

The effect of high fructose syrup concentration on physicochemical, microbiological, and sensory properties of goat milk kefir with *butterfly pea* extract

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ABSTRACT

Goat milk kefir is valued for its probiotic and nutritional benefits but exhibits suboptimal sensory qualities when combined with *butterfly pea* extract. The addition of HFS as a sugar source can ensure optimal fermentation and improve the sensory quality of kefir. This study aimed to determine the effect of HFS on the characteristics of goat milk kefir with *butterfly pea* flower extract in terms of physicochemical, microbiological, and sensory properties. A Completely Randomized Design (CRD) with five HFS concentrations (0%, 2.5%, 5%, 7.5%, and 10%) and four replications was used. Parameters measured included total acidity, pH, total yeast, total lactic acid bacteria (LAB), total soluble solids (TSS), antioxidant activity, and sensory attributes (color, aroma, taste, texture, soda sensation, and overall acceptance). Results showed that HFS addition significantly increased total acidity, TSS, and LAB counts, while pH and total yeast counts were unaffected. Total acidity ranged from 1.45 ± 0.11 to $1.96 \pm 0.11\%$; pH from 3.86 ± 0.099 to 3.95 ± 0.11 ; TSS from 8.18 ± 0.15 to 13.78 ± 0.21 °Brix; antioxidant activity decreased from 68.83% to 42.64%; LAB counts increased from 8.38 ± 0.10 to 9.15 ± 0.01 log CFU/mL; and total yeast ranged from 5.89 ± 0.22 to 6.49 ± 0.17 log CFU/mL. The optimal HFS concentration was 7.5%–10%, producing kefir with enhanced functional, physicochemical, and sensory qualities and highest panelist acceptance.

Introduction

The increasing consumer awareness regarding healthy lifestyles and the consumption of high-quality foods has spurred the development of functional foods. These foods are recognized not only for their nutritional value but also for providing various health benefits

(Vignesh *et al.*, 2024). A crucial role is played by the gut microbiota in maintaining overall health, which has further encouraged the development of functional foods containing prebiotics and probiotics to help preserve intestinal microbiota balance. Kefir is a probiotic drink made through fermentation by lactic acid bacteria (LAB) and yeasts (Yilmaz *et al.*, 2022). It is produced by inoculating milk with kefir grains, which contain LAB, acetic acid bacteria, and yeasts. These microorganisms work together to produce various metabolic compounds, including organic acids, ethanol, and bacteriocins. These compounds help provide probiotic and antibacterial effects against harmful organisms (Azizi *et al.*, 2021). Kefir has been shown to support digestive health, lower cholesterol levels, enhance the immune system, and reduce the risk of cardiovascular diseases (Satir and Seydim, 2023).

While kefir is usually made from cow's milk, goat milk is seen as having higher nutritional value (Turkmen, 2017). Goat milk contains certain amino acids and is made up of about 71 to 75% casein, 20 to 22% whey proteins, and 7% non-protein nitrogen (Satir and Seydim, 2023). Goat milk also has a lower potential for causing allergies (M'hiri *et al.*, 2024) and smaller fat globules, which makes it better for people with lactose intolerance (Santos *et al.*, 2023). Turning goat milk into fermented products not only improves its nutrition and shelf life but also helps remove its distinct goaty smell. In addition, minerals like magnesium, calcium, and potassium in goat milk add to the nutritional value of the kefir product (Singh *et al.*, 2021).

To improve both the look and function of the product, *butterfly pea* flower (*Clitoria ternatea*) extract was added to goat milk kefir. *Butterfly pea* flower contains anthocyanin compounds, mainly delphinidin gluco-

side, which gives it a unique bluish-purple color and acts as a strong antioxidant (Aksornsri *et al.*, 2023). This extract also shows anti-inflammatory, antidiabetic, antiproliferative, and antimicrobial effects, offering various health benefits (Jeyaraj *et al.*, 2021). The natural pigment in *butterfly pea* flowers can be used to enhance the visual appeal of goat milk kefir.

The kefir fermentation process requires sugar as a carbon source for microbial growth. Adding sugar speeds up fermentation and affects the final product's quality (Fiorda *et al.*, 2017). This study selected High Fructose Syrup (HFS) as an alternative sweetener because of its high solubility, non-crystalline form, and stability in acidic conditions. These factors make it suitable for fermented beverages (Kwan *et al.*, 2018). Additionally, HFS is commonly used in today's food and drink products (Surja *et al.*, 2019). While goat milk kefir with *butterfly pea* flower extract has been researched before, no studies have looked at using HFS as a sweetener in this mix. There has been no investigation into its optimal concentration or its effects on fermentation, quality, and sensory appeal. Therefore, this study aimed to examine how different concentrations of HFS affect the physicochemical, microbiological, and sensory aspects of goat milk kefir with *butterfly pea* flower extract. The results are expected to help create high-quality functional probiotic drinks that are safe, acceptable, and appealing to consumers.

Materials and methods

Materials

The materials used in this study comprised fresh goat milk, which was obtained from a private goat farm in Gunungpati owned by Mrs. Lis Pur; grain kefir; dried *Clitoria ternatea* (*butterfly pea*) flowers purchased online from "Aydaa Store Snack & Cemilan" in Surakarta; 55% High Fructose Syrup (HFS) acquired from the online shop "Powder Bubble Blast" in Bekasi; and chloramphenicol (Bernofarm capsules, 250 mg) obtained from the online pharmacy "Apotek Bintang Farma K24" in Bekasi with

a doctor's prescription. Additional reagents and chemicals included 1% phenolphthalein indicator (PP), Potato Dextrose Agar (PDA) medium, de Man Rogosa and Sharpe Agar (MRSA) medium, methanol, 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution, spiritus, distilled water, bottled mineral water, 0.85% physiological NaCl, 0.1 N NaOH, 70% ethanol, and 70% alcohol. The equipment utilized throughout the research consisted of a grinder (Maksindo, Indonesia), hot plate (IKA, Germany), refrigerator (GEA, China), freezer, analytical balance, hand refractometer (Atago, Japan), bacterial incubator (Mettler, Germany), UV-Vis spectrophotometer (Biobase, China), micropipette (DragonLab, China), micropipette tips, vortex mixer (DLAB, USA), autoclave (Hirayama, Japan), pH meter (Ohaus, Germany), laminar air flow cabinet, incubator bacteria (Mettler, Germany), incubator yeast (Mettler, Germany), magnetic stirrer, test tube rack, test tubes (Pyrex, France), beakers (Pyrex, France), Erlenmeyer flasks (Pyrex, France), volumetric flasks (Pyrex, France), Petri dishes (Pyrex, France), cuvette glasses, centrifuge tubes, burette, thermometer, Bunsen burner, stove, basin, tray, 60-mesh sieve, filter cloth, metal spatula, spoon, dropper pipette, plastic containers, plastic cups, plastic wrap, polyethylene (PE) plastic bags, aluminum foil, cotton, matches, mask, and marker.

Methods

The study was conducted through several stages, beginning with the preparation of *butterfly pea* flower (*Clitoria ternatea*) extract. Subsequently, goat milk kefir enriched with the *butterfly pea* flower extract was produced with varying concentrations of High Fructose Syrup (HFS). After producing the kefir, we evaluated the physicochemical, microbiological, and sensory parameters. The physicochemical tests measured total acidity, pH, total soluble solids, and antioxidant activity. We conducted microbiological assessments to count the total lactic acid bacteria (LAB) and total yeast. The sensory evaluations included both hedonic and organoleptic tests to check the quality and acceptability of the kefir products. We applied suitable statistical methods to analyze the effects of HFS concentration on the properties of goat milk kefir with *butterfly pea* flower extract.

Butterfly pea flower extraction

The extraction process was adapted from Jeyaraj *et al.* (2021) with modifications. Dried *butterfly pea* flowers were ground into a fine powder using a grinder. Next, the powder was passed through a 60-mesh sieve to get a uniform particle size. The *butterfly pea* powder was mixed with distilled water at a ratio of 5:1 (w/v). Then, the mixture was filtered with a cloth. The filtrate was stirred with a magnetic stirrer while being heated on a hot plate at 60°C for 30 minutes at a constant speed of 600 rpm to obtain a homogeneous mixture. Finally, the extract was allowed to cool, resulting in *butterfly pea* flower extract.

Preparation of goat milk kefir

The preparation of goat milk kefir was based on the method by Nur'Aini *et al.* (2023) with modifications. Goat milk was pasteurized at 80 °C for 15 minutes and then cooled to 30 °C. *Butterfly pea* flower extract was added at a concentration of 5% (v/v). Subsequently, kefir grains were added at 5% (w/v), along with High Fructose Syrup (HFS) according to the treatment groups: T0 (0%), T1 (2.5%), T2 (5%), T3 (7.5%), and T4 (10%) (v/v). The mixture was incubated at room temperature (25 – 28 °C) for 24 hours. After incubation, the kefir grains were removed by filtration, and the resulting kefir was stored in a refrigerator at 4°C.

Total acidity measurement

The total acidity was determined by titration using 0.1 N NaOH solution, referring to modification of the method by Manurung *et al.* (2022).

Initially, the kefir sample was diluted due to its thick texture, which hindered the titration process. A volume of 10 mL of the sample was transferred into a 100 mL volumetric flask, distilled water was added up to the meniscus mark, and the solution was mixed thoroughly to achieve homogeneity. From this solution, 20 mL was taken for titration. Prior to titration, the burette was rinsed with distilled water and filled with 0.1 N NaOH. The sample in the Erlenmeyer flask was supplemented with 2 – 3 drops of 1% phenolphthalein indicator and titrated slowly until a stable pink coloration appeared, indicating the titration endpoint. The titration results were used to calculate the total acidity. The titratable acidity was calculated using the following formula:

$$\text{Total acidity (\%)} = \frac{V1 \times N \times B \times FP}{V2 \times 1000} \times 100\%$$

Explanation:

V1= Volume of NaOH solution (mL);

V2= Initial volume of sample used for dilution (mL);

N= Normality of NaOH solution;

B= Molecular weight of lactic acid (90);

FP= Dilution factor 100/20 (total volume of dilution solution / titration volume)

pH value assay

The pH measurement was done using a digital pH meter from Ohaus, following the method by Gavriloska *et al.* (2023). Before use, we rinsed the probe with distilled water and dried it. We placed 60 mL of the kefir sample into a beaker and inserted the probe without letting it touch the bottom. Once the reading stabilized, we pressed the Read/Enter button and recorded the pH value when the number on the display stayed constant. Afterward, we cleaned the probe again to remove any leftover sample.

Total soluble solids measurement

Total soluble solids measurements refer to the method used by Praymono *et al.* (2025), using a hand refractometer. Instrument calibration was carried out by cleaning the refractometer prism using distilled water, which was dripped onto the prism surface then wiped with a soft cloth. Measurements were carried out by placing 1 – 2 mL of sample on the surface of the prism, then reading the °Brix value.

Antioxidant activity assay

Antioxidant activity was determined using the DPPH method, following the method by Birwal *et al.* (2025). The test solution was prepared by mixing 0.1 g of the sample with 10 mL of ethanol, and the mixture was centrifuged until a precipitate formed. Next, 0.2 mL of the supernatant was mixed with 1 mL of 0.05 mM DPPH solution in a light-tight test tube. The mixture was incubated for 30 minutes in the dark at 37°C. A color change in the DPPH solution from purple to pale yellow indicated the presence of antioxidant activity. The absorbance was measured using a spectrophotometer at a wavelength of 520 nm. A blank solution was prepared by mixing 1 mL of ethanol with 1 mL of DPPH solution, and its absorbance was measured at the same wavelength. The percentage of DPPH radical inhibition was calculated using the following formula.

$$\% \text{inhibition} = \frac{\text{absorbance blank} - \text{absorbance sampel}}{\text{absorbance blank}} \times 100\%$$

Determination of total lactic acid bacteria

The enumeration of total LAB referred to the method described by Nur'Aini *et al.* (2023), with modifications using the Total Plate Count (TPC) method. A 1 mL sample of kefir was added to 9 mL of 0.85% physiological NaCl solution and homogenized using a vortex mixer to obtain a 10⁻¹ dilution. Serial dilutions were performed by transferring 1 mL of the 10⁻¹

dilution into 9 mL of 0.85% physiological NaCl solution, resulting in a 10^{-2} dilution. This dilution process was continued until a final dilution of 10^{-8} was reached. Plating was conducted using the last three dilutions, namely 10^{-6} , 10^{-7} , and 10^{-8} . Each dilution was plated duplicate. A 1 mL aliquot from each selected dilution was transferred into a sterile Petri dish, followed by the addition of 15 mL of MRS Agar (MRSA) medium. The Petri dishes were gently rotated in a looping pattern motion to distribute the mixture evenly. The dishes were then incubated upside down at 37 °C for 48 hours. The total number of LAB was expressed as colony-forming units per milliliter (CFU/mL) and calculated using the following formula.

"Colonies/mL" = "number of colonies ×" "1" / "dilution factor"

Determination of total yeast counts

The total yeast count was determined using the dilution method with Potato Dextrose Agar (PDA) as the growth medium (McKernan *et al.*, 2021). To stop the growth of unwanted bacteria during the yeast count, the PDA medium was mixed with chloramphenicol at a concentration of 50 mg/L (Al Khatib *et al.*, 2025). The procedure for counting yeast followed the method described by Al-Baarri *et al.* (2020). A volume of 1 mL of the sample was added to 9 mL of 0.85% physiological NaCl solution and mixed using a vortex mixer. Serial dilutions were made up to 10^{-4} . For the last three dilutions, 1 mL of the suspension was transferred duplicate into Petri dishes, which were then covered to avoid contamination. Next, 15 mL of sterile PDA medium at 50°C was added with the lid slightly open. The Petri dishes were gently swirled in a looping pattern motion to spread the yeast cells evenly. After the medium solidified, the plates were incubated upside down at 25°C for 48 hours. After incubation, yeast colonies were counted using the Total Plate Count (TPC) method, considering only plates with colony counts between 30 and 300 per dilution series. The colony counts were then converted into logarithmic form ($\log_{10}$ CFU/mL) before statistical analysis.

"Total yeast (CFU/ml) = average number of colonies ×" "1" / "dilution factor"

Hedonic quality assessment

Hedonic quality assessment was based on a study by Pramono *et al.* (2025), which involved 25 semi-trained panelists aged 20 - 25 years. Panelists were given a series of samples, each labeled with a three-digit random code, along with an assessment form. They were asked to rate each hedonic attribute listed on the assessment form. The hedonic attributes assessed included color, aroma, taste, texture, freshness, and overall preference. A five-point hedonic scale was used, with the following categories: 1 = strongly dislike, 2 = dislike, 3 = slightly like, 4 = like, and 5 = strongly like.

Organoleptic quality assessment

The organoleptic evaluation used fresh kefir with *butterfly pea* flower extract added just before testing to keep the product quality high (Rizqiati *et al.*, 2025). A total of 30 trained panelists took part in the assessment. Each sample had a random three-digit code to reduce subjectivity during the evaluation. Panelists received questionnaires that included testing instructions, personal information forms, and details about the date and type of sample tested. Drinking water was available throughout the session to help neutralize tastes between samples. The questionnaire was thoughtfully designed. It included rating scales for each attribute and open-ended questions for panelists to share additional comments or feedback on the tested products. The organoleptic attributes assessed were color, aroma, taste, texture, and soda sensation. Color was rated on a scale of 1 to 5, where 1 meant "not blue at all" and 5 signified "very blue." Aroma and taste were judged based on acidity level, using the same scale, where 1 meant "not acidic at all" and 5 meant "very acidic."

Texture was rated by viscosity, with 1 meaning "not viscous at all" and 5 meaning "very viscous." Soda sensation was assessed on a scale from 1, "not carbonated at all," to 5, "very carbonated."

Statistical analysis

The data from physicochemical and microbiological tests were analyzed using parametric Analysis of Variance (ANOVA) at a 5% significance level. This was done with SPSS for Windows version 26.0. When significant differences were detected, Duncan's Multiple Range Test (DMRT) was subsequently performed to identify differences between treatments. Sensory data were divided into hedonic and organoleptic evaluations. Organoleptic attributes assessed included taste, aroma, color, texture, and soda sensation, while hedonic evaluation additionally included overall acceptance. These sensory data were analyzed using the non-parametric Kruskal-Wallis test at a 5% significance level. In cases where significant differences were observed, pairwise comparisons were conducted using the Mann-Whitney U Test (Margareth *et al.*, 2020). Antioxidant activity data were analyzed descriptively.

Results

Total acidity

The average total acidity values in goat milk kefir enriched with *butterfly pea* flower extract and supplemented with varying concentrations of High Fructose Syrup (HFS) exhibited an increasing pattern from treatment T0 to T2, ranging from 1.81% to 1.96%. However, a decrease was observed in treatments T3 and T4, with the lowest total acidity value recorded in treatment T4 at 1.45%. No significant differences in total acidity were detected among treatments T0, T1, T2, and T3, whereas treatment T4 showed a significant difference compared to T0, T1, T2, and T3. This indicates a significant effect of the highest HFS concentration (10%) on total acidity.

pH value

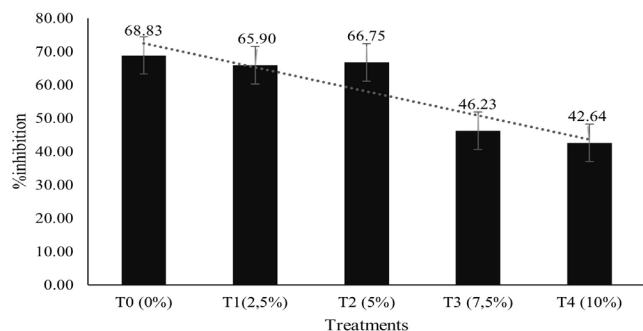
The pH values of goat milk kefir with *butterfly pea* flower extract and different amounts of HFS were quite similar, between 3.86 and 3.95. Even though there was a slight decrease in pH values across treatments, statistical analysis showed no significant differences among them ($P \geq 0.05$). This means that adding various HFS concentrations did not noticeably change the pH of the kefir.

Total soluble solids

The total soluble solids (TSS) of goat milk kefir with *butterfly pea* flower extract and various concentrations of high fructose syrup (HFS) ranged from 8.18 ± 0.15 to 13.78 ± 0.21 °Brix. The highest TSS value was observed with the addition of 10% HFS, while the lowest value was observed with the treatment of 0% HFS. Statistical analysis using ANOVA showed that various concentrations of HFS had a significant effect ($P < 0.05$) on the TSS values of goat milk kefir with *butterfly pea* flower extract. These results indicate that the TSS value increased progressively with increasing concentration of added HFS.

Antioxidant activity

The antioxidant activity of kefir showed different results for each treatment: T0 was 68.83%, T1 was 65.90%, T2 was 66.75%, T3 was 46.23%, and T4 was 42.64%. The antioxidant activity decreased significantly from 68.83% to 42.64%. These indicate that the antioxidant activity of goat milk kefir with *butterfly pea* flower extract decreased with concentrations of added HFS.

Fig 1. Histogram of antioxidant activity of goat milk kefir with *Butterfly pea* flower extract.

Total lactic acid bacteria

The total number of lactic acid bacteria (LAB) in goat milk kefir with *butterfly pea* flower extract and various concentrations of HFS ranged from 8.38 ± 0.10 to 9.15 ± 0.01 log CFU/mL. The lowest LAB count of 8.38 ± 0.10 log CFU/mL was observed in the treatment without HFS (0%), while the highest count of 9.15 ± 0.01 log CFU/mL was observed in the treatment with 10% HFS. The addition of various concentration of HFS had a significant effect ($P < 0.05$) on the total LAB count in goat milk kefir with *butterfly pea* flower extract. This indicates that the total LAB count increased proportionally with higher HFS concentrations.

Total yeast

The average total yeast counts in goat milk kefir with *butterfly pea* flower extract and different HFS concentrations ranged from 5.98 to 6.49 log CFU/mL. The Indonesian National Standard (2018) states that the minimum yeast culture requirement for fermented milk products like kefir is 1.0×10^4 CFU/mL. This indicates that all produced kefir samples met the microbiological quality standards and have potential as probiotic foods. Statistical analysis showed no significant differences among treatments T0, T1, T2, T3, and T4 ($P \geq 0.05$).

Hedonic quality

Sensory evaluation results showed that varying HFS concentrations had no significant effect ($P \geq 0.05$) on the color attribute of goat milk kefir enriched with *butterfly pea* flower extract. However, the addition of HFS at different concentrations significantly affected ($P < 0.05$) the aroma, taste, texture, carbonation sensation, and overall acceptance attributes. The mean panelist scores for color ranged from 3.64 ± 0.76 (T2) to 4.16 ± 0.80 (T4). The mean aroma scores ranged from 2.92 ± 0.76 (T0) to 3.68 ± 0.80 (T4). The highest taste score was obtained in T4 with a rating of 4.12 ± 0.93 , while the lowest was in T1 with a score of 2.36 ± 0.81 . For texture, the highest panelist score was 4.04 ± 0.73 (T4), and the lowest was 3.20 ± 0.91 (T1). The carbonation sensation was rated highest in T4 with a score of 4.00 ± 0.91 and lowest in T0 with a score of 2.80 ± 0.76 . Overall

Table 1. Chemical and Microbiological properties of goat milk kefir with *butterfly pea* flower extract.

Parameters	Treatments				
	T0	T1	T2	T3	T4
Total acidity (%)	1.81 ± 0.085^a	1.91 ± 0.11^a	1.96 ± 0.11^a	1.85 ± 0.12^a	1.45 ± 0.11^b
pH value	3.95 ± 0.11	3.92 ± 0.095	3.89 ± 0.095	3.87 ± 0.094	3.86 ± 0.099
Total Soluble Solids ($^{\circ}$ Brix)	8.18 ± 0.15^a	9.08 ± 0.15^b	11.10 ± 0.22^c	12.60 ± 0.18^d	13.78 ± 0.21^e
Total LAB (log CFU/mL)	8.38 ± 0.10^a	8.56 ± 0.18^b	8.84 ± 0.05^c	8.97 ± 0.06^c	9.15 ± 0.01^d
Total yeast (log CFU/mL)	5.89 ± 0.22	6.06 ± 0.62	5.99 ± 0.33	6.28 ± 0.27	6.49 ± 0.17

Superscript differences on the same basis indicate significant differences between treatments ($P < 0.05$) with data displayed as the mean value of 5 replications $\pm$ standard deviation. Hedonic quality assessment scale: (1) very dislike, (2) dislike, (3) somewhat like, (4) like, and (5) very like. T0, T1, T2, T3, and T4 are treatments with different HFS concentrations of 0%, 2.5%, 5%, 7.5%, and 10%.

Table 2. Organoleptic of goat milk kefir with *butterfly pea* flower extract.

Attributes	Treatments				
	T0	T1	T2	T3	T4
Color	3.37 ± 0.556	3.33 ± 0.606	3.40 ± 0.621	3.47 ± 0.629	3.40 ± 0.563
Aroma	3.10 ± 0.885	3.37 ± 0.890	3.63 ± 0.809	3.60 ± 0.968	3.33 ± 0.758
Taste	3.87 ± 0.900^{ab}	4.30 ± 0.651^a	3.67 ± 0.844^{bc}	3.63 ± 0.999^{bc}	3.17 ± 0.913^c
Texture	2.63 ± 0.850^a	2.83 ± 0.747^{ab}	2.97 ± 0.850^{abc}	3.30 ± 0.750^{bc}	3.13 ± 0.860^{bc}
Soda sensation	2.27 ± 0.907^a	3.27 ± 1.112^b	3.80 ± 1.095^b	3.33 ± 0.994^b	3.37 ± 0.890^b

Superscript differences on the same basis indicate significant differences between treatments ($P < 0.05$) with data displayed as the mean value of 5 replications $\pm$ standard deviation. Hedonic quality assessment scale: (1) very dislike, (2) dislike, (3) somewhat like, (4) like, and (5) very like. T0, T1, T2, T3, and T4 are treatments with different HFS concentrations of 0%, 2.5%, 5%, 7.5%, and 10%.

Table 3. Hedonic quality of goat milk kefir with *butterfly pea* flower extract.

Attributes	Treatments				
	T0	T1	T2	T3	T4
Color	3.96 ± 0.61	3.80 ± 0.82	3.64 ± 0.76	3.88 ± 0.73	4.16 ± 0.80
Aroma	2.92 ± 0.76^a	3.20 ± 0.71^a	3.20 ± 0.76^a	3.36 ± 0.95^{ab}	3.68 ± 0.80^b
Taste	2.36 ± 0.81^a	2.32 ± 0.90^a	2.92 ± 0.64^b	3.60 ± 0.96^c	4.12 ± 0.93^c
Texture	3.20 ± 0.91^a	3.28 ± 0.74^a	3.32 ± 0.63^a	3.68 ± 0.56^b	4.04 ± 0.73^b
Soda sensation	2.80 ± 0.76^a	3.00 ± 0.76^a	3.16 ± 0.80^{ab}	3.52 ± 0.87^{bc}	4.00 ± 0.91^c
Overall	2.68 ± 0.75^a	2.60 ± 0.71^a	3.24 ± 0.66^b	3.80 ± 0.82^c	4.44 ± 0.65^d

Superscript differences on the same basis indicate significant differences between treatments ($P < 0.05$) with data displayed as the mean value of 5 replications $\pm$ standard deviation. Hedonic quality assessment scale: (1) very dislike, (2) dislike, (3) somewhat like, (4) like, and (5) very like. T0, T1, T2, T3, and T4 are treatments with different HFS concentrations of 0%, 2.5%, 5%, 7.5%, and 10%.

hedonic acceptability was highest in T4 with a score of 4.44 ± 0.65 and lowest in T2 with a score of 2.60 ± 0.71 .

Organoleptic quality

The results on taste showed that adding High Fructose Syrup (HFS) to goat milk kefir enriched with *butterfly pea* flower extract did not greatly change the qualities of color and aroma. However, it did have a significant impact on taste, texture, and soda sensation. The average scores for organoleptic attributes were as follows: color ranged from 3.33 ± 0.606 (T1) to 3.47 ± 0.629 (T3), aroma from 3.10 ± 0.885 (T0) to 3.63 ± 0.809 (T2), taste from 3.17 ± 0.913 (T4) to 4.30 ± 0.651 (T1), texture from 2.63 ± 0.850 (T0) to 3.30 ± 0.750 (T3), and soda sensation from 2.27 ± 0.907 (T0) to 3.80 ± 1.095 (T2). The similar scores for color and aroma led to no clear sensory differences identified by the panelists. This indicates that changes in HFS concentration did not create noticeable visual or aromatic differences in the goat milk kefir with *butterfly pea* flower extract. However, taste, texture, and soda sensation were significantly affected.



Fig. 2. Color of goat milk kefir with *butterfly pea* flower extract in each treat.

Discussion

The rise in total acidity at low to moderate levels was due to the support of microbial fermentation activity by HFS. However, at high concentration (T4), microbial activity decreased because osmotic pressure disrupted cell balance (Sica et al., 2024). Despite the drop seen at T4, all treatments met the Codex (2003) standards for kefir acidity, which are $\geq 0.6\%$ and $0.5\text{--}2.0\%$. This shows that the products remained safe and suitable for consumption. The reduction in acidity at high concentrations was also backed by the study of Zielińska et al. (2021). This study found that large amounts of HFS can slow fermentation and lower lactic acid production. Since total acidity and pH are inversely related in fermented dairy products, changes in total acidity directly affected the pH profile of the kefir. Treatments with higher total acidity generally had lower pH values. The decrease in acidity at T4 explains the smaller pH change in this group compared to the others.

In general, an increase in total acidity in kefir is linked to a decrease in pH (Fiorda et al., 2017). However, in the treatment with the highest HFS addition, total acidity went down while pH still showed a downward trend across all treatments. This suggests that the drop in pH was not only due to lactic acid buildup, but could also be caused by the formation of other organic acids like acetic, formic, or succinic acids. Changes in the buffering system due to the high sugar concentration might also play a role (Lynch et al., 2021). Glucose and fructose from HFS are monosaccharides that microorganisms usually metabolize quickly. However, when sugar concentrations are high, osmotic stress can happen, which may suppress lactic acid bacteria activity and limit lactic acid production (Rizqiati et al., 2023). Additionally, microbial interactions during fermentation, such as competition for substrates between lactic acid bacteria and yeast, can affect metabolic pathways and the types of end-products formed. These changes in organic acid composition help

explain why pH continued to drop even when total acidity did not increase by the same amount.

Total soluble solids (TSS) indicate the sugar content of a substance, such as lactose in goat milk or fructose, the main component of high-fructose corn syrup (HFS). All inorganic and organic components, including organic acids, sucrose, and fermentation byproducts, are considered part of TSS (Suciati et al., 2024). The high fructose content in HFS contributes to the higher soluble solids concentration in goat milk kefir with *butterfly pea* flower extract. This increase in TSS is due to the ability of fructose to bind free water, thereby increasing the soluble solids concentration (Alfadila et al., 2020). The addition of excessive fructose as a sugar source can increase TSS levels but can also inhibit the metabolism of fermenting microorganisms. Microbial growth can be suppressed if the substrate concentration is too high, leading to the accumulation of soluble solids in the final product (Effendi and Parhusip, 2021).

Butterfly pea flowers (*Clitoria ternatea*) are an important source of antioxidants and contain various bioactive compounds such as flavonoids, flavonol glycosides, anthocyanins, tannins, saponins, and phenolic compounds. All of these compounds contribute to improving the functionality of goat milk kefir (Cahyaningsih et al., 2019). Experimental results showed that the addition of fructose syrup (HFS) at certain concentrations can reduce the antioxidant content of goat milk kefir enriched with *butterfly pea* flower extract. This decrease in antioxidant activity is likely caused by the degradation of anthocyanins in the *butterfly pea* flower extract. The decrease in pH caused by increasing fructose concentration can lead to the formation of 5-hydroxymethyl-2-furfural (HMF), a substance that contributes to anthocyanin degradation (Fajarwati et al., 2017). Furthermore, the acidic environment created by the production of organic acids can destabilize phenolic compounds, disrupt their interaction with DPPH, and reduce antioxidant activity (Puspitasari et al., 2017). Therefore, the addition of HFS should be optimized to maintain adequate antioxidant activity, as antioxidant activity is considered high if the inhibition rate exceeds 50% (Handayani et al., 2020).

The increase in total lactic acid bacteria (LAB) is due to the high fructose content in high-fructose corn syrup (HFS), which is readily metabolized by LAB. The addition of monosaccharides can increase the availability of carbon sources that support the growth of lactic acid bacteria (Chomphoosee et al., 2025). The addition of HFS encourages increased lactic acid bacteria activity on the fermentation substrate and lactic acid production, which in turn inhibits the growth of spoilage bacteria due to increased acidity. Increasing the HFS concentration provides more sugars, which stimulates the growth of LAB. This leads to an increase in total acidity due to lactic acid production during fermentation. The decrease in pH at higher HFS concentrations is consistent with the increase in total acidity, reflecting the metabolic activity of LAB. Kefir has shown antibacterial effects against various microorganisms, such as *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella* Enteritidis (Farag et al., 2020). Furthermore, the presence of phenolic compounds in *butterfly pea* flowers (*Clitoria ternatea*) can increase microbial viability and improve the functional properties of the product (Souza et al., 2019). According to Codex Alimentarius Commission (2003), fermented milk products should contain at least 1×10^7 CFU/ml of viable microorganisms. These results indicate that all goat milk kefir samples enriched with *butterfly pea* extract and various HFS concentrations met the required microbiological standards.

The lack of significant differences in total yeast counts among treatments shows that yeast was able to grow throughout the fermentation process, regardless of HFS concentration. In the early fermentation stage, when pH was still close to neutral, yeast could actively use HFS as a carbon source. However, as LAB activity increased, organic acid production gradually lowered the pH to below 4.0 by the end of fermentation. This change limited further yeast growth in the later stages. While high HFS concentrations might create hypertonic conditions that could hinder microbial activity due to osmotic stress (Rizqiati et al., 2023), the overall yeast population still increased compared to the initial stage. This stabil-

ity across treatments may also result from helpful interactions between yeast and LAB. Yeast produces growth factors that benefit LAB, and LAB creates anaerobic conditions that help yeast survive (Cheng *et al.*, 2024). The inverse relationship observed between final pH and total acidity in this study further indicates that microbial metabolism remained active in all treatments, despite the lack of statistically significant differences in yeast counts.

The analysis results showed that the addition of HFS significantly impacted the hedonic attributes of aroma, taste, texture, carbonation sensation, and overall acceptance. The color of goat milk kefir product was achieved by adding *butterfly pea* flower extract at a consistent concentration across all treatments, resulting in uniform coloration. The color produced by anthocyanins is known to vary with changes in the concentration of *butterfly pea* flower extract added to a product (Putri *et al.*, 2022). The addition of HFS to goat milk kefir with *butterfly pea* flower extract increases microbial activity in production of volatile compounds, which can help mask the goat odor typically associated with goat milk. Furthermore, the addition of sugar can prevent the development of undesirable flavors, as higher sugar concentration often increase the sourness of kefir products (Surja *et al.*, 2019).

HFS provides a sweet taste that can reduce the intensity of kefir's natural sourness. The sourness in kefir is caused by the production of lactic and acetic acids as a result of sugar metabolism (Rohman *et al.*, 2019). Panelists' preferences for specific products vary, with many preferring sweeter products. Although kefir viscosity is not always visually apparent, its texture varies between treatments, with panelists prefer a smooth and soft texture. Increasing the HFS concentration increases the water-sugar binding capacity, resulting in a thicker and creamier kefir texture (Raharjanti *et al.*, 2019). The carbonation sensation in goat milk kefir with *butterfly pea* flower extract is caused by the production of carbon dioxide and alcohol. The yeasts in kefir grains play a role in the production of these substances (Surja *et al.*, 2019). This carbonation contributes to a refreshing sensation that increases panelists' acceptance of the kefir product. The addition of HFS affects the taste, aroma, texture, and carbonation of kefir, thus influencing overall panelists' acceptance. Overall hedonic evaluation plays a critical role in consumer acceptance of goat milk kefir with *butterfly pea* flower extract.

The color was consistent because all treatments used a steady 5% concentration of *butterfly pea* flower extract. Additionally, HFS had no pigment. Similarly, the insignificance of aroma differences was due to the dominance of the characteristic goat milk aroma and the minimal contribution from HFS and *butterfly pea* extract. The taste attribute showed a decrease in sourness intensity with increasing HFS concentration. Treatment T1, with low HFS concentration, received the highest sourness score, indicating that the characteristic kefir flavor remained strong. Conversely, at high HFS concentrations, sourness tended to be masked, suggesting an interaction between fermentation-derived acidic compounds and HFS as a flavor balancer. The acidic conditions during the fermentation process caused the precipitation of milk proteins (casein), which played an important role in forming the thick texture of kefir. The kefir texture was influenced by the viscosity of HFS, which increased total dissolved solids (Bielska *et al.*, 2021). Treatment T3 produced the thickest and most balanced texture organoleptically. Meanwhile, the highest soda sensation was observed in treatment T2, which was attributed to the HFS level at this point, supporting optimal microbial fermentation for CO₂ production, thereby creating a pronounced carbonation sensation.

Conclusion

Based on the conducted research, the addition of High Fructose Syrup (HFS) to goat milk kefir enriched with *butterfly pea* flower extract was found to increase total acidity up to a certain concentration, while pH and total yeast counts remained consistent. Increasing HFS concentration caused a decrease in antioxidant activity and an increase in total soluble

solids and lactic acid bacteria (LAB) counts. Sensory evaluation showed consistent color and aroma across samples, with improved scores for aroma, taste, texture, soda sensation, and overall acceptance. The most favorable kefir characteristics were achieved at HFS concentrations between 7.5% and 10%, indicating that HFS concentration significantly affects the physicochemical, microbiological, and sensory properties of the product.

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Conflict of interest

The authors declare that no conflict of interest through any financial, personal, or other relationships with other people or organization related to the materials discussed in the manuscript.

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