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Water Kefir Based on Manalagi Apple and Sukari Date as a Novel Source of Bioactive Compounds with Antioxidant and Antidiabetic Activities

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ABSTRACT

Apple water kefir combined with Sukari dates and encapsulated probiotic bacteria is a new potential functional food that can provide antioxidant and antidiabetic effects. The research aims to develop a formula and characterise water kefir from a combination of manalagi apples, sukari dates, and nanoemulsion-encapsulated probiotic *Lactobacillus plantarum* as an alternative antioxidant and antidiabetic therapy. The water kefir formula in this study was developed using the response surface method. The best Response Surface Methodology formula was tested for characteristics (total acidity, pH, viscosity, vitamin C content, proximate composition, alcohol content, and phytochemical composition), antioxidant activity (DPPH, ABTS, FRAP), and antidiabetic activity (α -glucosidase and α -amylase inhibition). Metabolite compounds in the product were identified using UHPLC-MS. The results of antioxidant and antidiabetic activity testing showed that formula 1 (20% manalagi apple) had the highest antioxidant and antidiabetic activity. The antioxidant activity of formula 1 had IC₅₀ values of 0.0649 g/mL (DPPH method), 1.2648 g/mL (ABTS method), and 4.0373 g/mL (FRAP method). *In vitro* antidiabetic activity testing of formula 1 for inhibition of α -glucosidase and α -amylase enzyme activity showed IC₅₀ values of 38.46% and 5.73%, respectively. The general characteristics of water kefir from F1 had the highest values for total acidity, pH, viscosity, vitamin C content, proximate composition, phytochemical analysis, and alcohol content. Metabolite identification by UHPLC-MS revealed that 10 compounds matched those in the database. The research findings indicate that F1 exhibits the highest antioxidant and antidiabetic activity.

Keywords: Antidiabetic, Antioxidant, Water Kefir, Manalagi Apples, Sukari Dates.

Introduction

Diabetes mellitus (DM) is a metabolic disease characterised by chronic hyperglycemia due to impaired insulin secretion, insulin resistance, or both¹. One factor that worsens DM is increased oxidative stress, an imbalance between the production of free radicals and the body's antioxidant defence system. The accumulation of free radicals accelerates cell and tissue damage, thus worsening DM complications². Therefore, the development of natural materials with both antioxidant and antidiabetic potential has become a focus of research in functional foods and pharmaceuticals. Fermented products, such as water kefir, are recognised as a source of bioactive compounds that have the potential to exhibit antidiabetic and antioxidant properties^{3,4}. By fermenting a sugar solution with a consortium of lactic acid bacteria (LAB) and yeasts, water kefir is produced, which produces compounds such as organic acids, polysaccharides, bioactive peptides, and phenolic metabolites that have biological benefits⁵. Recent research has demonstrated that consuming water kefir can mitigate the risk of postprandial hyperglycemia by increasing antioxidant capacity and inhibiting the α -glucosidase enzyme⁶.

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Fruit substrates have the potential to enhance the bioactive content of kefir when utilised as a fermentation base. Manalagi apples are recognised for their antioxidant properties and their ability to inhibit the α -glucosidase enzyme, as they are abundant in phenolic compounds, flavonoids, and chlorogenic acid^{7,8}. The antioxidant activity of apples ranged from 24.8 μ mol Trolox equivalent (TE)/g to 41.6 μ mol TE/g when assessed by FRAP and from 29.2 μ mol TE/g to 71.7 μ mol TE/g when examined by the ABTS assay⁹. However, Sukari dates (*Phoenix dactylifera* L.) are a variety that contains ascorbic acid, flavonoids, and polyphenols, which have hypoglycemic effects and antioxidant activity¹⁰. In *in vitro* tests, date seed extract at 5 mg/mL had a moderate to good inhibitory impact on the α -glucosidase enzyme (range 5.91-51.71%). Meanwhile, date water extract exhibited the best antidiabetic efficacy by inhibiting α -glucosidase activity¹¹. It is anticipated that the water kefir fermentation system will generate bioactive synergy from the combination of Manalagi apples and Sukari dates. To improve the efficacy of probiotics in fermentation systems, bacterial encapsulation technology utilising nanoemulsion systems has been developed. Nanoemulsions serve as carriers that enhance the stability and viability of probiotics while protecting bacteria from environmental stressors during fermentation and digestion. Bioactive metabolite production during fermentation is significantly augmented by encapsulating bacteria in nanoemulsions, which enhances resilience to acidic and bile environments^{12,13}. One of the probiotic bacteria from the *Lactobacillus* genus commonly used in the food sector is *Lactobacillus plantarum*. *L. plantarum* is a probiotic bacterium that creates numerous metabolic compounds, such as organic acids, hydrogen peroxide, and bacteriocins, which can limit the formation of harmful bacteria. *L. plantarum* exhibited a good adaptation and adhesion ability in the gastrointestinal tract and the potential to alter host health through numerous positive activities, e.g., antibacterial, antioxidative, antigenotoxic, anti-inflammatory, and immunomodulatory, in several *in vitro* and *in vivo*

studies¹⁴. Encapsulation typically doesn't significantly alter the major metabolites produced by *L. plantarum*, but it can alter the rate, concentration, and stability of bacterial production by protecting it from harsh environments¹⁵. Encapsulation of *L. plantarum* can improve viability, thereby increasing the bacteria's bioactivity as an antidiabetic and antioxidant¹⁶.

Nevertheless, no research has specifically assessed the potential of a combination of water kefir made from Manalagi apples and Sukari dates, with the addition of nanoemulsion-encapsulated *L. plantarum* bacteria, for α -glucosidase enzyme inhibition and antioxidant activity. However, some studies have examined the effects of water kefir on antidiabetic and antioxidant activity. It is anticipated that this research will fill a gap in the development of fermented functional foods derived from local materials and formulated using a nanoencapsulation technology approach. The investigation aims to assess the antidiabetic and antioxidant activity of water kefir obtained by combining Manalagi apples (*Malus sylvestris*) and dates (*Phoenix dactylifera* L.) with nanoemulsion-encapsulated probiotic microbes. This study also evaluated the physicochemical characteristics and metabolite profile of water kefir using UHPLCMS.

Materials and Methods

This study utilises *Malus sylvestris* Mill. (Manalagi apple) sourced from Batu City, East Java, Indonesia; *Phoenix dactylifera* L. (Sukari dates) acquired from PT Timur Tengah Indonesia; and kefir grain starter from Aracaki, Indonesia. The kefir grains are composed of *Lactobacillus paracasei*, *Lactobacillus harbinensis*, *Lactobacillus hilgardii*, *Bifidobacterium psychraerophilum*, *Saccharomyces cerevisiae*, *Dekkera bruxellensis*, *Lactobacillus casei*, and *Bifidobacterium* spp. Manalagi apples and sukari dates were pre-identified at the Plant Determination Institute of PT. Palapa Muda Perkasa, bearing consecutive determination certificate numbers 056/IPH.1.10/If.7/V/2025 and 057/IPH.1.11/If.7/V/2025. The chemical substances employed were of analytical grade from Merck.

Sample Preparation

Manalagi apples and Sukari dates were prepared at a concentration of 10% (w/v) by weighing 100 g of manalagi apples and 100 g of sukari dates each. Each sample was mixed with 1000 mL of boiling water and blended. Apple juice and date juice were produced independently, each undergoing its own manufacturing, filtration, and pasteurisation processes. Combining apple juice and date juice did not necessitate a designated blending period; it was merely stirred manually until a uniform consistency was achieved. The resulting mixture was filtered, and the filtrate was pasteurised at 75°C for 15 seconds. The pasteurised Manalagi apple juice and Sukari date juice at 10% (w/v) concentration were cooled to 28°C before the addition of 3% (w/v) kefir grain starter. Fermentation was carried out at room temperature for 24 hours. The fermentation product was supplemented with a 1% nanoemulsion encapsulating *L. plantarum* bacteria. The sample formula used in this study is the result of screening for the best antioxidant activity using the Response Surface Methodology (RSM) method¹⁷.

Phytochemical Analysis

Phytochemical screening was performed qualitatively to test for the presence of alkaloids, flavonoids, saponins, tannins, triterpenoids/steroids, and phenols in test sample¹⁸. Quantitative analysis of flavonoids, phenols, and tannins was carried out according to previously reported procedures¹⁹⁻²¹.

Determination of Kefir Characteristics

The characteristics of the water kefir samples were analysed based on previous methods²². The characteristics studied were pH, viscosity, total lactic acid content, proximate composition, alcohol content, and vitamin C content.

Determination of Antioxidant Activity

Antioxidant activity was evaluated based on DPPH free radical scavenging capacity, ABTS free radical scavenging capacity, and ferric

reducing antioxidant power (FRAP). Antioxidant activity was determined using the method from a previous study²³.

Ascorbic acid standards at varying concentrations and water kefir sample solutions (80 μ L each) were pipetted into a 96-well plate. A multichannel pipette was used to dispense 80 μ L of 0.1 mM DPPH solution into the well plate that contained the standards and samples. The microplate was incubated at 25°C for 30 minutes, then covered with aluminium foil. A microplate reader was used to measure the solutions at 492 nm for 30 seconds, with medium vibration shaking, after 30 minutes²⁴.

A total of 10 μ L of the water kefir sample solution and gallic acid standards with varying concentrations were pipetted into a 96-well plate. ABTS solution (290 μ L) was pipetted into the well plate containing the standards and samples. The well plate was covered with aluminium foil and incubated for 6 minutes at room temperature (in the dark). After 6 minutes, the mixture was read on a microplate reader at 630 nm for 30 seconds with medium vibration shaking.

A 20 μ L sample solution of water kefir and gallic acid standard was pipetted into a 96-well plate. 280 μ L of FRAP solution was added to the well plate containing the standard and samples. The well plate was covered with aluminium foil and incubated for 10 minutes at room temperature (in the dark). After 10 minutes, the solution mixture was read on a microplate reader at 593 nm for 30 seconds with medium vibration shaking.

Determination of Antidiabetic Activity

The antidiabetic activity of water kefir was tested using alpha-glucosidase and amylase enzymes. The determination of antidiabetic activity was based on the method used in previous studies^{25,26}.

A total of 50 μ L of 0.1 M phosphate buffer saline (pH 6.9) was added to the plate, followed by 10 μ L of 1 ppm α -glucosidase enzyme and 20 μ L of water kefir sample or acarbose standard. The well plate was covered with aluminium foil and incubated for 15 minutes at 37°C. After 15 minutes of incubation, 20 μ L of 4-NPP was added to the well plate. The mixture was incubated for 20 minutes at 37°C, and 50 μ L of 0.1 M Na₂CO₃ was added. The absorbance of the solution was measured at 405 nm. A total of 500 μ L of sample and acarbose standard at varying concentrations were added to 500 μ L of α -amylase enzyme, and the mixture was incubated for 10 minutes at 37°C. Subsequently, 500 μ L of 0.5% starch was added to the samples, which were incubated for an additional 15 minutes at 37°C. After the incubation period, 10 μ L of 0.5% iodine was added. The absorbance was measured at 601 nm.

Metabolite Profile Identification

Identification of water kefir metabolite content was performed using UHPLC-MS Orbitrap (Thermo Scientific™ Q Exactive™ Hybrid Quadrupole). The eluent used is a mixture of water-0.1% formic acid and methanol-0.1% formic acid. The elution system used in this study is gradient elution. The sample solution was vortexed for 30 seconds, sonicated at 40 kHz for 30 minutes, and centrifuged at 13,000 \times g for 10 minutes at 4 °C. The sample supernatant was transferred to a sample container for UHPLC-MS analysis. A 3 μ L sample was injected into a ThermoScientific™ Accucore™ Phenyl-Hexyl 100 mm \times 2.1 mm ID \times 2.6 μ m column at a flow rate of 0.3 mL/min. The characteristics of the Mass Spectrometer used in this study are Electrospray Ionisation (ESI), mode: positive/negative, sheath gas: N₂, 32 AU, auxiliary gas: N₂, 8 AU, sweep gas: N₂, 4 AU, spray voltage: 3.30 kV_m, capillary temperature: 320 °C, and auxiliary gas heater temperature: 30 °C²⁷.

Results and Discussion

The formulation was created using response surface methodology (RSM). This study was designed using a split-plot mixture to optimise the formula for the best antioxidant activity. There is a proportional relationship between apples and dates, combined into a single factor, and another factor, the concentration of kefir grains and bacterial encapsulation. The ratio of apples to dates serves as the whole-plot factor, while the subplot factors are kefir grain concentration and bacterial encapsulation. Based on the split-plot mixture, 45 formula combinations were obtained. The RSM parameters are the kefir-to-apple ratio (0, 0.25, 0.5, 0.75, and 1), the concentration of kefir grains

(1%, 2%, 3%), and the concentration of bacterial encapsulation (1%, 2%, 3%). The selection of the best formula was based on antioxidant activity, specifically DPPH inhibition. The ANOVA test results for the RSM model showed significant effects for the linear, quadratic, and two-variable interaction models ($p < 0.05$). The linear models that influenced the extent of inhibition were the apple-to-date ratio ($p < 0.05$), kefir grains ($p < 0.05$), and bacterial encapsulation ($p < 0.05$). The quadratic models that influenced the extent of inhibition were the apple-to-date ratio ($p < 0.05$) and bacterial encapsulation ($p < 0.05$). Meanwhile, the two-variable interaction model that influenced the extent of inhibition was the interaction between the apple-to-date ratio and bacterial encapsulation. Thus, changes in inhibition are significantly influenced by changes in the ratio of apple to date administration and by the administration of bacterial encapsulation. The optimisation results for inhibition from the factors of the apple-to-date ratio, kefir grains, and bacterial encapsulation showed that to achieve maximum inhibition, the apple-to-date ratio was 0.4327 (apple: date = 1:2.31), kefir grains were 3%, and bacterial encapsulation was 1%. The estimated maximum inhibition from the RSM model, based on the optimal values of these variables, was 87.382%, with a composite desirability of 0.9108. This result indicates that the optimal solution generated is already optimal, achieving 91.08% of the ideal condition across all factors influencing inhibition. A total of 5 formulas were obtained based on the RSM method approach, which can be used in further research based on the comparison of apple and date ratios, namely F1: (2:8), F2: (4:6), F3: (6:4), F4: (8:2), F5: (10:0). The five formulas used kefir grain concentrations and bacterial encapsulation at 3% and 1%, respectively. The concentrations of manalagi apple juice and sukari dates were made at 10%. Phytochemical screening analysis showed that the apple extract tested positive for terpenoids, while the date extract contained compounds from the alkaloid, saponin, and terpenoid groups. Water kefir from all five formulas was also subjected to phytochemical screening, with the results showing that F1 tested positive for alkaloids, phenols, tannins, and saponins. Formulas 2, 3, and 5 tested positive for alkaloids, phenols, flavonoids, and tannins. Formula 4 tested positive for alkaloids, phenols, flavonoids, and saponins. The results of the quantitative phytochemical tests for all five formulas are presented in Figure 1. In the quantitative phytochemical content analysis, F1 had the highest total phenolic value (9.5 mg GAE/g), followed by F2 (7.32 mg GAE/g), while F5 had the lowest content (3.41 mg GAE/g). Conversely, the highest flavonoid content was found in F4 (0.99 mg QE/g) and F5 (0.94 mg QE/g), while F1 did not contain flavonoids. For tannin content, F1 showed the highest value (10.19 mg TAE/g), significantly greater than the other formulas, while F4 was not detected.

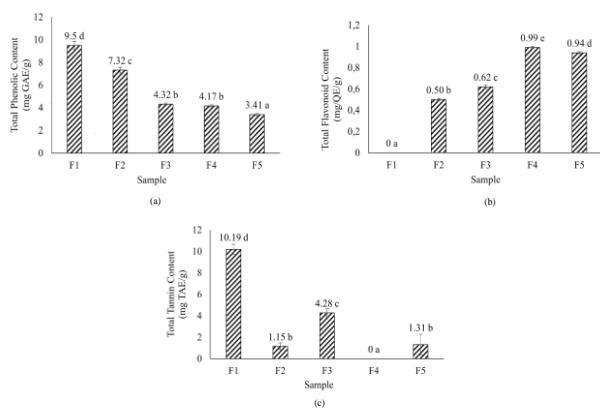


Figure 1: Total phenol (a), flavonoid (b), and tannin (c) values in apple and sukari date water kefir ($p < 0.05$)

The analysis results show that the kefir characteristics of the five formulas vary significantly across several parameters (Table 1). The highest total acidity was obtained in formula F1 (0.270%), while the lowest was in F5 (0.131%). The pH values ranged from 3.60 to 3.80, with the lowest pH in F1 (3.60) and the highest in F5 (3.80), indicating an inverse relationship with acidity. The highest vitamin C content was

found in F1 (7.099 mg/100 mL), while the lowest was in F4 (4.283 mg/100 mL). Meanwhile, the viscosity of the kefir was relatively uniform across all formulas, ranging from 0.941 to 0.964 cP. Proximate analysis showed that moisture content was relatively high across all formulas, with the lowest in F1 (98.2%) and the highest in F5 (99.8%). The highest ash content was found in F2 (0.18%), while the lowest was in F3 (0.07%). Protein content is relatively low, ranging from 0 to 0.02%, as is lipid content, which is present only in small amounts: 0.06% in F4 and 0.10% in F5. Carbohydrate content varies, with F1 having the highest level (1.65%) and decreasing to undetectable levels in F5. In the alcohol analysis, the highest ethanol content was found in F1 (2.32%), while the lowest was in F5 (0.48%). Meanwhile, methanol was not detected in any of the formulas (<0.05 %). The results of antioxidant activity testing using the DPPH, ABTS, and FRAP methods showed significant variations among the kefir formulas (Figure 2).

Table 1: Characteristics of apple and sukari date water kefir ($p < 0.05$).

Parameter	Formula				
	F1	F2	F3	F4	F5
Total acid (%)	0.270 e	0.250 d	0.233 c	0.205 b	0.131 a
pH	3.60 a	3.61 a	3.63 b	3.73 c	3.80 d
Vitamin C Content (%)	7.099 c	4.693 a	6.688 c	4.283 a	5.339 b
Viscosity (cP)	0.962 a	0.963 a	0.964 a	0.941 a	0.961 a
Proximate Analysis					
Water content (%)	98.2 a	99.1 b	99.4 bc	99.5 bc	99.8 c
Ash content (%)	0.15 c	0.18 c	0.07 a	0.11 b	0.08 ab
Protein (%)	0 a	0 a	0 a	0 a	0.02 b
Lipid (%)	0 a	0 a	0 a	0.06 b	0.10 c
Carbohydrates (%)	1.65 e	0.72 d	0.53 c	0.33 b	0 a
Alcohol					
Ethanol (%)	2.32 d	1.60 c	1.07 b	1.48 c	0.48 a
Methanol (%)	<0.05 a				

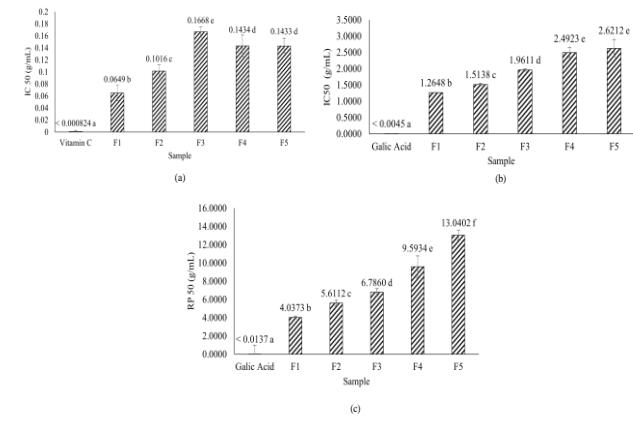


Figure 2: Antioxidant activity values of apple and sukari date water kefir ($p < 0.05$) using the DPPH (a), ABTS (b), and FRAP (c) methods

In the DPPH test (IC_{50}), formula F1 had the lowest value of 0.0649 g/mL, indicating the best antioxidant activity compared to the other formulas. Activity then decreased in F2 (0.1016 g/mL), F3 (0.1668 g/mL), F4 (0.1434 g/mL), and F5 (0.1433 g/mL). In the ABTS test (IC_{50}), the best activity was shown by F1 with a value of 1.2648 g/mL, followed by F2 (1.5138 g/mL), F3 (1.9611 g/mL), F4 (2.4923 g/mL), and F5 (2.6212 g/mL), so the higher the value, the lower the antioxidant activity. Meanwhile, in the FRAP test (% RRP), which measures reducing power, formula F1 also showed the lowest value of 4.0373 g/mL, followed by F2 (5.6112 g/mL) and F3 (6.7860 g/mL), while the highest value was found in F5 (13.0402 g/mL). Overall, the F1 formula consistently showed the lowest values across all three test methods, indicating the best antioxidant activity among the formulas. Conversely, the F5 formula had the highest values for ABTS and FRAP, indicating the lowest activity. The results of testing the antidiabetic activity of kefir, as measured by inhibition of α -glucosidase and α -amylase enzymes, showed significant differences between the formulas. In the α -glucosidase test (Figure 3a), the best activity was demonstrated by F1 with an IC_{50} value of 38.46%, followed by F2 (40.48%) and F5 (74.06%). Lower activity was observed in F3 (120.11%) while F4 had the highest value (253.21%), making it the formula with the weakest activity. Meanwhile, in the α -amylase assay (Figure 3b), F1 showed the highest activity, with an IC_{50} of 5.73%, followed by F5 (6.12%) and F4 (6.16%). Formula F2 showed a higher IC_{50} value of 18.9%, while F3 showed the lowest activity with an IC_{50} of 48.56%. Overall, kefir formula F1 consistently exhibited the best antidiabetic activity against both enzymes tested, α -glucosidase and α -amylase, compared to the other formulas. Conversely, F3 and F4 showed the lowest activity, with the highest IC_{50} values in each test. The identified metabolite separation results yielded 189 compounds. Screening based on similarity to the database library identified 10 compounds with 95% similarity: tryptophol, diethylhexyl phthalate, dimepranol, butanedione, kynurenic acid, dimethyladenine, dibutyl phthalate, tributyl phosphate, and tributylamine. There were 4 compounds identified in the natural material: tryptophol, butanedione, kynurenic acid, and dibutyl phthalate. The chromatogram of the best kefir formula separation is shown in Figure 4. This work uses response surface methodology (RSM), which integrates experimental design, statistical analysis, empirical modelling, and mathematical optimisation techniques. The quantity of experiments to be performed is determined logically, hence preventing information redundancy. Moreover, the implementation of optimisation techniques facilitates the examination of relationships among variables. A factor that appears not to affect the phenomenon under investigation may, in reality, exert an indirect influence through interaction¹⁷.

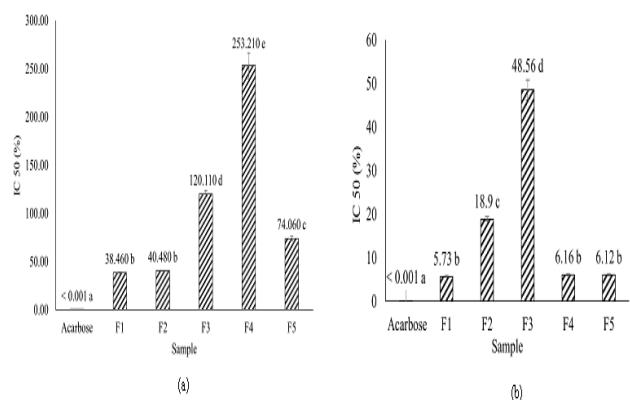


Figure 3: The antidiabetic activity values of Malang apple juice kefir and Sukari dates ($p < 0.05$) against α -glucosidase (a) and α -amylase (b) enzymes

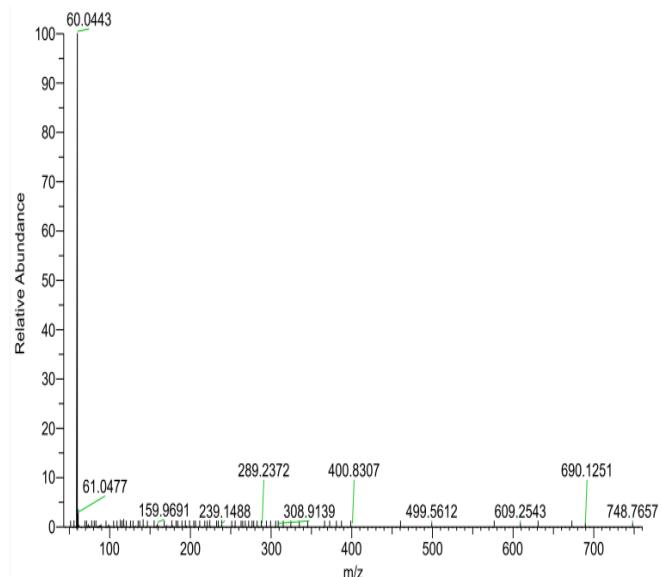


Figure 4: Results of UHPLC-MS separation of water kefir metabolites from Manalagi apple and Sukari dates

L. plantarum bacteria were encapsulated using the emulsion method. This study examines the emulsifiers gum arabic and Tween 80. Gum Arabic is an arabinogalactan polysaccharide that serves as a thickening and emulsifying agent in food products. Gum arabic consists of amino acids, polysaccharides, and polyglucuronic acid^{28,29}. Tween 80 is a non-ionic, hydrophilic surfactant that serves as an emulsifier. Gum arabic with Tween 80 can produce emulsions that are resilient to environmental stressors³⁰. This study examines the surface adsorption of *L. plantarum* in an emulsion formulation containing the coating agent soy protein isolate (SPI). SPI possesses foaming, water-binding, emulsification, and film-forming capabilities³¹. Pasteurisation at 75°C for 15 seconds was selected as it aligns with the parameters of HTST (High Temperature Short Time) techniques. This process effectively reduces microbial levels while preserving the integrity of heat-sensitive vitamin C, polyphenols, and bioactive compounds in the fruit. Therefore, this parameter is employed to ensure microbiological safety while preserving the functional constituents of the liquid³². A 3% concentration of kefir grains in this investigation is identified as the optimal concentration for producing water kefir products. Fermentation is conducted utilising glass canisters as small-scale fermentation vessels. Because *L. plantarum* is a facultative anaerobe capable of thriving in both the presence and absence of oxygen, fermentation is conducted using a cotton insert to facilitate air exchange while preventing the environment from becoming overly aerobic³³. Using a cotton plug also helps prevent excessive pressure from gas accumulation during fermentation. The pH of the fruit fluid before fermentation ranged from 5.91 to 4.25 (F1-F5), whereas after 24 hours of fermentation, it decreased to between 3.60 and 3.80, demonstrating typical lactic acid fermentation activity in water kefir. Nanoencapsulated probiotics were incorporated before fermentation, concomitant with the kefir grains. This aims to guarantee that the encapsulated *L. plantarum* can contribute during the fermentation process¹³. Measurements of total phenols, flavonoids, and tannins in water kefir exhibited differences across formulations (F1-F5). This change is determined by the ratio of input materials and the fermentation activity. Formulations with a greater amount of dates (F1 and F2) exhibit the highest quantity of phenols. This may be attributed to the elevated levels of natural polyphenols in dates, including gallic acid, catechins, and other phenolic derivatives. Furthermore, apples have lower phenol levels than dates. During fermentation, yeast and the enzymatic actions of lactic acid bacteria decompose complex substances into simpler phenolic compounds³⁴. The flavonoid concentration seems to differ among formulations. Formulas F4 and F5 exhibited the highest flavonoid concentrations among the formulations. The increased apple content led to elevated flavonoid levels, including

quercetin, a flavonoid prevalent in apples. Dates also contain flavonoids such as luteolin, apigenin, and other flavonoid derivatives. The largest concentration of tannins is present in F1. Apples possess a significant concentration of condensed tannins, while dates enhance the diversity of polyphenols. During fermentation, tannins may undergo partial breakdown by microbial enzymes, resulting in their quantity not being directly proportional to the amount of input materials³⁵. The total acid measurements of the five formulations yielded varying findings³⁶. The overall acid value may vary depending on the concentrations of the apple and date raw ingredients incorporated into the formula. The total acid results indicate that an increased proportion of dates (F1 and F2) correlates with higher total acid levels. This is due to dates containing moderate levels of sugars, such as glucose and fructose, which are higher than those found in manalagi apples. Sugar serves as a fermentation substrate for lactic acid bacteria (LAB) and yeast. The fermentation byproducts generated by these bacteria and yeast are organic acids, chiefly lactic acid³⁷. The pH test results differ from those of total acidity. A negative association exists between total acidity and pH. An increase in overall acidity corresponds to a decrease in the pH value of the kefir. Organic acid fermentation products can elevate the quantity of free H⁺ ions, thereby reducing the pH of kefir products³⁸. The resultant pH value aligns with prior research findings. The fermentation of kefir using fruit juice leads to a notable increase in total acidity, resulting in a reduction in pH to 3.5-4.2, thereby influencing the product's flavour and functional characteristics³⁹. The composition of raw materials influences the variation in vitamin C content in this investigation. The more dominant Sukari F1 dates offer a superior substrate for microbial activity during fermentation, potentially enhancing the enzymatic processes involved in vitamin C production or stabilisation. The polyphenols and bioactive compounds in dates provide natural protection against vitamin C breakdown. The comparatively lower sugar content of apples relative to dates leads to a slower fermentation process, resulting in a predominance of vitamin C breakdown over synthesis or stabilisation. Incorporating biopolymers such as SPI into the encapsulation system can enhance the solution's viscosity by creating a more compact molecular network. This aligns with the findings of F1-F5, which demonstrate increased viscosity relative to the blank. The elevated viscosity enhances emulsion stability by diminishing droplet mobility, hence mitigating the likelihood of coalescence and creaming. The viscosity values of F1-F5 range from roughly 0.961 to 0.964 cP, signifying that the system is stable and aligns with the properties of food nanoemulsions. The augmentation in water content from F1 to F5 corresponds with a reduction in carbs and ethanol. This suggests that an increase in water content in the fruit fermentation beverage results in a decrease in substrate fermentation by the microorganisms. Elevated moisture levels typically dilute the substrate, reducing fermentation. Variations in ash content are determined by the mineral composition of the raw materials (apples and dates) and by microbial interactions during fermentation. Fluctuations in ash content in fruit kefir reflect diverse mineral distributions resulting from the metabolic activities of yeasts and lactic acid bacteria⁴⁰. The protein level is comparatively low because the primary substrate is fruit juice, which inherently contains minimal protein. The protein in F5 likely originates from probiotic bacteria undergoing cellular lysis or metabolic byproducts. Analogous to protein, the low-fat content corresponds with the attributes of fruit substrates, which are deficient in lipids. The elevated fat content in F4-F5 may stem from microbial lipid molecules (phospholipid membranes) produced during fermentation⁴¹. The carbohydrate content considerably diminished from F1 (1.65%) to F5 (0%). This signifies that carbohydrates serve as the principal substrate for fermentation. This reduction aligns with the rise in ethanol concentrations in specific formulations. Simple carbohydrates in fruit are fermented by yeast into ethanol and carbon dioxide, whereas lactic acid bacteria ferment them into lactic acid. The ethanol concentration in kefir is affected by the presence of fermentable sugars⁴². The carbohydrate percentage in F1 remains elevated at 1.65%, resulting in the highest ethanol yield of 2.32%. Conversely, F5 has exhausted carbs (0%), resulting in minimal ethanol generation (0.48%). Methanol concentrations were not significantly identified in any of the formulations (<0.05%). Reduced methanol concentrations signify effective fermentation with little breakdown of plant cell walls⁴³. F1

(2:8) exhibited the highest antioxidant activity, while F5 (10:0) showed reduced activity. This is a result of the predominance of dates in F1, which is abundant in polyphenols, flavonoids, and vitamin C, which function as electron/hydrogen donors to reduce DPPH free radicals. The ABTS results pattern is relatively consistent with DPPH, with formulations containing a higher proportion of dates (F1) exhibiting greater ABTS radical-scavenging capacity. Apples are the sole source of flavonoids, including quercetin and catechins. However, dates are more potent due to their higher polyphenol content and greater stability during fermentation. Polyphenols can be fermented by lactic acid bacteria to produce more bioactive forms with enhanced antioxidant activity³⁴. The α -glucosidase and α -amylase inhibition experiments on apple kefir-sukari date water revealed variations among the five formulations (F1-F5), which were influenced by the proportion of raw materials and fermentation products present. F1 exhibited the most potent inhibitory activity. The higher proportion of dates is believed to have a substantial impact due to their high content of polyphenols, flavonoids, and tannins, which are natural inhibitors of the α -glucosidase enzyme. This compound inhibits the hydrolysis of complex carbohydrates into simple glucose by binding to the enzyme's active site. In comparison to dates, apples also contain quercetin and catechins, which possess inhibitory properties; however, their contribution is relatively minor. The inhibition pattern of α -amylase is comparatively similar to that of α -glucosidase, with higher activity at F1 and F2 than at F4 and F5. Acarbose, used as a positive control, showed higher percentage inhibition values for α -glucosidase and α -amylase than the five water kefir formulations (Figure 3). The α -amylase enzyme converts starch into oligosaccharides and glucose. The inhibitory mechanism significantly reduces the glycemic response. The enzyme-inhibition effect can be enhanced by the production of bioactive metabolites, including soluble polyphenols, organic acids, and peptides, during kefir fermentation. The synergistic effect of phenolic acids (chlorogenic acid) and flavonoids (quercetin, rutin) in suppressing α -glucosidase and α -amylase activity is achieved⁴⁴. The inclusion of iodine in the α -amylase test using starch as a substrate is based on the starch-iodine complex method, which measures the loss of substrate rather than the formation of product. This provides a distinct analytical approach compared to the dinitrosalicylic acid (DNS) method, which measures the reducing sugars produced by enzymatic reactions. This technique has the advantages of high sensitivity and can be very precise if reagents are prepared consistently⁴⁵. The optimal RSM formula results obtained in this study differed from the laboratory experimental results. Based on the RSM method, the formula with an apple-to-date ratio of 0.4 was the optimal result. Based on the apple-to-date ratio experiment, 0.2 provided the best antioxidant and antidiabetic activity. The discrepancy between the optimal apple-to-date ratio predicted by the Response Surface Methodology (RSM) model and the observed laboratory results can be attributed to various factors, including the inherent limitations of statistical modelling and the intricate nature of real-world biological systems⁴⁶. Kynurenic acid is a metabolite anticipated to function as an antidiabetic and antioxidant, as indicated by this study's data. Kynurenic acid is a derivative metabolite of the tryptophan degradation pathway that is produced through the kynurenine pathway. ROS scavenging, metal chelation, and antioxidant enzyme stimulation are the mechanisms by which this compound demonstrates antioxidant activity⁴⁷. Furthermore, it possesses antidiabetic potential by inhibiting digestive enzymes involved in carbohydrate digestion and modulating GPR35⁴⁸.

Conclusion

The research findings indicate that water kefir, derived from a blend of manalagi apples and sukari dates and enhanced with nanoemulsion-encapsulated probiotic *L. plantarum*, has significant potential as a functional food with antioxidant and antidiabetic properties. The ideal formula (F1) with an apple: date ratio of 2:8 has enhanced physicochemical properties, including superior total acidity, pH, vitamin C concentration, carbohydrate content, and ethanol content relative to alternative formulae. The antioxidant efficacy of F1 was consistently superior in DPPH, ABTS, and FRAP assays, while the lowest IC₅₀ values for inhibition of α -glucosidase and α -amylase evidenced its antidiabetic properties. The inclusion of bioactive

metabolites, including phenols and tannins, and the discovery of significant compounds via UHPLC-MS, such as kynurenic acid, enhance this kefir's potential to mitigate oxidative stress and prevent carbohydrate breakdown. This study demonstrates that combining manalagi apples, sukari dates, and encapsulated probiotics yields a bioactive synergy with antioxidant and antidiabetic properties, positioning it as a potential alternative nutraceutical therapy derived from local natural ingredients.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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