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Potential of White Jack Bean (*Canavalia ensiformis* L. DC) Kefir as a Microencapsulated Antioxidant

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ABSTRACT: Oxidative stress plays a major role in the pathogenesis and progression of noncommunicable diseases. Kefir is a fermented food that has been reported to repress oxidative stress. This study aimed to assess the antioxidant activity, bioactive composition, and encapsulation efficiency of white jack bean (WJB) kefir. The following procedures were conducted: WJB was prepared and converted into juice using water solvent. The sterilized WJB juice was then fermented with kefir grain (10%) for 24～72 h. Every 24 h, the kefir was evaluated for antioxidant activity, and the dominant bioactive component suspected to be the source of the antioxidant activity was identified. The final stage was the encapsulation process. WJB kefir showed high antioxidant activity, inhibiting DPPH radicals by 90.51±4.73% and ABTS radicals by 86.63±2.34% after 72 h of fermentation. WJB kefir contained 0.35±0.01 mg GAE/g total phenolics and 0.08 mg/g total flavonoids. The LC/MS identification suggested that the bioactive antioxidant components of the WJB kefir were from the alkaloid, saponin, phenolic, and flavonoid groups. The encapsulation with maltodextrin using freeze drying resulted in microencapsulation of WJB kefir with a particle size of 6.42±0.13 μm. The encapsulation efficiency was 79.61%, and the IC_{50} value was 32.62 ppm. The encapsulation method was able to maintain the antioxidant stability of the kefir and extend its shelf life. WJB kefir, a nondairy, lactose-free kefir, can be used as an antioxidant functional food.

Keywords: antioxidants, fermentation, functional food, kefir, noncommunicable diseases

INTRODUCTION

The number of sufferers of noncommunicable diseases (NCDs) has increased in the last 10～15 years (Bennett et al., 2018). NCDs can arise from conditions of oxidative stress, which are caused by higher exposure to free radicals compared to levels of antioxidants in the body. Preventive measures to reduce oxidative stress include the consumption of functional foods. Functional foods are broadly defined as foods that provide more than simple nutrition; they supply additional physiological benefits to the consumer (Tur and Bibiloni, 2016). Fermented food is a type of functional food that utilizes certain microorganisms to produce food that is rich in antioxidants.

Kefir is a lightly sparkling fermented food with a sour flavor, a thick viscosity, and an alcoholic aroma. Kefir differs from other fermented beverages because the starter, kefir grain, consists of a symbiotic culture of bacteria and yeasts. The number of microorganisms in kefir grain ranges from 7～9 log colony forming units (CFU)/g for lactic acid bacteria (LAB), 6～7 log CFU/g for yeast, and 5～7 log CFU/g for acetic acid bacteria (AAB) (Bengoa et al., 2019). *Lactobacillus*, *Gluconobacter*, and *Saccharomyces* are the dominant microorganisms in kefir grain (Garofalo et al., 2015; Gao and Li, 2016; Rosa et al., 2017). The microbial composition in kefir grain is dynamic; this is unique to kefir and an advantage that affects the characteristics of kefir products. During kefir fermentation, LAB play a role in converting sugar substrates into lactic acid, the AAB convert ethanol produced from the glycolysis process into acetic acid (Chakravorty et al., 2016), and the yeast convert sugar substrates into ethanol and carbon dioxide (CO_2), creating its carbonated property. Kefir grains are also a source of probiotics. Probiotics are live microorganisms that occur in certain foods, which, when consumed, benefit the health of the host by improving the in-

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testinal microflora composition. The microorganisms in kefir grains are also capable of producing bioactive compounds that impact human health, including food enzymes, bacteriocins, bioactive peptides, and compounds that are antioxidant, antihypertensive, anti-inflammatory, antitumor, antidiabetic, and antiallergic (Ruiz et al., 2010; Guzel-Seydim et al., 2021). Some reports have shown that kefir consumption can help lower cholesterol, boost the immune system, and relieve nervous disorders, insomnia, and anorexia (Azizi et al., 2021). Most importantly, the kefir grain microbiota has a high ability to adapt to various dietary substrates, including dairy and nondairy substrates, which can be used to create new beverages (Corona et al., 2016; Rodrigues et al., 2016; Koh et al., 2017; Guzel-Seydim et al., 2021).

The currently known type of kefir is dairy or milk kefir. In this research, nondairy, plant-based kefir will be produced. Plant-based kefir is thought to have several advantages, including being lactose-free and low in fat. Plant-based kefir is thought to retain the health effects of milk kefir. Previous research has provided strong evidence that legume-based kefirs, such as soy, cashew, hazelnut, peanut, and walnut kefirs, have functional benefits that are comparable to those of milk kefir (Tu et al., 2019; Comak Gocer and Koptagel, 2023). The advantages of plant-based kefirs include their high unsaturated fatty acid content, low saturated fatty acids content (Comak Gocer and Koptagel, 2023), and high antioxidant activity (Atalar, 2019). In addition, plant-based kefir is a nondairy fermented beverage that may prove to be an alternative for vegans and those who are lactose-intolerant or allergic to dairy.

One of the legumes that has the potential to be produced into kefir is white jack bean (WJB, *Canavalia ensiformis*). WJB are grown in Asia, Africa, and Latin America. WJB are simple to grow and have a high protein (32%) and carbohydrate (64%) contents (Akpapunam and Sefa-Dedeh, 1997). Concanavalin A, concanavalin B, canavalin, α mannosidase, legumain, and urease are the main proteins found in WJB. However, utilization of WJB as a food source is limited due to its content of phytic acid and cyanide acid, which are antinutritional and toxic compounds. According to research conducted by Ramli et al. (2021), the phytic acid and cyanide acid in WJB can be reduced by soaking the beans for 1～3 days in 1% sodium bicarbonate solution or autoclaving at 121°C.

WJB kefir is thought to have strong potential as a source of antioxidants. The antioxidant components that are produced after fermentation with kefir grain are thought to come from organic acid components, phenolics, flavonoids, and bioactive peptides. The high protein content of WJB is thought to stimulate the proteolytic activity of kefir grain microorganisms to produce peptides. To preserve and maximize the antioxidant activity of kefir, an encapsulation process can be performed. Encapsulation is a coating process that uses polymers to protect the components from the external environment (Kłosowska et al., 2023). One of the polymers used as encapsulant is maltodextrin. Maltodextrin is an encapsulant that has the advantages of low viscosity and good water solubility even at high concentrations (Rahman Mazumder and Ranganathan, 2020; Xiao et al., 2022). Another study reported that maltodextrin contains low sugar and is colorless (Kusmayadi et al., 2019), making it suitable for use in food. The use of maltodextrin as an encapsulant using the freeze-drying method creates microencapsulated WJB kefir with high encapsulation efficiency (EE).

MATERIALS AND METHODS

Kefir grains and WJBs

Kefir grains were purchased from the household-scale kefir industry in Bandung, West Java Province, Indonesia. Kefir grains were preserved in ultra-high temperature skim milk at 37±5°C for 24～48 h prior to fermentation in order to maintain the grains' viability. The kefir grains were separated from the supernatant by filtration through a sterile sieve. WJBs and soybeans were obtained from Central Java Province, Indonesia.

Chemicals

The microorganism growth media, de Man-Rogosa-Sharpe (MRS) and potato dextrose agar, were purchased from HiMedia Laboratories. The compounds, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) were purchased from Sigma-Aldrich. High performance liquid chromatography (HPLC)-grade methanol, acetonitrile, and 3-methyl-1-phenyl-2-pyrazolin-5-one were purchased from Merck Corp. All other chemicals and reagents were of analytical grade.

WJB extraction

The sorted and washed WJB were first soaked in clean water for 24 h. During the initial soaking, the water was changed once. After the soaking was complete, the skins were removed from the WJB. The skinless WJB were then soaked in 1% sodium carbonate ($NaHCO_3$) solution for 1 day. The WJB were then rinsed twice with clean water before autoclaving at 121°C for 15 min. After autoclaving, the WJB were cut into pieces and pulverized using a blender by mixing the beans with warm water (60～70°C) at a ratio of 1:3. The mixture was then filtered to obtain a liquid extract, which was used as the fermentation medium.

WJB kefir fermentation

WJB kefir fermentation was conducted in triplicate in 250

mL Erlenmeyer flasks containing 200 mL of the sterilized WJB and 3% sucrose (w/v). Each of the fermentation flasks was inoculated with 10% (w/v) kefir grain and incubated at 37°C for 72 h. Samples were collected every 24 h for analysis. A soybean extract and skim milk mixture was used as a control.

pH value measurement during fermentation

The pH was measured every 24 h in triplicate using a pH meter (F-71S Desktop pH meter, Horiba Scientific).

Enumeration of WJB kefir microorganisms

The enumeration of WJB kefir microorganisms was conducted using the spread plate method following Yusuf et al. (2020). LAB and yeasts were enumerated on MRS agar and potato dextrose agar, respectively. NaCl solution (0.85%, w/v) was used for the serial dilutions of the samples. The bacteria and yeast plates were incubated at 37°C and 30°C, respectively, for 24~48 h. The viable microbial cells were counted and expressed as log CFU/mL.

Preparation of cell-free supernatant (CFS)

The preparation of CFS was based on the method developed by Chen et al. (2014). WJB kefir was produced and the supernatant was separated from the curd and cells by centrifugation (8,000 *g*; 5 min) and filtration through 0.22-μm membrane filters (Minisart Syringe Filter, Sartorius). CFS obtained was used in the next assay.

DPPH radical scavenging ability assay

This assay was performed referring to the method described by Chen et al. (2014). In brief, 1 μL of CFS was mixed with 1 mL of DPPH solution, and the sample was incubated in the dark for 30 min. The DPPH free radical scavenging activity was observed by measuring the sample's absorbance at a wavelength of 517 nm using UV-Visible spectrophotometry (BioSpec-1601, Shimadzu Corp.). The sample's capability to scavenge DPPH radicals was calculated using the following equation:

$$\text{DPPH radical scavenging activity (\%)} = [1-(A_{sample}-A_{blank})/A_{DPPH}]\times 100\%$$

ABTS scavenging ability assay

The test method was performed referring to research by Chen et al. (2014). ABTS solution (0.5 mL) was dissolved in 0.5 mL of CFS. The mixture was incubated in the dark for 6 min at 37°C. Then, the absorbance value of the mixed solution was calculated using UV-Visible spectrophotometry at a wavelength of 345 nm. The sample's capability to scavenge the ABTS radicals was calculated using the following equation:

$$\text{ABTS radical scavenging activity (\%)} = [(A_{control}-A_{sample})/A_{control}]\times 100\%$$

where $A_{control}$ represents the absorbance of the blank without the sample, and A_{sample} represents the absorbance in the presence of the sample.

Quantification of total phenolic content (TPC)

The TPC was determined using the Folin-Ciocalteu method following Kumar et al. (2021) with some adjustments. In brief, 1 mL of CFS was dissolved in distilled water until the volume reached 4 mL. Then 250 μL of Folin-Ciocalteu reagent was added to the solution. The mixed solution was homogenized and incubated for 8 min at room temperature (25~30°C) before adding 750 μL of 20% Na_2CO_3 solution. The mixed solution was homogenized and incubated for 2 h at room temperature. After incubation, the absorbance was read at 765 nm using an ELISA plate reader (Bio-Tek Instruments, Agilent Technologies). The TPC was expressed as the gallic acid equivalent (mg GAE/g). This experiment was carried out in triplicate.

Quantification of total flavonoid content (TFC)

The TFC was measured following the method described by Kumar et al. (2021) with some adjustments. In brief, 9 μL of 5% $NaNO_3$ was mixed with 150 μL of CFS or catechin (50 mg/mL) as standard and incubated for 6 min in the dark. Then, 9 μL of 10% $AlCl_3$ was added, and the sample was incubated for 5 min. After incubation, 60 μL of 1 M NaOH and 72 μL of distilled water were added to the sample, and the sample was mixed using a vortex. The absorbance was then read at 430 nm using an ELISA plate reader. TFC of the sample was expressed as the catechin equivalent (mg CATE/g). This experiment was carried out in triplicate.

Nano liquid chromatography-mass spectrometry (LC-MS) scanning

Bioactive compound analysis was carried out using a LC-MS system (Nano LC Ultimate 3000 Series Tandem Q System Exactive Plus Orbitrap HRMS, Thermo Fisher Scientific Corp.) following the method described by Daliri et al. (2018) with some adjustments. The Detector MS Orbitrap type Q Exactive Plus (Thermo Fisher Scientific Corp.) was used. Samples were trapped in a Phenomenex HPLC Column (Kinetex C18, 100 mm L×2.1 mm ID, 2.6 μm, 100 A). The capillary column had a size of 75 μm× 15 cm, a particle size of 3 μm, and a pore size of 100 (PepMap RSLC C18, ES 800, Thermo Fisher Scientific Corp.). For the eluent, H_2O/acetonitrile 98:2 formic acid (A) and H_2O/acetonitrile 2:98 0.1% formic acid (B) were used. The flow rate was set at 300 μL/min with a gradient of 2~35% (B) for 30 min, 30~90% (B) for 15 min,

90% (B) for 15 min, and 5% (B) for 30 min. The mass range was 200~2,000 m/z. The results obtained were analyzed using the MassBank program (MassBank Corp.). In the initial stage, screening was carried out at peak intensities of >100, then the m/z value at the peak was entered into the program as the exact mass value. This value was then analyzed using the electrospray ionization method. The program shows the name, formula/structure, and exact mass of the analyzed compound. For compound group data, a manual search was carried out using Google search.

Encapsulation process

Samples were encapsulated using maltodextrin as a coating material, referring to research by Akdeniz et al. (2017). The encapsulation process was carried out by reducing the water content of the kefir using a rotary evaporator (R-300 System, Buchi Corp.) at a temperature of 40~55°C and a pressure of 125~175 bar for 3 h. The sample was then mixed with 15% (w/v) maltodextrin at a ratio of 1:1. The mixing process was carried out using a homogenizer at a speed of 500 *g* for 20 min. Then, the mixture was frozen −80°C. After freezing, the sample was dried using a freeze-drying machine (Lyovapor L-200, Buchi Corp.) for 72 h at −50°C. The resulting kefir capsules were analyzed for antioxidant activity using the DPPH method to determine the level of EE.

Particle size determination

The particle size determination method was based on research by Akdeniz et al. (2017). Encapsulant size particle determination was performed in triplicate using a particle size analyzer (Partica LA-960, Horiba Scientific Corp.). The measurement conditions were in the range of 0.01~5,000 μm. Flow cells in water were used as accessories, and the static light scattering method was employed. A sample weighing 0.25 mg was put into a cuvette, then aqua pro injection was added to increase the volume to 2.5 mg. The cuvette was inserted into the holder.

Encapsulation efficiency evaluation

The EE (%) was calculated based on the antioxidant activity before encapsulation divided by the initial antioxidant activity following encapsulation.

Statistical analysis

All data were presented as the mean±standard deviation. Data were evaluated using one-way ANOVA in IBM SPSS ver. 24 (IBM Corp.). Differences between means were assessed using Duncan's test. Statistical significance was considered at a probability value (*P*) of <0.05.

RESULTS AND DISCUSSION

Characterization of WJB kefir

WJB kefir showed a pH of 3.75±0.02 after 24 h, a decrease from the previous pH of 6.25±0.02. The pH value tends to decrease as the incubation time increases. This value was relatively similar to that in the comparison treatments of soybean kefir and skim milk kefir, which showed pHs of 4.10±0.04 and 3.80±0.03, respectively (Table 1). The fermentation time was extended to 48 and 72 h to determine the effect of the microbiological and chemical properties produced. WJB kefir showed a significant decrease in pH at 48 and 72 h. The decrease in pH correlated with a significant increase in the percentage of total lactic acid. This result indicates that kefir grain microorganisms continue to actively metabolize in WJB media. The legume extraction process that was applied also succeeded in eliminating the toxic and antinutritional effects that are thought to inhibit the growth of kefir microbes.

Table 1. Characteristics of WJB, soybean, and skim milk kefirs after different fermentation times

Substrate	Fermentation time (h)	pH	Lactic acid (%)	Peptides (mg/mL)
WJB	0	6.25±0.02^{a}	0.60±0.20^{d}	0.48±0.19^{c}
	24	3.75±0.02^{b}	5.60±0.26^{c}	6.48±0.13^{b}
	48	3.61±0.02^{c}	6.50±0.26^{b}	7.11±0.27^{a}
	72	3.54±0.03^{d}	7.40±0.45^{a}	7.25±0.06^{a}
Soybean	0	6.60±0.04^{a}	0.62±0.20^{d}	0.88±0.39^{c}
	24	4.10±0.04^{c}	9.15±0.06^{b}	7.70±0.41^{a}
	48	4.16±0.04^{c}	10.20±0.32^{a}	7.19±0.46^{b}
	72	4.35±0.03^{b}	7.10±0.38^{c}	7.06±0.72^{b}
Skim milk	0	6.78±0.02^{a}	0.80±0.40^{d}	1.20±0.70^{d}
	24	3.80±0.03^{b}	14.55±1.13^{a}	9.39±0.19^{a}
	48	3.66±0.02^{b}	19.00±0.52^{b}	7.37±2.61^{b}
	72	3.67±0.06^{b}	17.40±0.45^{c}	4.85±0.22^{c}

Values are presented as mean±SD from three repetitions.
Different superscript letters within the same column (a-d) indicate that the treatment results were significantly different (P<0.05) according to Duncan's multiple range test.
WJB, white jack bean.

The elimination of the toxic and antinutritional components was also supported by the results of the total colony counts of LAB and yeast in the WJB kefir, which were relatively constant after 3 days of fermentation at around 7～8 log CFU/mL and 5～7 log CFU/mL, respectively. The addition of sucrose as a substrate is also thought to affect microbial growth in nondairy kefir (Randazzo et al., 2016; Patel et al., 2022; Tavares et al., 2023).

Peptide content testing was carried out on the WJB, soybean, and skim milk kefirs. The results showed that, in all the kefirs, the highest peptide content was produced within 24～48 h of fermentation. When fermentation was extended to 72 h, the peptide content decreased (Table 1). During fermentation, the dominant microbes in kefir grain, LAB and yeast, produce protease enzymes that break down soluble WJB proteins into oligopeptides, dipeptides, and tripeptides, which may have potential as bioactive peptides (Singh et al., 2014; Daliri et al., 2018). Both groups of microbes have been reported to produce intracellular and extracellular protease enzymes (Kieliszek et al., 2021). They can also produce bioactive peptides with functional characteristics during the fermentation process, such as peptides with antioxidant activity (Pessione and Cirrincione, 2016; Yusuf et al., 2021). For example, peptides with antioxidant activity have been produced by *Lactobacillus plantarum* from traditional Chinese foods (tofu and kefir) (Li, 2012). Mixed cultures of LAB and yeast have also been reported to produce antioxidant peptides in certain media (Li et al., 2015).

Antioxidant activity

WJB kefir showed high antioxidant activity, inhibiting DPPH radicals by 90.51±4.73% and ABTS radicals by 86.63±2.34% (Table 2). The highest antioxidant activity was produced after 72 h of incubation. The antioxidant activity was found to increase significantly according to the length of the fermentation time. The metabolite components created during fermentation increase the overall antioxidant effect (Winarti et al., 2021). The results showed that the WJB kefir had higher antioxidant activity than the soybean kefir.

Kefir grain bacteria and yeast are thought to be the sources of antioxidants in WJB kefir. One of these antioxidants, superoxide dismutase (SOD), comes from the cellular antioxidant system (Najmuldeen et al., 2019; Pinmanee et al., 2023). Superoxide is one of the most abundant reactive oxygen species (ROS) produced by the mitochondria, while SOD catalyzes the breakdown of superoxide into hydrogen peroxide and water and is therefore a central regulator of ROS levels (Najmuldeen et al., 2019). In addition, LAB can produce various metabolites that have antioxidant activity, such as glutathione (Al-Madboly et al., 2017), butyrate (LeBlanc et al., 2017), folate (Rossi et al., 2011), and exopolysaccharides (EPS) (Dilna et al., 2015). AAB can also produce EPS, increase the levels of polyphenolic compounds, increase the antioxidant activity from oxidative fermentation, and lower the acidity of the environment (Neffe-Skocińska et al., 2023). For yeast, especially *Saccharomyces*, which is predominant in kefir grain, the components that contribute to the antioxidant properties include glutathione, sulfur-containing amino acids, and Maillard reaction products (Makky et al., 2021).

Bioactive peptides are another source of antioxidants in WJB kefir. Kefir and other LAB fermentation products have been widely reported to contain antioxidant peptides (Conway et al., 2013; Punaro et al., 2014; Daliri et al., 2018; Sonklin et al., 2018). These bioactive peptides result from proteolytic activity. Ramesh et al. (2012) reported a correlation between proteolytic activity and antioxidant activity during LAB fermentation. Antioxidant peptides are generally characterized by containing the hydrophobic amino acid residues valine or leucine at the N-terminus and proline, histidine, or tyrosine in the amino acid sequence (Sabeena Farvin et al., 2010).

Table 2. Antioxidant activities of WJB, soybean, and skim milk kefir

Sample	Fermentation time (h)	DPPH (%)	ABTS (%)	TPC (mg GAE/g sample)	TFC (mg CATE/g sample)
WJB kefir	0	30.81±4.51^{c}	31.36±3.51^{c}	0.16±0.20^{a}	0.01±0.08^{a}
	24	40.47±1.78^{b}	44.91±2.66^{bc}	0.20±0.08^{a}	0.01±0.06^{a}
	48	46.29±3.84^{b}	45.25±2.08^{bc}	0.23±0.10^{a}	0.05±0.06^{ab}
	72	90.51±4.73^{a}	86.63±2.34^{a}	0.28±0.00^{b}	0.08±0.06^{b}
Soybean kefir	0	17.66±4.21^{ab}	15.24±2.06^{b}	0.14±0.03^{a}	0.01±0.00^{a}
	24	19.37±0.72^{ab}	15.77±1.06^{b}	0.18±0.07^{a}	0.01±0.00^{a}
	48	21.00±2.06^{ab}	16.61±2.80^{b}	0.21±0.02^{a}	0.01±0.03^{a}
	72	27.54±1.69^{a}	19.35±0.04^{a}	0.35±0.01^{b}	0.01±0.04^{a}

Values are presented as mean±SD from three repetitions.
Different superscript letters in the same column (a–c) indicate that the results were significantly different (P<0.05) according to Duncan's multiple range test. TPC and TFC (a,b) is not significanly different.
WJB, white jack bean; DPPH, 2,2-diphenyl-1-picrylhydrazyl; ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt; TPC, total phenolic content; GAE, gallic acid equivalent; TFC, total flavonoid content; CATE, catechin equivalent.

Total phenolic content

TPC of the WJB kefir essence supernatant that was fermented for 72 h was 0.35±0.01 mg GAE/g, meaning each gram of sample was equivalent to 0.35 mg of gallic acid. During the fermentation process, microbes synthesize enzymes that are able to hydrolyze ester bonds and glycosidic bonds and distort hydroxyl groups so that they can release bound phenolics, thereby increasing the level of free phenolic compounds (Adebo and Medina-Meza, 2020). TPC of the WJB kefir was higher than that of the soybean kefir, which showed a TPC of 0.28 mg GAE/g. This result was due to the fact that natural WJB has a high phenolic content (Sutedja et al., 2020). The structure of the phenolic groups in WJB, including the arrangement of hydroxyl groups, glycans, and other substituents, change the free radical scavenging capacity, consequently influencing the overall antioxidant activity (Sutedja et al., 2020). An increase in total phenol can also occur due to the addition of carbon sources such as sucrose and the quantity of microorganisms in the kefir grain. The added carbon source acts as a substrate for LAB to produce many metabolites, one of which is phenol. Meanwhile, the quantity of microorganisms can affect the total phenol content because they can trigger enzymatic reactions (Azizi et al., 2021). Enzymatic reactions break bonds in the substrate, causing the release of phenolic compounds from their conjugate forms. According to Melini et al. (2019) the hydrolytic activity of enzymes can damage the cell wall structure of WJB seeds, increasing the bioaccessibility and bioavailability, resulting in greater levels of phenolic compounds. The fermentation process stimulates a decrease in pH so that several enzymes involved in the hydrolysis of polyphenolic complexes are activated. During fermentation, β-glucosidase and esterase enzymes from microbes hydrolyze phenolics and flavonoids (Wijayanti et al., 2017).

Total flavonoid content

TFC of the WJB kefir was 0.08 mg CATE/g. In comparison, TFC of the soybean kefir was 0.01 mg CATE/g (Table 2). This value is quite low when compared to TFC of the WJB kefir. It is suspected that WJB kefir's antioxidant activity is not dominantly derived from flavonoid compounds. The antioxidant potential of flavonoid compounds occurs due to the presence of hydroxyl groups attached to the carbon of the aromatic ring so that they have the ability to scavenge free radicals from lipid peroxidation reactions. Flavonoid compounds will donate one hydrogen atom to stabilize the radicals (Papuc et al., 2017). One flavonol that has been identified and reported as a source of antioxidants in WJB is kaempferol in the form of glycosides (Sutedja et al., 2022). Soybeans contain lots of flavonoids, including isoflavones, flavones, flavonols, flavonones, proanthocyanidins, and anthocyanins (Panche et al., 2016; Chen et al., 2021). Isoflavones, which are abundant in legumes, have strong antioxidant activity, especially in their free forms, namely genistin, genistein, daidzein, glycitein, and daidzin (Król-Grzymała and Amarowicz, 2020). However, these compounds may lose all or some of their antioxidant activity during the extraction and/or fermentation processes.

Identification of potential antioxidant compounds in WJB kefir

In addition to testing for total phenolics and flavonoids, we also attempted to identify components in the WJB ke-

Table 3. White jack bean kefir components based on LC-MS scanning

m/z[1)]	Retention time[1)]	Molecular formula[2)]	Component name[2)]	Type[3)]
248.45	18.333	$C_{15}H_{24}N_2O$	Lupanine	Alkaloids
248.35	19.255	$C_{15}H_{24}N_2O$	Lupanine	Alkaloids
365.3	36.971	$C_{16}H_{19}N_3O_5S$	Amoxicillin	Antibiotics/antitoxic
780.95	43.585	$C_{42}H_{68}O_{13}$	Saikosaponin	Saponin
394.55	45.662	$C_{23}H_{22}O_6$	Rotenone	Flavonoids
201.2	48.849	$C_{11}H_{23}NO_2$	11-Aminoundecanoicacid	Fatty acid and phenol
407.7	49.917	$C_{23}H_{37}NO_5$	Isotalitizidine	Diterpenoid alkaloids
384.15	18.308	$C_{22}H_{28}N_2O_4$	Rhynchophylline	Alkaloids
325.3	19.014	$C_{19}H_{23}N_3O_2$	Ergonovine	Alkaloids
446	36.967	$C_{21}H_{18}O_{11}$	Baicalin	Flavonoid glycosides
224.2	48.758	$C_{14}H_8O_3$	1-Hydroxyanthraquinone	Phenol
219.1	49.224	$C_6H_6NO_6P$	4-Nitrophenyl phosphate	Phenol
314.1	49.464	$C_{14}H_{10}N_4O_5$	Dantrolene	Phenol
780.55	50.016	$C_{41}H_{64}O_{14}$	Digoxin	Cardiac glycosides
207.1	51.204	$C_{11}H_{13}NO_3$	N-acetylphenylalanine	Phenol
216	51.637	$C_{11}H_{20}O_4$	Undecanedioic acid	Phenol
356.4	55.497	$C_{20}H_{20}O_6$	Isoflavone base+30, 1-P-renyl	Isoflavones

[1)]Data collected by nano liquid chromatography-mass spectrometry (LC-MS) scanning procedures.
[2)]Data collected by MassBank program.
[3)]Data collected by Google search.

fir with antioxidant potential. The WJB kefir CFS was analyzed using nano LC-MS. The results showed that phenolic, flavonoid, alkaloid, and saponin compounds were present (Table 3). These compounds have also been reported to be present in other plant-based kefirs (Tu et al., 2019; Azizi et al., 2021). All compounds had an alternative intensity value of 100 (counts $\times 10^6$). This value was also shown by a single peak on the chromatogram. The m/z value of each compound was then entered on the MassBank website for classification. The results suggested that WJB kefir has strong potential as an antioxidant.

The results obtained cannot clearly show the role of bacteria and yeast in producing antioxidant compounds. As previously explained, bacteria and yeast are thought to be able to break down proteins into bioactive peptides, some of which are antioxidants (Tu et al., 2019). In addition, the concentration of each identified component was not successfully obtained due to database limitations in the instrument used. For this reason, further testing methods are needed that can show the sequences and concentrations of the peptides or other components in the kefir.

WJB kefir encapsulation

***Particle size of encapsulant*:** The encapsulation process was successfully carried out and produced white encapsulants of uniform size (Fig. 1). The average size of a WJB kefir encapsulant was 6.42±0.13 μm (Table 4), which is in the microencapsulation range (Krajišnik, 2017). Particle size is one of the most important indices of the colloidal stability, bioavailability, and solubility of a bioactive compound (Sarabandi et al., 2019). Maltodextrin is an efficient coating agent for antioxidant bioactive compounds such as isoflavones (Rahman Mazumder and Ranganathan, 2020). The use of 10% maltodextrin resulted in an EE value of approximately 80.59% in this study. Phenolic compounds and flavonols may form complexes with polysaccharides depending on the solubility, molecular size, mobility, and shape of the polyphenols (Xiao et al., 2022). Nevertheless, the success of encapsulation is dependent on attaining high core material retention and minimum core material retention on the surface of the powder particles. Several factors, including the chemical properties of the coating and the core materials, the emulsion characteristics, and the drying parameters (particularly spray drying conditions such as inlet and outlet temperatures, feed flow rate, air flow and humidity, powder particle size) can influence the EE. The important advantage of this microencapsulation approach is protecting the compound from decreasing activity for a relatively longer time. Microencapsulation also increases bioavailability, and for food-related compounds, this method can mask unwanted aromatic tastes and odors (Bodade and Bodade, 2020).

Fig. 1. White jack bean kefir encapsulated with maltodextrin using the freeze-dry method.

***Encapsulation efficiency*:** The antioxidant activity of the CFS of WJB kefir against DPPH radical decreased after encapsulation. Encapsulation increased the IC_{50} value of the WJB kefir against DPPH radicals (from 31.79 to 32.62 ppm). Meanwhile, the WJB kefir encapsulant which was analyzed using the ABTS method experienced an increase in IC_{50} of 1.57 ppm. The results supported the initial hypothesis that the antioxidant value of the kefir encapsulant does not decrease when compared to the supernatant form, so this technique is an alternative to maintain the antioxidant content and shelf life of kefir. The antioxidant activity of WJB kefir essence encapsulants can be affected by the thermal processes involved in encapsulation, especially when evaporating samples using a rotary evaporator.

WJB kefir was encapsulated to maintain product stability, to avoid a reduction in the antioxidant content of the kefir supernatant, and to maintain the physical properties of the sample. The success of encapsulant formation can be influenced by several factors, including the length of evaporation time for the emulsion formation and the

Table 4. Characteristics of encapsulated white jack bean kefir

Sample	DPPH (%)	IC_{50} (μg/g)	Average size (μm)	Encapsulation efficiency (%)
Nonencapsulant	90.51±1.80	31.79±0.70	–	–
Encapsulant	72.06±4.91	32.62±0.50	6.42±0.13	79.61±3.60

Values are presented as mean±SD from three repetitions.
DPPH, 2,2-diphenyl-1-picrylhydrazyl.

speed of stirring in the homogenizer (Sharma et al., 2017). The choice of evaporation time and temperature, as well as the homogenization speed, will affect the particle size and EE (Sharma et al., 2017). The length of the evaporation time will affect the concentration and water content of the sample. The encapsulants were analyzed for the content of antioxidant activity and particle size.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Concept and design: DY. Analysis and interpretation: DY, RK, MN. Data collection: DY, RK. Writing the article: DY, RK. Critical revision of the article: DY, MN, RHBS, LA, S. Final approval of the article: all authors. Statistical analysis: DY, RK. Overall responsibility: all authors.

REFERENCES

Adebo OA, Gabriela Medina-Meza I. Impact of fermentation on the phenolic compounds and antioxidant activity of whole cereal grains: a mini review. Molecules. 2020. 25:927. https://doi.org/10.3390/molecules25040927

Akdeniz B, Sumnu G, Sahin S. The effects of maltodextrin and gum arabic on encapsulation of onion skin phenolic compounds. Chem Eng Trans. 2017. 57:1891-1896.

Akpapunam MA, Sefa-Dedeh S. Jack bean (*Canavalia ensiformis*): nutrition related aspects and needed nutrition research. Plant Foods Hum Nutr. 1997. 50:93-99.

Al-Madboly LA, Khedr EG, Ali SM. Optimization of reduced glutathione production by a *Lactobacillus plantarum* isolate using Plackett-Burman and Box-Behnken designs. Front Microbiol. 2017. 8:772. https://doi.org/10.3389/fmicb.2017.00772

Atalar I. Functional kefir production from high pressure homogenized hazelnut milk. LWT. 2019. 107:256-263.

Azizi NF, Kumar MR, Yeap SK, Abdullah JO, Khalid M, Omar AR, et al. Kefir and its biological activities. Foods. 2021. 10:1210. https://doi.org/10.3390/foods10061210

Bengoa AA, Iraporda C, Garrote GL, Abraham AG. Kefir micro-organisms: their role in grain assembly and health properties of fermented milk. J Appl Microbiol. 2019. 126:686-700.

Bennett JE, Stevens GA, Mathers CD, Bonita R, Rehm J, Kruk M, et al. NCD Countdown 2030: worldwide trends in non-communicable disease mortality and progress towards Sustainable Development Goal target 3.4. Lancet. 2018. 392:1072-1088.

Bodade RG, Bodade AG. Microencapsulation of bioactive compounds and enzymes for therapeutic applications. In: Pal K, Banerjee I, Sarkar P, Kim D, Deng WP, Dubey NK, et al., editors. Biopolymer-Based Formulations: Biomedical and Food Applications. Elsevier. 2020. p 381-404.

Chakravorty S, Bhattacharya S, Chatzinotas A, Chakraborty W, Bhattacharya D, Gachhui R. Kombucha tea fermentation: Microbial and biochemical dynamics. Int J Food Microbiol. 2016. 220:63-72.

Chen P, Zhang Q, Dang H, Liu X, Tian F, Zhao J, et al. Screening for potential new probiotic based on probiotic properties and α-glucosidase inhibitory activity. Food Control. 2014. 35:65-72.

Chen YT, Hsieh SL, Gao WS, Yin LJ, Dong CD, Chen CW, et al. Evaluation of chemical compositions, antioxidant capacity and intracellular antioxidant action in fish bone fermented with *Monascus purpureus*. Molecules. 2021. 26:5288. https://doi.org/10.3390/molecules26175288

Comak Gocer EM, Koptagel E. Production of milks and kefir beverages from nuts and certain physicochemical analysis. Food Chem. 2023. 402:134252. https://doi.org/10.1016/j.foodchem.2022.134252

Conway V, Gauthier SF, Pouliot Y. Antioxidant activities of buttermilk proteins, whey proteins, and their enzymatic hydrolysates. J Agric Food Chem. 2013. 61:364-372.

Corona O, Randazzo W, Miceli A, Guarcello R, Francesca N, Erten H, et al. Characterization of kefir-like beverages produced from vegetable juices. LWT. 2016. 66:572-581.

Daliri EBM, Lee BH, Park MH, Kim JH, Oh DH. Novel angiotensin I-converting enzyme inhibitory peptides from soybean protein isolates fermented by *Pediococcus pentosaceus* SDL1409. LWT. 2018. 93:88-93.

Dilna SV, Surya H, Aswathy RG, Varsha KK, Sakthikumar DN, Pandey A, et al. Characterization of an exopolysaccharide with potential health-benefit properties from a probiotic *Lactobacillus plantarum* RJF4. LWT. 2015. 64:1179-1186.

Gao X, Li B. Chemical and microbiological characteristics of kefir grains and their fermented dairy products: A review. Cogent Food Agric. 2016. 2:1272152. https://doi.org/10.1080/23311932.2016.1272152

Garofalo C, Osimani A, Milanović V, Aquilanti L, De Filippis F, Stellato G, et al. Bacteria and yeast microbiota in milk kefir grains from different Italian regions. Food Microbiol. 2015. 49:123-133.

Guzel-Seydim ZB, Gökırmaklı Ç, Greene AK. A comparison of milk kefir and water kefir: Physical, chemical, microbiological and functional properties. Trends Food Sci Technol. 2021. 113:42-53.

Kieliszek M, Pobiega K, Piwowarek K, Kot AM. Characteristics of the proteolytic enzymes produced by lactic acid bacteria. Molecules. 2021. 26:1858. https://doi.org/10.3390/molecules26071858

Kłosowska A, Wawrzyńczak A, Feliczak-Guzik A. Microencapsulation as a route for obtaining encapsulated flavors and fragrances. Cosmetics. 2023. 10:26. https://doi.org/10.3390/cosmetics10010026

Koh WY, Utra U, Rosma A, Effarizah ME, Rosli WIW, Park YH. Development of a novel fermented pumpkin-based beverage inoculated with water kefir grains: a response surface methodology approach. Food Sci Biotechnol. 2017. 27:525-535.

Krajišnik D, Čalija B, Cekić N. Polymeric microparticles and

inorganic micro/nanoparticulate drug carriers: an overview and pharmaceutical application. In: Čalija B, editor. Microsized and Nanosized Carriers for Nonsteroidal Anti-Inflammatory Drugs: Formulation Challenges and Potential Benefits. Academic Press. 2017. p 31-67.

Król-Grzymała A, Amarowicz R. Phenolic compounds of soybean seeds from two European countries and their antioxidant properties. Molecules. 2020. 25:2075. https://doi.org/10.3390/molecules25092075

Kumar MR, Yeap SK, Lee HC, Mohamad NE, Nazirul Mubin Aziz M, Khalid M, et al. Selected kefir water from Malaysia attenuates hydrogen peroxide-induced oxidative stress by upregulating endogenous antioxidant levels in SH-SY5Y neuroblastoma cells. Antioxidants. 2021. 10:940. https://doi.org/10.3390/antiox10060940

Kusmayadi A, Adriani L, Abun A, Muchtaridi M, Tanuwiria UH. The microencapsulation of mangosteen peel extract with maltodextrin from arenga starch: formulation and characterization. J Appl Pharm Sci. 2019. 9:33-40.

LeBlanc JG, Chain F, Martín R, Bermúdez-Humarán LG, Courau S, Langella P. Beneficial effects on host energy metabolism of short-chain fatty acids and vitamins produced by commensal and probiotic bacteria. Microb Cell Fact. 2017. 16:79. https://doi.org/10.1186/s12934-017-0691-z

Li G. Intestinal probiotics: interactions with bile salts and reduction of cholesterol. Procedia Environ Sci. 2012. 12:1180-1186.

Li Y, Liu T, He G. Antioxidant activity of peptides from fermented milk with mix culture of lactic acid bacteria and yeast. Adv J Food Sci Technol. 2015. 7:422-427.

Makky EA, AlMatar M, Mahmood MH, Ting OW, Qi WZ. Evaluation of the antioxidant and antimicrobial activities of ethyl acetate extract of *Saccharomyces cerevisiae*. Food Technol Biotechnol. 2021. 59:127-136.

Melini F, Melini V, Luziatelli F, Ficca AG, Ruzzi M. Health-promoting components in fermented foods: an up-to-date systematic review. Nutrients. 2019. 11:1189. https://doi.org/10.3390/nu11051189

Najmuldeen H, Alghamdi R, Alghofaili F, Yesilkaya H. Functional assessment of microbial superoxide dismutase isozymes suggests a differential role for each isozyme. Free Radic Biol Med. 2019. 134:215-228.

Neffe-Skocińska K, Karbowiak M, Kruk M, Kołożyn-Krajewska D, Zielińska D. Polyphenol and antioxidant properties of food obtained by the activity of acetic acid bacteria (AAB)—A systematic review. J Funct Foods. 2023. 107:105691. https://doi.org/10.1016/j.jff.2023.105691

Panche AN, Diwan AD, Chandra SR. Flavonoids: an overview. J Nutr Sci. 2016. 5:e47. https://doi.org/10.1017/jns.2016.41

Papuc C, Goran GV, Predescu CN, Nicorescu V, Stefan G. Plant polyphenols as antioxidant and antibacterial agents for shelf-life extension of meat and meat products: classification, structures, sources, and action mechanisms. Compr Rev Food Sci Food Saf. 2017. 16:1243-1268.

Patel SH, Tan JP, Börner RA, Zhang SJ, Priour S, Lima A, et al. A temporal view of the water kefir microbiota and flavour attributes. Innov Food Sci Emerg Technol. 2022. 80:103084. https://doi.org/10.1016/j.ifset.2022.103084

Pessione E, Cirrincione S. Bioactive molecules released in food by lactic acid bacteria: encrypted peptides and biogenic amines. Front Microbiol. 2016. 7:876. https://doi.org/10.3389/fmicb.2016.00876

Pinmanee P, Sompinit K, Jantimaporn A, Khongkow M, Haltrich D, Nimchua T, et al. Purification and immobilization of superoxide dismutase obtained from *Saccharomyces cerevisiae* TBRC657 on bacterial cellulose and its protective effect against oxidative damage in fibroblasts. Biomolecules. 2023. 13:1156. https://doi.org/10.3390/biom13071156

Punaro GR, Maciel FR, Rodrigues AM, Rogero MM, Bogsan CS, Oliveira MN, et al. Kefir administration reduced progression of renal injury in STZ-diabetic rats by lowering oxidative stress. Nitric Oxide. 2014. 37:53-60.

Rahman Mazumder MA, Ranganathan TV. Encapsulation of isoflavone with milk, maltodextrin and gum acacia improves its stability. Curr Res Food Sci. 2020. 2:77-83.

Ramesh V, Kumar R, Singh RRB, Kaushik JK, Mann B. Comparative evaluation of selected strains of lactobacilli for the development of antioxidant activity in milk. Dairy Sci Technol. 2012. 92:179-188.

Ramli NAM, Chen YH, Mohd Zin Z, Abdullah MAA, Rusli ND, Zainol MK. Effect of soaking time and fermentation on the nutrient and antinutrients composition of *Canavalia ensiformis* (Kacang Koro). IOP Conf Ser Earth Environ Sci. 756:012033. https://doi.org/10.1088/1755-1315/756/1/012033

Randazzo W, Corona O, Guarcello R, Francesca N, Germanà MA, Erten H, et al. Development of new non-dairy beverages from Mediterranean fruit juices fermented with water kefir microorganisms. Food Microbiol. 2016. 54:40-51.

Rodrigues KL, Araújo TH, Schneedorf JM, de Souza Ferreira C, de Oliveira Isac Moraes G, Coimbra RS, et al. A novel beer fermented by kefir enhances anti-inflammatory and anti-ulcerogenic activities found isolated in its constituents. J Funct Foods. 2016. 21:58-69.

Rosa DD, Dias MMS, Grześkowiak ŁM, Reis SA, Conceição LL, Peluzio MDCG. Milk kefir: nutritional, microbiological and health benefits. Nutr Res Rev. 2017. 30:82-96.

Rossi M, Amaretti A, Raimondi S. Folate production by probiotic bacteria. Nutrients. 2011. 3:118-134.

Ruiz B, Chávez A, Forero A, García-Huante Y, Romero A, Sánchez M, et al. Production of microbial secondary metabolites: regulation by the carbon source. Crit Rev Microbiol. 2010. 36:146-167.

Sabeena Farvin KH, Baron CP, Nielsen NS, Otte JAHJ, Jacobsen C. Antioxidant activity of yoghurt peptides: Part 2—Characterisation of peptide fractions. Food Chem. 2010. 123:1090-1097.

Sarabandi K, Mahoonak AS, Hamishehkar H, Ghorbani M, Jafari SM. Protection of casein hydrolysates within nanoliposomes: Antioxidant and stability characterization. J Food Eng. 2019. 251:19-28.

Sharma M, Mann B, Sharma R, Bajaj R, Athira S, Sarkar P, et al. Sodium caseinate stabilized clove oil nanoemulsion: Physicochemical properties. J Food Eng. 2017. 212:38-46.

Singh BP, Vij S, Hati S. Functional significance of bioactive peptides derived from soybean. Peptides. 2014. 54:171-179.

Sonklin C, Laohakunjit N, Kerdchoechuen O. Assessment of antioxidant properties of membrane ultrafiltration peptides from mungbean meal protein hydrolysates. PeerJ. 2018. 6:e5337. https://doi.org/10.7717/peerj.5337

Sutedja AM, Ito A, Yanase E, Batubara I, Fardiaz D, Lioe HN. Influence of jack bean (*Canavalia ensiformis* (L) DC) milk processing on bioactive compounds and its antioxidant activity. Food Sci Technol. 2022. 42:e11521. https://doi.org/10.1590/fst.11521

Sutedja AM, Yanase E, Batubara I, Fardiaz D, Lioe HN. Identification and characterization of a-glucosidase inhibition flavonol glycosides from jack bean (*Canavalia ensiformis* (L.) DC. Molecules. 2020. 25:2481. https://doi.org/10.3390/molecules25112481

Tavares PPLG, Mamona CTP, Nascimento RQ, dos Anjos EA, de Souza CO, de Castro Almeida RC, et al. Non-conventional sucrose-based substrates: development of non-dairy kefir beverages with probiotic potential. Fermentation. 2023. 9:384. https://doi.org/10.3390/fermentation9040384

Tu C, Azi F, Huang J, Xu X, Xing G, Dong M. Quality and meta-

genomic evaluation of a novel functional beverage produced from soy whey using water kefir grains. LWT. 2019. 113:108258. https://doi.org/10.1016/j.lwt.2019.108258

Tur JA, Bibiloni MM. Functional foods. In: Caballero B, Finglas PM, Toldrá F, editors. Encyclopedia of Food and Health. Academic Press. 2016. p 157-161.

Wijayanti ED, Setiawan NCE, Cristi JP. Effect of lactic acid fermentation on total phenolic content and antioxidant activity of fig fruit juice (*Ficus carica*). Adv Health Sci Res. 2017. 2:282-289. https://doi.org/10.2991/hsic-17.2017.44

Winarti S, Sarofa U, Islami BY. Physicochemical properties of black soygurt made from black soybeans (BS) and black sticky rice (BR). IOP Conf Ser Mater Sci Eng. 2021. 1125:012103. https://doi.org/10.1088/1757-899X/1125/1/012103

Xiao Z, Xia J, Zhao Q, Niu Y, Zhao D. Maltodextrin as wall material for microcapsules: A review. Carbohydr Polym. 2022. 298:120113. https://doi.org/10.1016/j.carbpol.2022.120113

Yusuf D, Nuraida L, Dewanti-Hariyadi R, Hunaefi D. *In vitro* antioxidant and α-glucosidase inhibitory activities of *Lactobacillus* spp. isolated from Indonesian kefir grains. Appl Food Biotechnol. 2021. 8:39-46. https://doi.org/10.22037/afb.v8i1.30367

Yusuf D, Nuraida L, Dewanti-Hariyadi R, Hunaefi D. Lactic acid bacteria and yeasts from Indonesian kefir grains and their growth interaction. Asian J Microbiol Biotech Environ Sci. 2020. 22:44-49.