatives, including palm milk, rice milk, yellow split pea milk and almond milk. Investigating this area could provide valuable insights into refining the fermentation process, improving nutritional profiles, and fostering probiotic development across a range of non-dairy bases. Additionally, there is an opportunity to explore different pre-treatment methods aimed at preserving snake fruit puree. Given the potential nutrient loss during processing, identifying effective pre-treatment approaches is crucial for safeguarding the nutritional integrity of snake fruit puree throughout its journey from processing to consumption.

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Author Contributions

Syaza Farhanim Hamran, Khairin Isyraq Kamarul Zaman: Conceptualization, Methodology, Software, Writing- Original Draft Preparation; **Nabilla Huda Baharuddin**: Data Curation, Validation, Supervision; **Harinee Devaraj**: Software, Validation, Writing-Reviewing and Editing.

Conflicts of Interest

The manuscript has not been published elsewhere and is not under consideration by other journals. All authors have approved the review, agree with its submission and declare no conflict of interest in the manuscript.

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