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Effect of Date Palm (*Phoenix dactilyfera* L.) Juice Concentration with Mixed Culture on Product Quality and Antioxidant Activity of Synbiotic Yoghurt

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Abstract: The development of functional fermented foods with improved nutritional and antioxidant properties has attracted increasing interest in food science. This study examined the effect of adding date palm juice at different concentrations with a mixed culture on product quality and antioxidant activity of synbiotic date yogurt. The date palm juice concentrations used were 0%, 10%, 15%, and 20% (v/v). The fermentation process was carried out for 8 hours at 37°C with a mixed starter culture consisting of two yogurt starter bacteria and three probiotic strains. Then, chemical quality testing was carried out in the form of pH values using a pH meter and total titratable acidity (TTA) with acid-base titration, microbiological quality (total LAB) using the Total Plate Count (TPC) method, organoleptic testing using the hedonic test, and antioxidant activity testing using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method expressed as IC_{50}. Test data were analyzed using SPSS software to determine the effect of date palm juice concentration on test results. Synbiotic date yogurt has met the yogurt quality requirements in the form of pH, TTA, and total LAB according to SNI 2981:2009 at date palm juice concentrations of 0% to 20% (v/v). Increasing the concentration of date juice in synbiotic date yogurt affects the pH value, TTA value, total LAB and IC_{50} value as the results of antioxidant activity testing. The 20% Ajwa date juice treatment produced the best overall results, with a pH of 3.87, a TTA of 0.9394%, a total LAB count of 3.61×10^8 CFU/mL, and the strongest antioxidant activity (IC_{50} = 60.785 ppm). The results of this study indicate that Ajwa date juice can be utilized as a functional ingredient in the development of synbiotic yogurt with good quality and antioxidant activity.

Keywords: Antioxidant Activity; Date Palm Juice; Product Quality; Synbiotic.

Introduction

Data from the International Diabetes Federation (IDF) for 2019 show that the number of people with diabetes worldwide reached 463 million, resulting in 1.5 million deaths. This figure is projected to rise to 578 million by 2030, then increase to 700 million by 2045. The increasing prevalence of diabetes mellitus is associated with changes in dietary patterns, reduced physical activity, and rising obesity rates. In addition to insulin resistance, diabetes mellitus is characterized by alterations in the composition of the gut microbiota that contribute to inflammation and impaired glucose metabolism, thereby exacerbating insulin resistance [1]. Functional foods containing dietary fiber and prebiotics may support diabetes management because these components are not digested in the human gastrointestinal tract but are fermented by the gut microbiota, producing short-chain fatty acids (SCFAs) that help maintain gut health and metabolic homeostasis. Therefore, the development of synbiotic functional foods represents a promising strategy for supporting diabetes management.

Yogurt is a milk-based fermented product produced by lactic acid bacteria. Milk is a good medium for the growth of lactic acid bacteria because it contains lactose, protein, fat, minerals, and vitamins [2]. Yogurt also has a relatively complete nutritional profile, including protein, unsaturated fatty acids, calcium, magnesium, and B vitamins [3]. However, yogurt naturally contains relatively low levels of dietary fiber, vitamin C, carotenoids, and iron. Therefore, adding fruits or vegetables can enhance its nutritional and functional value [4]. As food science and technology advance, innovations in yogurt are rapidly evolving toward synbiotic products. Synbiotics are a combination of probiotics and prebiotics.

Ajwa dates contain glucose and fructose as the main reducing sugars (20–70% dry weight), which can be utilized by lactic acid bacteria during fermentation, thereby reducing the free sugar content in the final product [5]. Dates (*Phoenix dactilyfera* L.) are a type of fruit with potential as a source of prebiotics because they contain carbohydrates, fiber, and flavonoid polyphenols. Among various date cultivars, Ajwa dates contain fructooligosaccharides (FOS), a natural prebiotic [6]. FOS is resistant to digestion by human gastrointestinal enzymes because its β-(2→1) glycosidic bonds cannot be hydrolyzed by α-amylase [8]. Instead, FOS is fermented by lactic acid bacteria and the gut microbiota to produce short-chain fatty acids (SCFAs), while β-fructofuranosidase produced by several lactic acid bacteria hydrolyzes FOS into glucose and fructose for bacterial growth and metabolism [7][8].

Yogurt production, the type of starter culture used significantly influences the characteristics of the final product [9]. The fermentation process can be carried out using either a single-strain culture or a mixed culture. However, the use of a single-strain culture carries risks because it requires optimal and stable environmental conditions to maintain microbial purity. Unsuitable conditions can inhibit microbial growth and increase the risk

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of contamination. To minimize these risks, mixed cultures can be used [10].

The starter cultures commonly used in yogurt production are *L. bulgaricus* and *S. thermophilus*. During fermentation, these two bacteria play different roles. *L. bulgaricus* contributes to the aroma, while *S. thermophilus* influences the flavor and acidity level [11]. However, both bacteria grow best at an optimal temperature of 37°C with a pH of 5.5–6.8 and tend to be less resistant to the acidic conditions of the stomach, their role is limited to initial fermentation rather than acting as probiotics capable of surviving all the way through the digestive tract [12]. In contrast, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, and *Lactobacillus casei* are more tolerant to acidic conditions and bile salts, allowing them to survive in the gastrointestinal tract and function as probiotics [13]. These species also possess FOS-utilization mechanisms via specific transport systems and β-fructofuranosidase, enabling them to metabolize FOS as an energy source and to support bacterial growth [14][15][16].

Free radicals can come from cigarette smoke, pollution, industrial waste, and medications, posing health risks. When the body is continuously exposed to free radicals, it creates an imbalance between free radicals and antioxidants, leading to oxidative stress [17]. Ajwa dates contain high levels of carotenoids, including β-carotene and lutein, as well as polyphenols such as quercetin, isoquercetin, luteolin, apigenin, rutin, and anthocyanins, compared to other varieties [18]. Ajwa dates contain phenolics (19.37–23.9 g/100 g) and flavonoids (12.24–18.44 g/100 g), giving them potential as natural antioxidants [19]. However, most flavonoids in Ajwa dates occur as glycosides, thereby limiting their bioavailability and antioxidant activity [20]. Fermentation enhances antioxidant activity by converting phenolic glycosides into free aglycones via microbial β-glucosidase [21]. In addition, *Lactobacillus plantarum* exhibits antioxidant activity through its resistance to hydrogen peroxide and its ability to scavenge hydroxyl, superoxide, and DPPH free radicals.

The production of synbiotic yogurt using Ajwa dates as a prebiotic was undertaken to enhance its nutritional and functional value. As a food product, yogurt must comply with the Indonesian National Standard (SNI 2981:2009), which specifies the chemical, microbiological, and organoleptic quality requirements to ensure product safety and quality. Although previous studies have demonstrated the potential health benefits of dates, most have investigated fermented date-based beverages rather than yogurt. In addition, studies on date-enriched yogurt have generally used conventional yogurt starter cultures and have mainly evaluated physicochemical, microbiological, or sensory properties. Studies investigating synbiotic yogurt fermented with mixed probiotic cultures supplemented with different concentrations of Ajwa date juice and evaluating both product quality and antioxidant activity using IC_{50} values remain limited. Therefore, this study evaluated the effect of different concentrations of Ajwa date juice on the chemical quality (pH and total titratable acidity), microbiological quality (total lactic acid bacteria), organoleptic properties (color, aroma, taste, and texture), and antioxidant activity of synbiotic yogurt fermented with a mixed probiotic culture. The findings of this study are expected to provide scientific evidence to support the development of synbiotic yogurt as a functional food with improved quality and health-promoting properties.

Research Methods

Experimental Design

This study employed a Completely Randomized Design (CRD) with one factor: the concentration of Ajwa date juice. Four treatment groups were prepared, consisting of 0% (control), 10%, 15%, and 20% (v/v) Ajwa date juice. Each treatment was independently prepared in triplicate. Samples from each experimental unit were collected immediately after fermentation for chemical, microbiological, and antioxidant analyses. Freshly prepared yogurt samples from each treatment were used for organoleptic evaluation.

Tools and Material

The tools used in this study were analytical balance (Denver S1-234), a laminar air flow cabinet (Thermo Scientific 1300-series A2), an autoclave (Hirayama HVE-50), an incubator (Memmert), a shaker (D-lab SK 220 Pro), a water bath (Labtech LWB 122D), a UV-Vis spectrophotometer (Shimadzu UV-1700), spray bottles, watch glasses, glass funnels, measuring cups, test tube racks, Erlenmeyer flasks, test tubes, centrifuge tubes, measuring cups, pH meter, sterile glass jars, beakers, burettes, stands & clamps, volumetric flasks, juicer, stirring rods, dropper pipettes, petri dishes, volumetric pipettes, micropipettes.

The materials used in this study included Ajwa dates, liquid skim milk, sucrose, powdered yogurt starter culture, distilled water, 0.1 N NaOH, 1% PP indicator, oxalic acid, DPPH solids, analytical-grade methanol (p.a.), 70% alcohol, oxalic acid, $CaCO_3$, 0.85% NaCl, and de Man, Rogosa, and Sharpe agar (MRSA). A commercial powdered starter culture containing two yogurt starter bacteria (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) and three probiotic bacteria (*Lactobacillus acidophilus*, *Lactobacillus plantarum*, and *Lactobacillus casei*) was used. The manufacturer did not specify the proportion of each bacterial strain.

Research Procedure

Date Palm Juice

The dates are sorted, cleaned, and separated into flesh and pits. The date flesh is pureed with water in a 1:3 (w/v) ratio to produce date juice with pulp. Next, the mixture is filtered to separate the pulp from the date juice, yielding brown date juice.

Yoghurt Making

500 mL of liquid skim milk was pasteurised at 75°C for 15 minutes, then cooled to 40–45°C. The pasteurized milk was transferred into four separate containers, and date juice was added at varying concentrations of 0%, 10%, 15%, and 20% (v/v) to bring the total volume in each container to 100 mL. Each container was then supplemented with 7 grams of sucrose and 0.2 grams of commercial powdered

starter culture. The mixtures were then homogenized and labeled. They were incubated for 8 hours at 37°C. The date juice synbiotic yogurt was stored in a refrigerator at 4°C.

Adity (pH)

The pH value measurements were performed using a pH meter that had been previously calibrated with pH 4 and pH 7 buffer solutions and rinsed with distilled water. A 10 mL sample was placed in a beaker, and the pH meter electrode was immersed in it until a stable pH value was obtained.

Total Titratable Acidity (TTA)

A 10 mL aliquot of each sample was taken and diluted in a 100 mL volumetric flask with distilled water to the graduation mark. The mixture was homogenized, and the residue was separated from the filtrate. A 10 mL portion of the resulting filtrate was transferred to an Erlenmeyer flask, 2–3 drops of phenolphthalein indicator were added, and the mixture was titrated with 0.1 N NaOH solution until a soft pink color persisted for 20 seconds. The Total Titratable Acidity (TTA) value was calculated using the following formula:

$$\text{TTA} = \frac{\text{V x N x fp x BM}}{\text{W x 1000}} \text{ x } 100\%$$

Where:
V : volume of NaOH solution for titration (mL)
N : normality of NaOH
BM : molecular weight of lactic acid (90.08 g/mol)
Fp : dilution factor
W : sample weight

Antioxidant Activity Test

Preparation of DPPH solution 40 ppm

4 mg of DPPH crystals were weighed and placed into a 100 mL volumetric flask. The crystals were then dissolved in 100 mL of analytical-grade (p.a.) methanol and homogenized to obtain a 40 ppm DPPH solution.

Determination of DPPH maximum wavelength

1 mL of 40 ppm DPPH solution was added to a centrifuge tube containing 2 mL of analytical-grade (p.a.) methanol. The mixture was homogenized using a vortex mixer for 1 minute and incubated for 30 minutes in a dark room at room temperature. Subsequently, absorbance was measured using a UV-Vis spectrophotometer over the 400–700 nm wavelength range, yielding a maximum at 517 nm for the antioxidant assay.

Preparation of DPPH blank

1 mL of a 40 ppm DPPH solution was added to a centrifuge tube containing 2 mL of p.a. methanol solution. The mixture was then homogenized using a vortex mixer for 1 minute and incubated for 30 minutes in a dark room at room temperature. Absorbance measurements were taken in triplicate using a UV-Vis spectrophotometer at the maximum wavelength obtained.

Measurement of Antioxidant Potential of Sampel

10 mg of the sample and dissolve it in 10 mL of p.a. methanol to obtain a 1,000 ppm solution. Next, concentration variations of 50, 100, 150, 200, and 250 were prepared from the stock solution. For each concentration, 2 mL was dispensed with a micropipette into a centrifuge tube, and then 1 mL of a 40 ppm DPPH solution was added. Next, the solutions were vortexed for 1 minute to homogenize them and stored for 30 minutes in a dark room at room temperature until a color change due to DPPH activity occurred. Afterwards, absorbance measurements were taken in triplicate using a UV-Vis spectrophotometer at the maximum wavelength obtained.

Calculation

$$\% \text{ inhibition} = \frac{A_c - A}{A_c} \text{ x } 100\%$$

Description:
Ac: Absorbance value of blank
A: Absorbance value of sample

The IC_{50} value was determined from the linear regression equation between sample concentration (x) and percentage inhibition (y):

$$y = ax + b$$

Description:
y = Percent inhibition (%)
x = sample concentration (ppm)
a = slope of the curve
b = gradient

Total Lactic Acid Bacteria (LAB)

The Total Plate Count (TPC) method was used to determine the number of lactic acid bacteria (LAB) present in synbiotic yogurt fortified with date extract. For this test, the equipment was first sterilized in an autoclave for 15 minutes at 121°C. Ten test tubes were prepared and labeled 101 through 108. For dilution 10^1, a 1 mL sample was taken and placed into a test tube containing 9 mL of 0.85% NaCl solution. Next, for dilution 10^2, 1 mL of the sample from dilution 10^1 was added to a test tube already containing 9 mL of 0.85% NaCl solution; this process was repeated for dilutions 10^3 through 10^8. From dilutions 10^6 through 10^8, 1 mL was taken from each test tube and added to a Petri dish. Approximately 15 mL of the still-warm MRSA medium was poured into the petri dish and spread evenly to cover the bottom. Incubation was then continued for 48 hours at 37°C after the medium had solidified.

Organoleptic

Organoleptic testing was conducted using a hedonic questionnaire. The hedonic test was conducted to assess preferences for the colour, aroma, taste, and texture of synbiotic date juice yogurt at concentrations of 0%, 10%,

15%, and 20%. The testing was conducted with 20 untrained panellists.

Data Analysis Technique

The research data were analyzed using IBM SPSS Statistics 25 at a 95% confidence level ($\alpha = 0.05$). Data were tested for normality and homogeneity before being analyzed using one-way ANOVA. Significant differences ($p < 0.05$) were further analyzed using the Least Significant Difference (LSD) post hoc test. The IC_{50} value was determined by linear regression analysis of the relationship between sample concentration and DPPH radical-scavenging activity (% inhibition). Organoleptic data were analyzed using the Kruskal–Wallis test, followed by the Mann–Whitney U test when significant differences were found.

Results and Discussion

Effect of Date Palm Juice Concentration on Total LAB

The Total Lactic Acid Bacteria (LAB) test aims to determine the effect of varying concentrations of Ajwa date extract on the growth of LAB in date-based synbiotic yogurt using the Total Plate Count (TPC) method. The formation of a clear zone around colonies on Petri dishes can identify LAB growth. The clear zone that forms results from an interaction between the lactic acid produced by LAB and $CaCO_3$ during incubation, yielding calcium lactate. The addition of $CaCO_3$ to the medium serves as an indicator of the bacterial colonies' ability to produce [22].

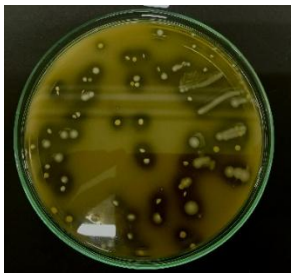


Figure 1. LAB colonies on Agar Media

Figure 1 shows that LAB colonies produce clear zones on agar medium. The medium consists of a mixture of de Man, Rogosa, and Sharpe (MRS) agar and $CaCO_3$, with 0.85% NaCl as the solvent.

Table 1. Effect of Date Palm Concentration on Total LAB of Date Palm Synbiotic Yoghurt

Date Palm Juice Concentration (% v/v)	Total LAB (CFU/mL)
0	$1.36 \times 10^8 \pm 0.040^a$
10	$2.15 \times 10^8 \pm 0.017^b$
15	$3.01 \times 10^8 \pm 0.018^c$
20	$3.61 \times 10^8 \pm 0.102^d$

Description: Values followed by the same letter indicate no significant difference ($\alpha > 0.05$).

The total LAB data were normally distributed and homogeneous ($p > 0.05$). One-way ANOVA showed that Ajwa date juice concentration had a significant effect on the total LAB count ($p < 0.05$). Furthermore, the LSD post hoc test indicated significant differences among treatments (Table 1).

The increase in LAB count with increasing Ajwa date juice concentration is mainly attributed to the higher availability of fermentable carbohydrates in the date juice. Ajwa dates contain glucose and fructose as the major reducing sugars (approximately 20–70% of dry weight), which are readily utilized by lactic acid bacteria as carbon and energy sources through glycolysis, thereby supporting bacterial growth and metabolic activity [23]. In addition, dates contain FOS and dietary fiber, which act as prebiotics that promote the growth of *Lactobacillus* and *Bifidobacterium* [24]. Several *Lactobacillus* species used in this study can utilize FOS via β-fructofuranosidase, which hydrolyses it into glucose and fructose for bacterial metabolism. Fermentation of FOS also produces short-chain fatty acids (SCFAs), mainly acetate, propionate, and butyrate, as metabolic end products [7]. LAB growth can also be influenced by the sucrose added to the yogurt. Sucrose serves as a source of carbon and energy for LAB during fermentation [24]. These findings are consistent with those of [6], who reported that increasing date juice concentration significantly increased LAB counts because dates provide fermentable sugars and prebiotic components that support bacterial proliferation during fermentation. Similarly, enhanced LAB growth was observed following date supplementation, confirming the role of date-derived carbohydrates in promoting LAB growth.

Effect of Date Palm Juice Concentration on pH Value and TTA

pH value and TTA tests were conducted to determine the effect of date concentration on the pH and TTA values of date synbiotic yogurt. The pH was measured with a pH meter, and TTA was determined by acid-base titration.

Table 2. Effect of Date Palm Concentration on the pH Value of Date Palm Synbiotic Yoghurt

Date Palm Juice Concentration (% v/v)	pH
0	4.26 ± 0.015^a
10	4.05 ± 0.026^b
15	3.93 ± 0.020^c
20	3.87 ± 0.015^d

Description: Values followed by the same letter indicate no significant difference ($\alpha > 0.05$).

Table 3. Effect of Date Palm Concentration on TTA of Date Palm Synbiotic Yoghurt

Date Palm Juice Concentration (% v/v)	TTA
0	0.5925 ± 0.011^a
10	0.7446 ± 0.016^b
15	0.8807 ± 0.038^c
20	0.9394 ± 0.023^d

Description: Values followed by the same letter indicate no significant difference ($\alpha > 0.05$).

pH value and TTA data were normally distributed and homogeneous ($p > 0.05$). One-way ANOVA showed that Ajwa date juice concentration significantly affected the pH

and TTA of synbiotic yogurt ($p < 0.05$), followed by the LSD post hoc test (Tables 2 and 3).

The addition of Ajwa date juice resulted in a gradual decrease in pH from 4.26 to 3.87, accompanied by an increase in TTA from 0.616% to 0.939%. This indicates that increasing the concentration of Ajwa date juice enhanced acid production during fermentation. During fermentation, LAB used glucose and fructose from Ajwa date juice, along with lactose from skim milk, as fermentable substrates to produce organic acids, primarily lactic acid. The accumulation of organic acids increased hydrogen ion (H^+) concentration, resulting in a decrease in pH and an increase in TTA. The increase in total LAB observed in this study was consistent with the changes in pH and TTA, indicating that higher bacterial growth contributed to greater organic acid production during fermentation. The availability of fermentable sugars and prebiotic components in Ajwa dates supported LAB metabolism, resulting in a proportional increase in acid production across treatments. These findings are consistent [6], who reported that date supplementation increased LAB growth, decreased pH, and increased TTA due to enhanced fermentation activity.

Effect of Date Palm Juice Concentration on Organoleptic

The organoleptic test aimed to determine the panelists preference for the date synbiotic yogurt product using human senses. The organoleptic test was conducted using a hedonic scale questionnaire distributed to 20 panelists. The organoleptic test was conducted to determine the panelists responses to the sensory characteristics of the date synbiotic yogurt, including color, aroma, taste, and texture. Rating scales ranged from 1 to 4, where a score of 1 indicated strongly dislike, 2 indicated dislike, 3 indicated like, and 4 indicated strongly like.

The Organoleptic Test of Color

Figure 2 shows the results of the organoleptic aroma evaluation of date synbiotic yogurt. Based on the *Kruskal–Wallis* test, the addition of Ajwa date juice did not significantly affect panelists preference for aroma ($p > 0.05$). The highest mean aroma preference score was observed in the 15% Ajwa date juice treatment (3.20), while the lowest was in the 20% treatment (3.00). These results indicate that panelists tended to prefer the aroma of yoghurt with a 15% addition of Ajwa date juice compared with other treatments, although the differences were not statistically significant. The addition of date juice may influence the aroma characteristics of yogurt due to the presence of volatile compounds in dates and fermentation metabolites produced by lactic acid bacteria, including organic acids and other aromatic compounds. However, the variation in date juice concentration was not sufficient to produce a significant difference in aroma acceptance among treatments.

The Organoleptic Test of Aroma

Figure 3 shows the results of the organoleptic aroma evaluation of date synbiotic yogurt. Based on the Kruskal–Wallis test, the addition of Ajwa date juice did not significantly affect panelists' preference for aroma ($p > 0.05$). The highest mean aroma preference score was observed for the 15% treatment (3.10), while the lowest was for the 0% treatment (2.80). The characteristic sour aroma of yogurt is produced by organic acids, primarily lactic acid, generated during fermentation by lactic acid bacteria. The addition of Ajwa date juice provided fermentable substrates for bacterial metabolism; however, the differences in date juice concentration were insufficient to produce significant differences in aroma acceptance among the panelists.

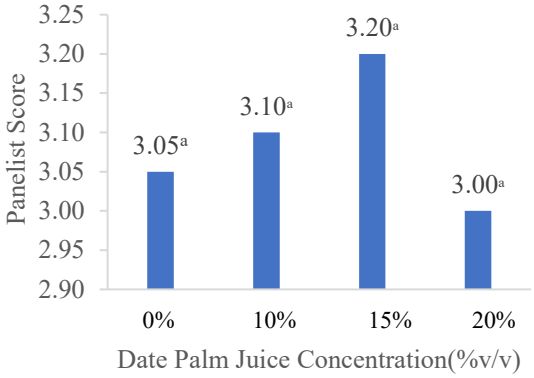


Figure 2. Organoleptic evaluation results of Color in Date Palm Synbiotic Yoghurt

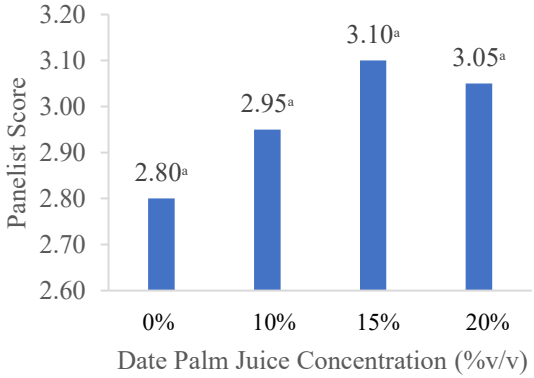


Figure 3. Organoleptic evaluation results of Aroma in Date Palm Synbiotic Yoghurt

The Organoleptic Test of Taste

Figure 4 shows the results of the organoleptic evaluation of taste in date synbiotic yogurt. Based on the Kruskal–Wallis test, Ajwa date juice concentration significantly affected panelists' taste preference ($p < 0.05$). Pairwise comparisons using the Mann–Whitney U test showed significant differences among treatments, particularly at the 15% and 20% Ajwa date juice concentrations. The highest mean taste preference score was obtained for the 20% treatment (3.25), whereas the lowest score was observed in the control treatment (0% Ajwa date juice) (2.10). The lower preference score of the control yogurt was associated with its more pronounced sour taste resulting from lactic acid production during milk fermentation. The addition of Ajwa date juice provided glucose and fructose, which not only supported lactic acid bacterial metabolism but also contributed natural sweetness, balancing the yogurt's acidic taste. Consequently, increasing the concentration of Ajwa date juice improved taste acceptance despite the increase in acidity. This finding indicates that the natural sugars present in Ajwa dates

enhanced consumer acceptance while maintaining the fermentation process.

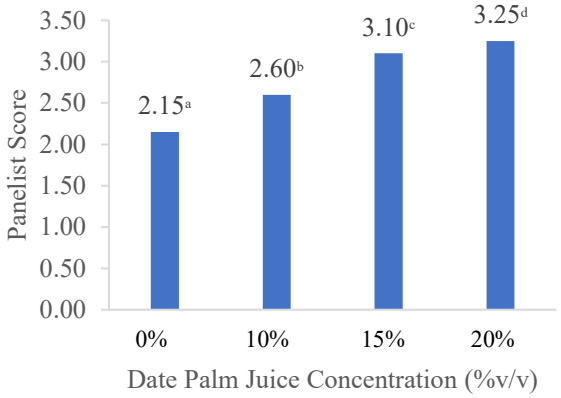


Figure 4. Organoleptic evaluation results of Taste Date Palm Synbiotic Yoghurt

The Organoleptic Test of the Texture

Figure 5 shows the results of the organoleptic evaluation of texture in date synbiotic yogurt. Based on the Kruskal–Wallis test, the addition of Ajwa date juice did not significantly affect panelists' preference for texture ($p > 0.05$). The highest mean texture preference score was observed for the 15% treatment (3.40), while the lowest was observed for the 10% treatment (3.20). Although the 15% treatment received the highest preference score, the differences among treatments were not statistically significant. The relatively high texture acceptance in all treatments indicates that the addition of Ajwa date juice did not adversely affect the typical semisolid consistency of yogurt. During fermentation, lactic acid bacteria produce organic acids that lower the pH and promote casein coagulation, resulting in the formation of a stable gel structure responsible for the characteristic thick and homogeneous texture of yogurt. In addition, the soluble solids and dietary fiber present in Ajwa date juice may contribute to improving water retention and gel stability, thereby helping to maintain yogurt consistency.

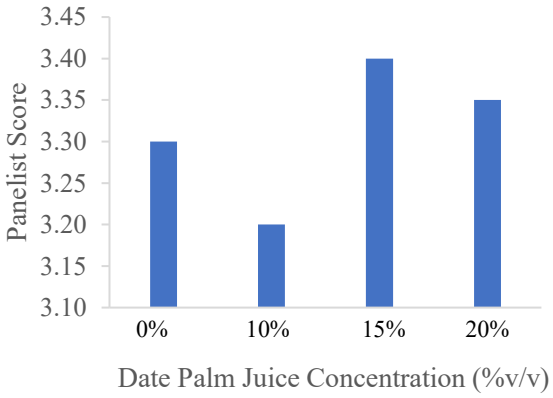


Figure 5. Organoleptic evaluation results of Texture Date Palm Synbiotic Yoghurt

Table 4. Comparison of Quality Test Results of Date Palm Synbiotic Yoghurt with Yoghurt Quality Requirements Based on SNI 2981:2009

Test Criteria	Unit	SNI 2981: 2009	Result
Display	-	Thick-Solid Liquid	Thick-Solid Liquid
Aroma	-	Normal /typical	Normal
Taste	-	Sour/typical	Sour
TTA	%	0,5-2,0	0,5923-0,9394
pH	-	3,80-4,50	3,87-4,26
Bacteria	CFU/mL	Min 10^7	10^8

Based on Table 4, the test results for total LAB, pH, and TTA at date juice concentrations ranging from 0% to 20% met the requirements of SNI 2981:2009.

Effect of Date Palm Juice Concentration on IC_{50} Value

Antioxidant activity was tested to determine the effect of date extract concentration on the antioxidant activity of date synbiotic yogurt. Antioxidant activity can be determined by the ability of the antioxidant compounds in the product to scavenge DPPH (2,2-diphenyl-1-picrylhydrazyl) free radicals, as measured using a UV-Vis spectrophotometer. The level of antioxidant activity can be expressed as the IC_{50} value, which is the concentration of the antioxidant compound required to inhibit 50% of free radicals, determined by linear regression of percentage inhibition versus concentration.

Table 5. Effect of Date Palm Juice Concentration on IC_{50} of Date Palm Synbiotic Yoghurt

Date Palm Juice Concentration (% v/v)	IC_{50} Value (ppm)
0	104.978 ± 0.625^a
10	86.522 ± 1.369^b
15	72.310 ± 1.230^c
20	60.785 ± 1.837^d

Description: Values followed by the same letter indicate no significant difference ($\alpha > 0.05$).

Antioxidant activity data were statistically analyzed; the results showed a normal and homogeneous distribution with a significance level of $p > 0.05$. Next, a one-way ANOVA revealed that the concentration of date extract significantly affected the antioxidant activity of date-synbiotic yogurt ($p < 0.05$). Subsequently, a post-hoc LSD test showed significant differences among all treatments (Table 5). The addition of Ajwa date juice decreased the IC_{50} value from 104.978 ppm to 60.785 ppm, indicating a significant increase in antioxidant activity.

The addition of date extract also influenced the decrease in the IC_{50} value of date-synbiotic yogurt. The higher the concentration of date extract, the lower the resulting IC_{50} value. Yogurt without Ajwa date juice (0%) showed an IC_{50} value of 104.978 ppm, which is classified as moderate antioxidant activity, whereas yogurt supplemented with 10–20% Ajwa date juice exhibited strong antioxidant activity, with IC_{50} values ranging from 86.522 to 60.785 ppm.

The increase in antioxidant activity is mainly attributed to the higher content of bioactive compounds in Ajwa dates, particularly phenolic compounds, flavonoids, and carotenoids such as β-carotene and lutein. Phenolic compounds and flavonoids act as antioxidants by donating hydrogen atoms or electrons to neutralize DPPH free radicals, whereas carotenoids stabilize free radicals through their conjugated double-bond system [25]. During fermentation, β-glucosidase produced by lactic acid bacteria hydrolyzes flavonoid glycosides into free aglycones, which possess higher antioxidant activity than their glycoside forms [26]. In addition, pectinolytic activity during fermentation facilitates the degradation of pectin in date tissues, promoting the release of bound phenolic compounds, while the acidic conditions generated by lactic acid bacteria further enhance phenolic liberation [27]. Consequently, the combined effects of bioactive compounds in Ajwa dates and microbial biotransformation during fermentation increase the availability of antioxidants that can scavenge DPPH free radicals. These findings are consistent with previous studies reporting that increasing the concentration of date extract enhances the antioxidant activity of fermented products [27].

Conclusion

Based on the results of this study, it can be concluded that increasing the concentration of Ajwa date juice affected the chemical, microbiological, organoleptic, and antioxidant properties of synbiotic yogurt. Higher concentrations of date juice decreased the pH from 4.26 to 3.87, while total titratable acidity (TTA) increased from 0.5925% to 0.9394%. Total lactic acid bacteria (LAB) also increased from 1.36×10^8 CFU/mL to 3.61×10^8 CFU/mL, and all treatments met the requirements of SNI 2981:2009. In the organoleptic evaluation, the 15% date juice treatment was the most preferred in terms of color, aroma, and texture, whereas the 20% treatment received the highest preference score for taste. Statistical analysis showed that date juice concentration significantly affected taste, whereas no significant differences were observed for color, aroma, and texture. Furthermore, the IC_{50} value decreased from 104.978 ppm to 60.785 ppm, indicating enhanced antioxidant activity; the 20% date juice treatment exhibited the highest activity among all treatments. These findings demonstrate the potential of Ajwa date juice as a natural prebiotic ingredient for the development of functional synbiotic yogurt. Therefore, a 20% Ajwa date juice concentration is recommended when enhanced functional properties are the primary objective, whereas a 15% concentration is more suitable when consumer acceptance is prioritized.

Author's Contribution

Nita Abelia: The author conducted the experiments, performed data analysis, interpreted the results, and prepared and revised the manuscript. The author was also responsible for sample preparation, laboratory testing, and data collection. Rudiana Agustini: contributed to the discussion and finalization of the manuscript.

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