Trop J Nat Prod Res, November 2025; 9(11): 5520 - 5528
ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Antioxidant and Antidiabetic Activities of Manalagi Apple (*Malus sylvestris* Mill) Juice and Water Kefir: An *In Vitro* and *In Silico* Study

Harry Noviardi, Sitaresmi Yuningtyas*, Amalia Adzani, Rumi M. Alawiyah

Department of Pharmacy, Sekolah Tinggi Teknologi Industri dan Farmasi Bogor, Bogor 16128, West Java, Indonesia

ARTICLE INFO

Article history:
Received 30 August 2025
Revised 29 October 2025
Accepted 02 November 2025
Published online 01 December 2025

ABSTRACT

Diabetes mellitus is associated with oxidative stress, which is linked to an increased production of reactive oxygen species, potentially resulting in cellular damage. Antioxidant compounds have been shown to effectively reduce this damage in individuals with diabetes mellitus. Among the prominent sources of these antioxidants are apple juice and water kefir. The aim of this research was to asses the antioxidant and antidiabetic activities of Manalagi apple juice (MAJ) and Manalagi apple water kefir (MAWK) using *in vitro* and *in silico* methods. Antioxidant and antidiabetic activities were determined using 2,2-diphenyl-1-picrilhydrazyl (DPPH) assay and α-glucosidase inhibition. The interactions of the active compounds were analyzed *in silico* using molecular docking techniques. MAJ and MAWK contain flavonoids, saponins, and tannins. MAWK showed a higher potential with a pH of 4.7, total flavonoid content of 0.014 – 0.025 mg QE/g, total phenolic content of 224.270 – 413.973 mg GAE/g, vitamin C content of 56.320–166.613 mg/100 mL, antioxidant activity with an IC_{50} value of 6.48% (w/v), and α-glucosidase inhibition activity with an IC_{50} value of 23.82% (w/v). Chlorogenic acid, as a phytoconstituent of plants, is reported to exhibit both antioxidant and antidiabetic properties. This assertion is substantiated by molecular docking studies, which reveal Gibbs free energy values of -6.9 kcal/mol for its antioxidant activity and -6.7 kcal/mol for its antidiabetic activity. Consequently, MAWK has potential as a functional food that serves as an antioxidant and aids in the regulation of blood glucose levels in diabetes mellitus.

***Keywords*:** Antidiabetic, Antioxidant, Apple, Chlorogenic Acid, Water Kefir

Introduction

Diabetes mellitus (DM) is a chronic endocrine disorder characterised by disruption of carbohydrate, protein, and lipid metabolism due to either insufficient insulin production by pancreatic beta cells or increased insulin resistance in peripheral tissues.[1] Chronic hyperglycemia in DM leads to complications associated with tissue damage. Hyperglycemia increases the formation of reactive oxygen species (ROS) through various mechanisms, increases the expression of tumor necrosis factor-α (TNF-α), and induces oxidative stress. Oxidative stress results from an imbalance of free radicals and endogenous antioxidants. This condition exacerbates ROS formation in the mitochondria of patients with DM, causing oxidative damage, and complications such as systemic vascular disease (accelerated atherosclerosis), cardiovascular disease, diabetic retinopathy, and diabetic neuropathy.[2] In addition, decreased activity of antioxidant enzymes such as catalase (CAT), glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD) contributes to oxidative stress in DM patients.[3] Oxidative stress damage can be mitigated by antioxidant compounds, which may help to prevent DM complications.

*Corresponding author. E mail: sitaresmi.yuningtyas@gmail.com
Tel.: + 62-81310379033

Citation: Noviardi H, Yuningtyas S, Adzani A, Alawiyah RM. Antioxidant and Antidiabetic Activities of Manalagi Apple (*Malus sylvestris* Mill) Juice and Water Kefir: An *In Vitro* and *In Silico* Study. Trop J Nat Prod Res. 2025; 9(11): 5520 – 5528 https://doi.org/10.26538/tjnpr/v9i11.35

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Apples are rich in phytochemicals that act as antioxidants, antiproliferative agents, and cell-signaling agents. These properties help prevent several diseases, including cardiovascular disease, Alzheimer's disease, cancer, asthma, diabetes mellitus, weight loss, lung disease, bone disease, and gastrointestinal disorders.[4] Apples contain high levels of phenolic compounds, including polyphenols, condensed tannins (procyanidins), chlorogenic acid, epicatechin, and derivatives of p-coumaric acid, flavan-3-ols, caffeic acid, flavonols, and dihydrochalcones.[5] The antioxidant capacity of apple extracts using the FRAP (Ferric Reducing Antioxidant Power) method is 518.7±42.9 mg TE/g extract, while the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method yields a capacity of 56.7±10.2 mg TE/g extract or 53.6±9.7%.[6] *In vitro* studies have suggested that apples have antidiabetic potential because of their high polyphenol content. Polyphenols inhibit carbohydrate-hydrolyzing enzymes, thereby reducing the blood glucose levels. Inhibition of dipeptidyl peptidase-4 (DPP-4) enhances postprandial insulin secretion and mitigates the formation of advanced glycation end-products (AGEs), thereby diminishing the risk of diabetes-related complications. [7]

Kefir is a traditional drink made by fermenting kefir grains in either milk or water through a symbiotic process. Kefir grains contain symbiotic associations between bacteria and yeast, including *Kluyveromyces*, *Saccharomyces*, *Acetobacter* spp., *Lactobacillus kefiri*, *Lactobacillus casei*, *Lactobacillus acidophilus*, *Lactobacillus fermentum*, *Lactobacillus brevis*, *Lactobacillus helveticus*, *Leuconostoc mesenteroides*, *Kefiranofaciens lactis*, and *Kefiranofaciens parakefiri*. Fermentation of fruit juice with kefir grains produces bioactive compounds, including glutathione, lactic acid, acetic acid, tartaric acid, citric acid, succinic acid, butyric acid, oxalic acid, malic acid, and phenolic compounds.[8] Moreover, water kefir contains chlorogenic acid at concentrations of 0.03–0.05 mg/L[9], which have been shown to possess antioxidant activity.[10] Under optimal conditions, specifically with 7.56% kefir grains (w/v) and an incubation temperature of 24.82°C, apple water kefir exhibited the highest levels of antioxidant

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

activity and total phenolic content. In addition, apple water kefir showed a free radical scavenging capacity of 46.12%, linoleic acid autoxidation inhibition of 65.33%, and ascorbic acid autoxidation inhibition of 21%, highlighting its potential as a powerful antioxidant.[11] Supplementation with antioxidant-enriched fruit juice or kefir offers additional health benefits, particularly by enhancing the antioxidant activity and contributing to the development of functional foods. The development of antioxidant-rich apple-based functional beverages has the potential to offer an alternative therapeutic approach for metabolic syndromes. Currently, the potential of the Manalagi apple as a functional food with antioxidant and antidiabetic properties has not been extensively explored through *in vitro* and *in silico* studies. This study integrated *in vitro* and *in silico* methodologies to facilitate a comprehensive understanding of the antioxidant and antidiabetic properties of Manalagi apple juice and water kefir. *In vitro* studies have provided empirical data on their efficacy. Molecular docking is a method used to predict the optimal binding conformation between ligands and protein-binding sites.[12] This enhances the ability of this study to translate these findings into potential health benefits. The aim of this study is to assess the antioxidant and antidiabetic activities of Manalagi apple juice (MAJ) and Manalagi apple water kefir (MAWK) using *in vitro* and *in silico* methods.

Materials and Methods

Materials

The materials used in this study were *Malus sylvestris* Mill (Manalagi apple) obtained from Batu City (GPS coordinates of 7° 52′ 0″ S, 112° 31′ 0″ E), East Java, Indonesia. Kefir grain starter from Aracaki Indonesia SMEs (Small and Medium Enterprises) (GPS Coordinate 6°11'40.1"S 106°53'50.1"E) with voucher number 34562/P.1/31.75.02.1007.02.017.R.4/4/-1.828.2/e.r/2021.

Morphological and biochemical characteristics from kefir grain were used for identification of the microbes, the kefir grain consists of *Lactobacillus paracasei*, *Lactobacillus harbinensis*, *Lactobacillus hilgardii*, *Bifidobacterium psychraerophilum*, *Saccharomyces cerevisiae*, *Dekkera bruxellensis*, *Lactobacillus casei*, and *Bifidobacterium* spp. Analytical grade chemicals were used in this study. The software used was a Lenovo IdeaPad 320 laptop, with AMD A9-9420 Radeon R5 processor specifications, Microsoft® Windows® 10 Pro operating system, an NVIDIA GeForce Graphic Card, and Avogadro for energy minimization. Other software used in this study included BIOVIA Discovery Studio Visualizer 21.1.0.20298, AutoDockTools-1.5.7, AutoDock Vina, and PyMOL™ 2.5.2 Edu.

Preparation of Manalagi Apple Juice (MAJ)

MAJ was prepared at concentrations of 5, 10, 15, 20, and 25% (w/v) by weighing 50, 100, 150, 200, and 250 g, respectively. Each sample was mixed with 1000 mL of boiled water and blended. The resulting mixture was filtered and the filtrate was pasteurized at 75℃ for 15 s.

Preparation of Manalagi Apple Water Kefir (MAWK)

Pasteurized MAJ at concentrations of 5, 10, 15, 20 and 25% (w/v) was allowed to cool to 28℃ before adding 5% (w/v) kefir grain starter. Fermentation was performed at room temperature for 24 h.

Phytochemical Screening

Qualitative phytochemical screening of MAJ and MAWK was performed to detect alkaloids, flavonoids, phenols, saponins, tannins, terpenoids, and steroids.[13]

Determining of pH Value

The pH values of MAJ and MAWK were measured at room temperature using a calibrated pH meter (Laqua, Japan) with a measurement range of 0–14.

Total Flavonoid Analysis

Total flavonoid content was analyzed using the sodium acetate colorimetric method. A total of 1 mL of the sample was mixed with 0.1 mL of 10% $AlCl_3$ (Merck, Germany), 0.1 mL of 1 M sodium acetate (Merck, Germany), 2.8 mL of distilled water, and 6 mL of methanol (Sigma-Aldrich, USA). The solution was homogenized and incubated for 30 min. The absorbance of the mixture was measured using a UV-Vis spectrophotometer (Shimadzu Series UV mini-1240, Japan) at a wavelength of 435 nm.[14] Absorbance values were plotted against a quercetin standard curve. Total flavonoid content was expressed as milligrams of quercetin equivalents per gram of sample (mg QE/g).

Total Phenolic Analysis

The total polyphenol content was determined using the Folin-Ciocalteu method. A total of 0.5 mL of the sample was pipetted into a test tube and mixed with 2 mL of ethanol (Merck, Germany) and 0.2 mL of Folin-Ciocalteu reagent (Merck, Germany). The mixture was allowed to stand for 4–8 min. Then 1.5 mL of 7% sodium carbonate (Sigma-Aldrich, USA) and 0.8 mL of distilled water were added until the solution turned blue. The mixture was then incubated in the dark for 2 h. The absorbance of the solution was measured using a UV-Vis spectrophotometer (Shimadzu Series UV mini-1240, Japan) at a wavelength of 730 nm.[14] Absorbance values were plotted against a gallic acid standard curve. Total phenolic content was expressed as milligrams of gallic acid equivalent per gram of sample (mg GAE/g).

Determining Vitamin C Content

Five grams of the sample were placed in a volumetric flask and diluted to a final volume of 100 mL with distilled water. Subsequently, 25 mL of each sample was transferred to an Erlenmeyer flask. Then, 2 mL of 1% starch indicator solution (Merck, Germany) was added, and the solution was titrated with 0.01 N iodine solution (Merck, Germany) until a blue color appeared.[15] The vitamin C content was calculated using the following formula (Equation 1).

$$\text{Vitamin C (mg/100 mL)} = \frac{A(mL) \times 0{,}88 \times 100 \times FP}{W\ (gram)} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ (1)$$

Description

A = Volume of iodine used for titration (mL)

0.88 = 0.88 mg of ascorbic acid equivalent to 1 mL of 0.01 N iodine solution

FP = Dilution factor

W = Sample weight (grams)

Antioxidant Activity

The antioxidant activity was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method (Sigma-Aldrich, USA). A total of 2 mL of the sample was mixed with 2 mL of 0.1 mM DPPH solution. The mixture was incubated at room temperature for 30 min, and the absorbance was measured at 517 nm using a UV-Vis spectrophotometer (Shimadzu Series UV mini-1240, Japan).[16] A blank solution containing DPPH (0.1 mM) was used as a control. The antioxidant activity of the samples was calculated as a percentage of DPPH radical scavenging using the following formula (Equation 2).

$$\%\ \text{inhibition} = \frac{\text{blank absorbance-sample absorbance}}{\text{blank absorbance}} \times 100\% \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ (2)$$

The IC_{50} value was determined by plotting the percentage inhibition against the sample concentration.

Inhibition of α-Glucosidase

An *in vitro* antidiabetic assay was conducted to evaluate α-glucosidase enzyme activity. A reaction mixture was prepared by combining 50 µL of 0.1 M phosphate buffer (pH 7.0) (Oxoid, USA), 25 µL of 0.5 mM 4-nitrophenyl α-D-glucopyranoside (Sigma-Aldrich, USA), 10 µL of the sample, and 25 µL of α-glucosidase enzyme solution (Sigma-Aldrich, USA). The reaction mixture was then incubated at 37℃ for 30 min. The reaction was terminated by adding 100 µL of 0.2 M sodium carbonate solution (Merck, Germany). Absorbance was measured at 410 nm

using a UV-Vis spectrophotometer (BioTek Epoch Microplate Spectrophotometer, Agilent, USA).[17] The percentage of enzyme inhibition was calculated using the following formula (Equation 2). The IC_{50} value was obtained by plotting the percentage inhibition against the sample concentration.

Molecular Docking

Molecular docking analysis was conducted in several steps: protein and ligand preparation, validation of the docking method, and molecular docking simulation.[18] The protein structure files were obtained from the RCSB PDB website. Antioxidant proteins (PDB ID: 1DNU) and antidiabetic proteins (PDB ID: 4X9Y) were downloaded in PDB format. Water molecules were removed, and energy minimization was performed on the proteins. Processed protein files were saved .pdbqt format. The ligands used in this study were selected from the literature.[8,19] The ligand structure files were obtained from the PubChem database. The PyMOL software was used to convert the ligand files .sdf to .pdb format. Further optimization of the ligands was performed using AutoDock Tools 1.5.7, and the optimized ligands were saved in .pdbqt format. Docking validation was performed using reference ligands, with validation criteria based on a root-mean-square deviation (RMSD) of <2 Å. Molecular docking simulations were performed at the active site of the protein using AutoDock Vina software. The active site of the protein was covered by a grid box defined by the dimensions x, y, and z with a spacing of 1.0. The grid box coordinates used were 1DNU (26.044; 0.098; 15.067) and 4X9Y (0.250; -10.639; 29.278). The protein-ligand interactions were visualized using Discovery Studio. The ADMET predictions for the ligands were performed using an online tool available at ADMETLab (http://www.swissadme.ch/index.php).

Data Analysis

Experimental data were analysed using the Kruskal-Wallis test, followed by the Dunn-Bonferroni post-hoc test at a significance level of 0.05. Statistical analysis was conducted using SPSS software version 24 (IBM Analytics, USA) with a sample size of n = 3.

Results and Discussion

Characteristics and metabolite content of MAJ and MAWK

During the fermentation of MAWK, apple juice served as a substrate for the growth of microorganisms, including *Lactobacillus casei*, *Lactobacillus harbinensis*, *Lactobacillus paracasei*, *Lactobacillus hilgardii*, *Bifidobacterium psychraerophilum*, *Bifidobacterium* spp, *Saccharomyces cerevisiae*, and *Dekkera bruxellensis*. Manalagi apples contain fiber (3.2%), pectin (1.3%), total phenolic (109.01 mg GAE), flavonoid (76.19 mg QE), and vitamin C (12.08 mg).[20] These nutrients support microbial growth during the fermentation of kefir grains. MAJ has a distinctive apple flavor, while fermented water kefir has a sour and alcoholic flavor. The pH of MAJ was higher than that of the resulting kefir. The average pH of MAJ was 6.7, whereas that of MAWK decreased to 4.7. The pH of water kefir ranges from 4.2-4.7.[21] Water kefir contains various metabolites, including sugars and fruit-derived components, acetic acid, lactic acid, ethanol, carbon dioxide, mannitol, vitamins (especially B-complex vitamins), amino acids (such as arginine), glycerol, esters, and other organic acids.[22,23] In addition, polysaccharides, such as glucans (glucose polymers) and levans (fructose polymers), are synthesized by microorganisms in kefir grains.[24] Under favorable conditions, microorganisms in water kefir synthesize glucans, leading to an increase in the kefir grain biomass. During the first 24 h of fermentation, the concentration of sucrose decreased by up to 98%[25] due to fermentation to ethanol, primarily by *Saccharomyces cerevisiae*, which contains the invertase enzyme responsible for sucrose hydrolysis. Lactic acid and acetic acid bacteria present in kefir grains also metabolize sucrose, leading to increased fructose and glucose levels. The presence of yeast in water kefir enhances its sensory quality, contributing to a refreshing taste and distinctive sharp flavor.[26] Fermentation of water kefir using 17% kefir grains and 7.1% sugar for 72 h resulted in the production of 2% ethanol, 0.5% lactic acid, 0.1% acetic acid, 0.2% glycerol, and 0.08% mannitol.[22] Therefore, the primary products of water kefir fermentation include lactic acid, acetic acid, and ethanol.

Analysis of the secondary metabolite content revealed that both MAJ and MAWK contained flavonoids, tannins, and saponins (Table 1). Apples contain high levels of flavonoids, anthocyanins, phenolic acids, and dihydrochalcones, all of which contribute to their antioxidant properties. The antioxidant capacity of apples results from the combined effects of vitamins A and E, copper (Cu), anthocyanins (such as cyanidin and malvidin), and several phytochemical compounds, including quercetin, rutin, D-glutaric acid, epicatechin, and myricetin. Among these, myricetin and quercetin play the most important roles as antioxidants. Quercetin is predominantly found in apple peels.[27] Quercetin has the potential to enhance antioxidant capacity by modulating glutathione (GSH) levels. This is attributable to the production of free radicals during metabolic processes, which can lead to genetic mutations and damage to the cell membranes. Oxidative stress is implicated in the pathogenesis of various diseases, including cardiovascular disease, liver disease, and diabetes, and also contributes to the acceleration of the aging process.[28] The presence of flavonoids, tannins, and saponins in both MAJ and MAWK indicates their potential antioxidant activity and ability to inhibit α-glucosidase enzymes.

Table 1: Phytocemical component of Manalagi Apple Juice (MAJ) and Manalagi Apple Water Kefir (MAWK)

No.	Phytocemical Component	MAJ	MAWK
	Alkaloid	-	-
	Flavonoid	+	+
	Tannin	+	+
	Phenol	-	-
	Saponin	+	+
	Steroid	-	-
	Terpenoid	-	-

Description: (+): present; (-): absent

Total flavonoid, total phenolic, and vitamin C content in MAJ and MAWK

The total flavonoid content in MAWK was higher than that in MAJ, and the total flavonoid content in MAJ samples (5–25% w/v) ranged from 0.013 to 0.021 mg QE/g. In MAWK (5–25% w/v), it ranged from 0.014 to 0.025 mg QE/g (Figure 1). Apples are rich in phenolic compounds and have high antioxidant potential because of their polyphenols, condensed tannins (procyanidins), chlorogenic acid, and epicatechin. They also contain caffeic acid derivatives, p-coumaric acid derivatives, flavan-3-ols, flavonols, and dihydrochalcones.[29] Specifically, Manalagi apples contain a total flavonoid content of 76.19 mg QE/100 g.[20] Fermentation of MAJ by kefir grains increased total flavonoid content.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

The difference in total flavonoid content between MAJ and MAWK was statistically significant (p<0.05) at concentrations of 15% and 20%, indicating that this increase can be attributed to the enzymatic activity of lactic acid bacteria present in kefir grains. These bacteria produce enzymes capable of hydrolyzing carbohydrates, degrading complex phenolic compounds, and releasing them into the substrate. This process increases the availability of phenolic groups for flavonoid formation.[30] A similar phenomenon has been observed in goji berry juice fermented with Tibetan kefir grains, which exhibited a higher total flavonoid content due to enzyme production by the bacteria in the kefir grains. Fermentation of goji berry juice results in the formation of 16 flavonoid compounds, eight phenolic acid compounds, phenolic acid derivatives, and coumarins. In addition, enzymes such as amylase and xylanase contribute to the release of flavonoid compounds, including mangiferin, rutin, hyperoside, isoquercetin, and quercetin, during fermentation.[31]

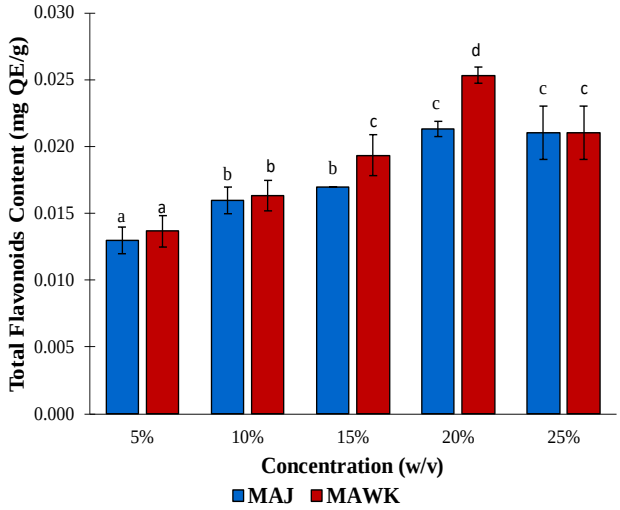

Figure 1: Total flavonoid content of Manalagi Apple Juice (MAJ) and Manalagi Apple Water Kefir (MAWK). Note: different letter notations indicate significant differences (p<0.05) based on the Kruskal-Wallis test followed by the Dunn-Bonferroni test.

These results indicated that fermentation can increase the bioavailability of flavonoid compounds, thereby improving the antioxidant potential of the product. The total phenolic content in MAJ ranged from 304.213 to 370.067 mg GAE/g. In MAWK, it ranged from 224.270 to 413.973 mg GAE/g (Figure 2). At concentrations ranging from 5% to 20% (b/v), the total phenolic content in MAWK was significantly lower (p<0.05) compared to that in MAJ. However, at 25% (w/v) MAWK had a higher total phenolic content than MAJ. The total phenolic content in Manalagi apples is approximately 109.01 mg GAE/100 g[20]. Phenolic compounds, mainly phenolic acids, can decrease during fermentation because of processes such as co-pigmentation, oxidation, condensation, and adhesion to yeast cell walls.[32] Several enzymes produced by lactic acid bacteria in kefir grains, such as β-glucosidase, phenolic acid reductase, phenolic acid decarboxylase, and tannase, play roles in the metabolism of phenolic compounds. The reduction of total phenolic content during fermentation is influenced by factors such as substrate composition, microbial starter, and fermentation conditions.[33]

The vitamin C content in MAWK was significantly higher than that in the juice (Figure 3). The vitamin C content in MAJ ranged from 37.547 to 70.400 mg/100 mL, whereas that in MAWK ranged from 56.320 to 166.613 mg/100 mL. Manalagi apples contain approximately 12.08 mg of vitamin C per 100 g.[20] Increasing the concentration of Manalagi apples in the juice led to elevated vitamin C content. The significantly higher vitamin C content in MAWK (p<0.05) than MAJ is likely attributable to microbial activity during fermentation. During MAWK fermentation, *Saccharomyces cerevisiae* converts glucose to arabinose. This microorganism produces arabinose dehydrogenase, which catalyses the conversion of arabinose to arabinono-1,4-lactone. Subsequently, arabinono-1,4-lactone oxidase oxidizes it to erythroascorbic acid, an analog of ascorbic acid (vitamin C).[34] This pathway contributes to the observed increase in vitamin C content in MAWK.

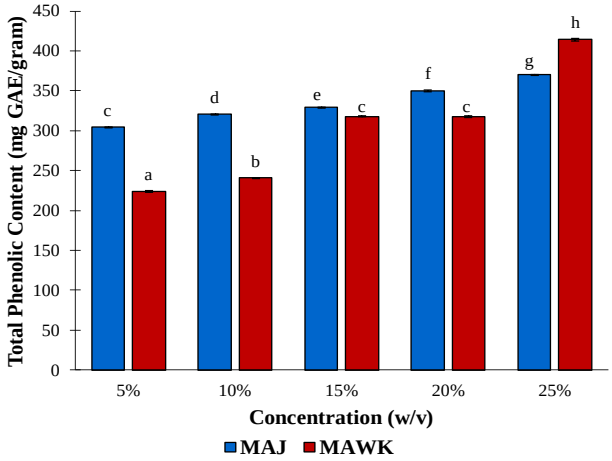

Figure 2: Total phenolic content of Manalagi Apple Juice (MAJ) and Manalagi Apple Water Kefir (MAWK). Note: different letter notations indicate significant differences (p< 0.05) based on the Kruskal-Wallis test followed by the Dunn-Bonferroni test.

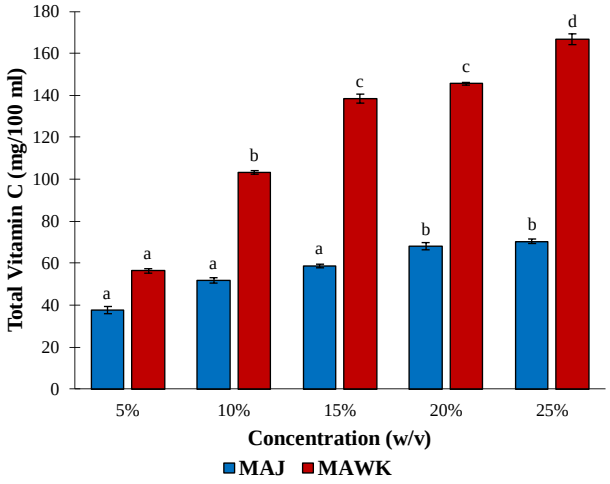

Figure 3: Vitamin C content of Manalagi Apple Juice (MAJ) and Manalagi Apple Water Kefir (MAWK). Note: different letter notations indicate significant differences (p< 0.05) based on the Kruskal-Wallis test followed by the Dunn-Bonferroni test.

Antioxidant activity of MAJ and MAWK

Both MAJ and MAWK demonstrated effective scavenging of DPPH free radicals at increasing concentrations, resulting in a statistically significant increase in radical scavenging activity (p<0.05; Figure 4). Apples are rich in flavonoids, anthocyanins, phenolic acids, dihydrochalcones, myricetin, and quercetin, which are all natural antioxidants. Quercetin, which is predominantly found in apple peels, is a major contributor to antioxidant activity.[27] The DPPH radical scavenging activity of MAJ ranged from 47.37% to 61.30%, whereas that of MAWK ranged from 49.30% to 68.27%. The IC_{50} values for MAJ was 10.20% (w/v), whereas the IC_{50} value for MAWK was 6.48% (w/v), respectively. The antioxidant activity of MAWK was significantly higher (p<0.05) than that of MAJ. The higher antioxidant

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

potential of MAWK can be attributed to its higher total flavonoid and vitamin C content than those of MAJ.

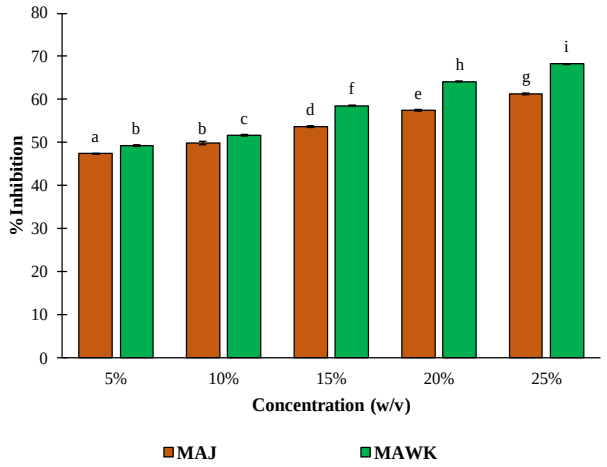

Figure 4: Antioxidant activity from Manalagi Apple Juice (MAJ) and Manalagi Apple Water Kefir (MAWK). Note: different letter notations indicate significant differences (p< 0.05) based on the Kruskal-Wallis test followed by the Dunn-Bonferroni test.

The antioxidant activity observed in both samples was correlated with the total flavonoid, total phenolic, and vitamin C content. The correlation coefficients (R^2) observed at a 95% confidence level for total flavonoids content were 0.8992 for MAJ and 0.9653 for MAWK, for total phenolic content were 0.9848 for MAJ and 0.9106 for MAWK, and for vitamin C content were 0.9138 for MAJ and 0.8759 for MAWK. These results suggest that flavonoids, phenolic compounds, and vitamin C significantly contribute to the DPPH radical-scavenging capacity of both MAJ and MAWK. The antioxidant properties of polyphenols and flavonoids are attributed to their ability to act as reducing agents, hydrogen or electron donors, and singlet oxygen quenchers.[10,35] Vitamin C enhances antioxidant potential by interrupting the oxidative chain reactions of free radicals and acting as a radical scavenger, preventing further damage to cellular components.[36]

MAWK has a higher antioxidant potential than MAJ. Several antioxidant molecules in MAWK are produced by lactic acid bacteria (LAB), including glutathione, organic acids, and phenolic compounds.[10] LAB are a group of bacteria with antioxidant potential that can tolerate oxidative stress. These bacteria can scavenge free radicals, chelate metal ions, produce antioxidant enzymes, and modulate the gut microbiota.[37] Microorganisms of the *Lactobacillus* genus found in kefir grains can produce cysteine, which donates hydrogen atoms to DPPH free radicals, thereby neutralizing their activity. In addition, the synergistic effect of phenolic compounds further enhanced the antioxidant activity of water kefir by improving free radical scavenging activity.[11] Glutathione produced by kefir grains acts as a powerful antioxidant by scavenging free radicals and preventing their formation.[38] The organic acids and enzymes produced by microorganisms in kefir grains influence antioxidant activity by releasing bound phenolic compounds and enhancing their bioavailability. Enzymes, such as β-glucosidase, hydrolyze complex phenolic compounds into simpler forms, thereby increasing their antioxidant potential. In addition, enzymatic activity can break down fruit fibers, releasing more fermentable sugars, which in turn lowers the acidity of the water kefir and optimizes microbial growth. Organic acids produced during fermentation, such as citric acid, malic acid, and vitamin C, contribute to the antioxidant capacity of water kefir.[10,35]

Inhibition of α-glucosidase from MAJ and MAWK

MAJ and MAWK inhibit α-glucosidase activity. Increasing the concentration of both products significantly (p<0.05) increased their α-glucosidase-inhibitory effects (Figure 5). The inhibition activity of α-glucosidase by MAJ ranges from 8.84% to 56.81%, while MAWK from 6.35% to 53.82%, respectively. The IC_{50} values for MAJ and MAWK were 20.30% and 23.82% (w/v), respectively. This indicates that the inhibitory potential of MAJ was higher than that of MAWK.

The inhibitory potential of α-glucosidase is affected by its bioactive components. The apple extract contained high concentrations of total phenolics and various phenolic compounds, including procyanidin B dimer, chlorogenic acid, epicatechin, 4-p-coumaroyl quinic acid, and procyanidin B trimer. The presence of condensed tannins, such as procyanidin B dimer and procyanidin B trimer, contributes to the inhibition of α-glucosidase activity.[39] Specifically, procyanidin B3 dimer had an IC_{50} value of 139 µg/mL for inhibiting α-glucosidase.[40] Flavonoids and tannins, along with hydroxyl groups in their chemical structures, plays a critical role in α-glucosidase inhibition.[41] The interaction between tannins and α-glucosidase is attributed to hydrogen bonding and hydrophobic interactions, which lead to conformational change in the catalytic site of the enzyme, thereby reducing its affinity for the substrate.[42] In addition, the inhibitory activity may be influenced by flavan-3-ol monomers and oligomers, where an increase in molecular weight enhances the number of hydroxyl groups responsible for enzyme binding.[43] Quercetin and phloretin are also known to contribute to α-glucosidase inhibition. Quercetin, predominantly found in apple peel, has been reported to inhibit α-glucosidase activity up to 100 times more effectively than acarbose.[44] The hydroxyl group at the C-3 position in the ring structure of quercetin is particularly important for enzyme inhibition mechanism.[45]

Other compounds found in apples, such as glucosyl-O-pentosyl-O-glucoside, phloretin-2-O-xylosyl-glucoside, and phloretin-2-O-glucoside (phlorizin), play a role in inhibit the sodium-dependent glucose transporter in the intestinal lumen. Among these glucoside compounds, phloretin-2'-O-xylosylglucoside has been shown to reduce postprandial glucose levels.[46,47] This effect is highly beneficial for individuals with diabetes mellitus, as it slows glucose absorption in the digestive tract, thereby reducing the postprandial glycemic index and helping to regulate fluctuating blood glucose levels. An increase in α-glucosidase inhibitory activity has been observed in soy milk kefir fermented with kefir grains supplemented with Rhodiola extract.[46] Moreover, a study showed that soy milk kefir has the ability to inhibit α-amylase activity with an IC50 value of 52.71 µg/mL.[47] Several compounds found in apples have been identified as α-glucosidase inhibitors. However, fermentation by kefir grain microbes may result in interactions with different effects on the α-glucosidase enzyme involved in glucose digestion and absorption. Thus, MAJ and MAWK may serve as potential options for the prevention and management of diabetes mellitus. In diabetic patients, elevated levels of free radicals can cause damage to pancreatic and liver cells. The inhibition of α-glucosidase is critical for reducing postprandial hyperglycemia.

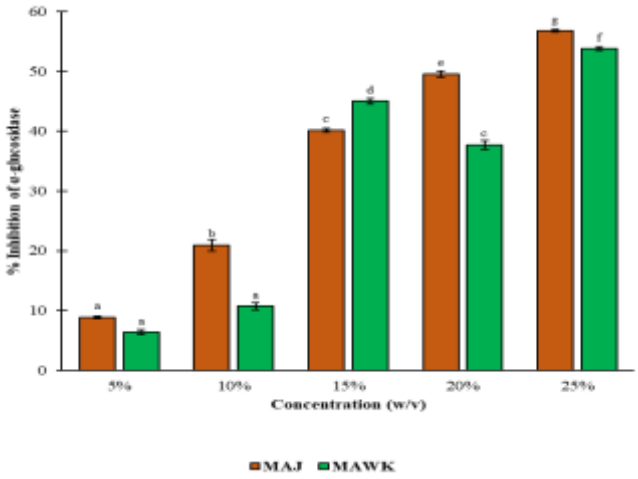

Figure 5: The inhibition activity of α-glucosidase from Manalagi Apple Juice (MAJ) and Manalagi Apple Water Kefir (MAWK). Note: different letter notations indicate significant differences (p< 0.05) based on the Kruskal-Wallis test followed by the Dunn-Bonferroni test.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Molecular docking

Validation of the protein-ligand molecular docking method yielded a root mean square deviation (RMSD) value of 1.256 Å. An RMSD value of less than 2 Å indicated that the molecular docking method used in this study was valid.[48] Table 2 shows the calculated binding free energy (ΔG) and inhibition constant (Ki) obtained from flexible ligand docking simulations. A negative and lower ΔG value indicates stronger binding affinity between the enzyme and ligand. The relationship between ΔG and Ki suggests that an increase in the negative ΔG value corresponds to stronger enzyme-ligand complex formation.[49] Chlorogenic acid (CGA), identified in both MAJ and MAWK, had low binding energy values. CGA is a polyphenol with several critical therapeutic effects, including antioxidant, antibacterial, hepatoprotective, cardioprotective, and anti-inflammatory activities.[9] The antioxidant properties of CGA, facilitated by the Nrf2-heme oxygenase-1 signaling pathway, were found to increase the levels of antioxidant enzymes, including superoxide dismutase, catalase, glutathione-S-transferases, glutathione peroxidase, and glutathione reductase, as well as increase the glutathione content.[50]

Table 2: Gibbs Free Energy (ΔG) and Inhibition Constant (Ki) values between ligands and proteins

No	Name	Energy (kcal/mol)		Ki (μM)	
		Antioxidant	Antidiabetic	Antioxidant	Antidiabetic
MAWK (Manalagi apple water kefir)					
1	Acetic acid	-2.5	-2.9	0.990	0.989
2	Lactic acid	-3.2	-4.1	0.988	0.984
3	Citric acid	-4.7	-5.5	0.982	0.979
4	Tartaric acid	-3.9	-5.2	0.985	0.980
5	Butyric acid	-3.2	-3.5	0.988	0.986
6	Succinic acid	-3.9	-4.2	0.985	0.984
7	Malic acid	-3.8	-4.6	0.985	0.982
8	Oxalic acid	-3.2	-3.9	0.988	0.985
9	Propionic acid	-2.9	-3.4	0.989	0.987
10	chlorogenic acid	-6.9	-6.7	0.974	0.974
MAJ (Manalagi Apple Juice)					
1	Chlorogenic acid	-6.9	-6.7	0.974	0.974
2	p-hydroxybenzoic acid	-7.5	-7.1	0.971	0.973
3	Procyanidin B2	-5	-5.2	0.981	0.980
4	Epicatechin	-7.8	-6.9	0.970	0.974
5	Caffeic acid	-7.7	-7.8	0.971	0.970
6	Rutin	-5.3	-5.8	0.980	0.978
7	Hyperoside	-7.4	-6.3	0.972	0.976
8	Protocatechuate	-2.6	-7.1	0.990	0.973
9	Phloridzin	-4.6	-4.8	0.982	0.982
Ligand Control					
1	Acarbose	-7.3	-5.8	0.972	0.978
2	Vitamin C	-7.2	-5.8	0.972	0.978

The two-dimensional interaction between CGA and the protein is shown in Figure 6. CGA exhibits both covalent and non-covalent interactions. Covalent interactions occured in the form of hydrogen bonds. Hydrogen bonds are defined as the intermolecular or intramolecular forces that occur between atoms with high electronegativity and a hydrogen atom covalently bonded to an electronegative atom. Non-covalent or non-binding interactions between a protein and ligand can increase the affinity of the ligand for the protein. The non-covalent interactions that occur with chlorogenic acid include electrostatic and van der Waals interactions. These interactions can cause changes in the potential energy.[49] The ligand-protein interactions are expected to disrupt protein stability and functionality. The ADMET analysis of CGA is shown in Table 3.

(a) (b)

Figure 6: Two-dimensional interactions between chlorogenic acid and proteins 1DNU (a) and 4X9Y (b).

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 3: ADMET analysis of chlorogenic acid.

Model	Result	Probability
Absorption		
Blood-Brain Barrier	BBB^+	0.5663
Human Intestinal Absorption	HIA^+	0.7433
Caco-2 Permeability	$CaCO_2^-$	0.8005
P-glycoprotein Substrate	Substrate	0.6363
P-glycoprotein Inhibitor	Non-inhibitor	0.8766
	Non-inhibitor	0.9451
Renal Organic Cation Transporter	Non-inhibitor	0.9206
Distribution		
Subcellular localization	Mitochondria	0.6770
Metabolism		
CYP450 2C9 Substrate	Non-substrate	0.7901
CYP450 2D6 Substrate	Non-substrate	0.8976
CYP450 3A4 Substrate	Non-substrate	0.5493
CYP450 1A2 Inhibitor	Non-inhibitor	0.9045
CYP450 2C9 Inhibitor	Non-inhibitor	0.9071
CYP450 2D6 Inhibitor	Non-inhibitor	0.9388
CYP450 2C19 Inhibitor	Non-inhibitor	0.9069
CYP450 3A4 Inhibitor	Non-inhibitor	0.8744
CYP Inhibitory Promiscuity	Low CYP Inhibitory Promiscuity	0.9686
Excretion Toxicity		
Human Ether-a-go-go-Related Gene Inhibition	Weak inhibitor	0.9862
	Non-inhibitor	0.8460
AMES Toxicity	Non AMES toxic	0.9132
Carcinogens	Non-carcinogens	0.9341
Fish Toxicity	High FHMT	0.9959
Tetrahymena Pyriformis Toxicity	High TPT	0.9971
Honey Bee Toxicity	High HBT	0.6825
Biodegradation	Not ready biodegradable	0.8204
Acute Oral Toxicity	III	0.7775
Carcinogenicity (Three-class)	Non-required	0.6128

Conclusion

MAJ and MAWK exhibit antioxidant and antidiabetic effects *in vitro*. MAWK showed the highest levels of total flavonoids and vitamin C, as well as the highest antioxidant activity, with an IC_{50} value of 6.48% (w/v). However, the highest *in vitro* antidiabetic activity was found in MAJ, with an IC_{50} = 20.30% (w/v). Several compounds in apples act as antioxidants and α-glucosidase inhibitors. The differences in the antioxidant and antidiabetic activities of MAJ and MAWK may be attributed to interactions among compounds during microbial fermentation in kefir grains, resulting in distinct effects on α-glucosidase activity. Overall, these results suggest that MAWK has the potential to serve as an antioxidant agent for managing blood glucose levels and oxidative stress associated with type 2 diabetes mellitus. Chlorogenic acid was predicted to act as an antioxidant and antidiabetic agent *in silico*. These findings demonstrate the potential of MAWK as a functional food with antioxidant and anti-diabetic properties, indicating its applicability in dietary interventions for the management of diabetes mellitus. Further research is recommended to evaluate the long-term effects of MAWK consumption on glycemic control and oxidative stress markers in individuals with diabetes.

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.

Acknowledgments

This research was funded by a grant from Sekolah Tinggi Teknologi Industri dan Farmasi Bogor, Indonesia, in 2024, and by The Indonesian Ministry of Higher Education, Science, and Technology through the Regular Fundamental Research Grant scheme in 2025, under the grant number 0419/C3/DT.05.00/2025.

References

1. Ogurtsova K, Guariguata L, Barengo NC, Ruiz PLD, Sacre JW, Karuranga S, Sun H, Boyko EJ, Magliano DJ . IDF Diabetes Atlas: Global estimates of undiagnosed diabetes in adults for 2021. Diabetes Res Clin Pract. 2022; 183(109118): 1-8. doi:doi.org/10.1016/j.diabres.2021.109118
2. Kumar R, Saha P, Kumar Y, Sahana S, Dubey A, Praksh O. A Review on diabetes mellitus : type1 & type2. World J Pharm Pharm Sci. 2020; 9(10): 838-850. doi:10.20959/wjpps202010-17336
3. Gusti AMT, Qusti SY, Alshammari EM, Toraih EA, Fawzy MS. Antioxidants-related superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutathione-s-transferase (GST), and nitric oxide synthase (NOS) gene variants analysis in an obese population: A preliminary case-control study. Antioxidants. 2021; 10(595): 1-16. doi:10.3390/antiox10040595
4. Oyenihi AB, Belay ZA, Mditshwa A, Caleb OJ. An apple a day keeps the doctor away: The potentials of apple bioactive constituents for chronic disease prevention. J Food Sci. 2022; 87(6): 2291-2309. doi:10.1111/1750-3841.16155
5. Feng S, Yi J, Li X, Wu X, Zhao Y, Ma Y, Bi J. Systematic review of phenolic compounds in apple fruits: compositions, distribution, absorption, metabolism, and processing stability. J Agric Food Chem. 2021; 69(1): 7-27. doi:10.1021/acs.jafc.0c05481
6. Mustafa B, Nebija D, Hajdari A. Evaluation of essential oil composition, total phenolics, total flavonoids and antioxidant activity of *Malus sylvestris* (l.) Mill. fruits. Research. 2018; 23: 71-85.
7. Yu CHJ, Migicovsky Z, Song J, Rupasinghe HPV. (Poly)phenols of apples contribute to *in vitro* antidiabetic properties: assessment of Canada's Apple Biodiversity Collection. Plants People Planet. 2022; 5(2): 225-240. doi:10.1002/ppp3.10315
8. Viana RO, Magalhães-Guedes KT, Braga RA, Dias DR, Schwan RF. Fermentation process for production of apple-based kefir vinegar: microbiological, chemical and sensory analysis. Brazilian J Microbiol. 2017; 48(3): 592-601. doi:10.1016/j.bjm.2016.11.006
9. Li Q, Chen F, Luo Z, Wang M, Han X, Zhu J, Li JE, Liu J, Li K, Gong P. Determination of nine bioactive phenolic components usually found in apple juice by simultaneous UPLC-MS/MS. Food Sci Nutr. 2023; 11(7): 4093-4099. doi:10.1002/fsn3.3399
10. Cai Y, Sounderrajan A, Serventi L. Water kefir: a review of its microbiological profile, antioxidant potential and sensory quality. Acta Sci Nutr Heal. 2020; 4(6): 10-17. doi:10.31080/asnh.2020.04.0706
11. Sabokbar N, Khodaiyan F, Moosavi-Nasab M. Optimization of processing conditions to improve antioxidant activities of apple juice and whey based novel beverage fermented by kefir grains. J Food Sci Technol. 2014; 52(6): 3422-3432. doi:10.1007/s13197-014-1397-4
12. Trott O, Olson AJ. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem. 2010; 31(2): 455-461. doi:10.1002/jcc.21334
13. Balamurugan V, Sheerin FMA, Velurajan S. A Guide to phytochemical analysis. Int J Adv Res Innov. 2019; 5(1): 236-245. www.ijariie.com
14. Constantin EA, Popa-Tudor I, Matei F, Constantinescu-Aruxandei D, Oancea F. Evaluation of polyphenol content and antioxidant activity of standard water kefir. Chem Proceesings. 2023; 13(1): 1-7. doi:10.3390/chemproc2023013007
15. Satpathy L, Pradhan N, Dash D, Baral PP, Parida SP. Quantitative determination of vitamin C concentration of common edible food sources by redox titration using iodine solution. Lett Appl NanoBioScience. 2021; 10(3): 2361-2369. doi:10.33263/LIANBS103.23612369
16. Yuningtyas S, Roswiem AP, Azahra D, Alfarabi M. Antioxidant activity and characterization of arrowroot (*Maranta arundinacea*) tuber yogurt. Biodiversitas. 2023; 24(5): 2850-2854. doi:10.13057/biodiv/d240539
17. Yuningtyas S, Alfarabi M, Lestari Y, Noviardi H. The *In Vitro* and *In Silico* Study of α-glucosidase Inhibition by Kombucha Derived from *Syzygium polyanthum* (Wight) Walp. Leaves. Hayati J Biosci. 2024; 31(5): 951-963. doi:10.4308/hjb.31.5.951-963
18. Noviardi H, Iswantini D, Mulijani S, Wahyudi ST, Khusniati T, Sulistiani. Anti-inflammatory activity of the metabolite extract of *Lactobacillus plantarum* Su-ls29 on lipopolysaccharide-induced RAW 264.7 macrophage cells. J Appl Pharm Sci. 2024; 14(1): 148-158. doi:10.7324/JAPS.2024.141691
19. Tschida A, Stadlbauer V, Schwarzinger B, Maler M, Pitsch J, Stubl F, Muller U, Lanzerstorfer P, Himmelsbach M, Wruss J, Klanert G, Schurr J, Wurm L, Rosner F, Hoglinger O, Winkler S, Weghuber J. Nutrients, bioactive compounds, and minerals in the juices of 16 varieties of apple (*Malus domestica*) harvested in Austria: A four-year study investigating putative correlations with weather conditions during ripening. Food Chem. 2021; 338(128065): 1-9. doi:10.1016/j.foodchem.2020.128065
20. Harsono RDA, Dewi YLR, Lestari A. The effect of Manalagi and Fuji apple juice on triglyceride level in elderly. J Int Conf Proc. 2021; 4(1): 279-289. doi:10.32535/jicp.v4i1.1155
21. Cufaoglu G, Erdinc AN. An alternative source of probiotics: water kefir. Food Front. 2023; 4(1): 21-31. doi:10.1002/fft2.200
22. Laureys D, De Vuyst L. Microbial species diversity, community dynamics, and metabolite kinetics of water Kefir fermentation. Appl Environ Microbiol. 2014; 80(8): 2564-2572. doi:10.1128/AEM.03978-13
23. Laureys D, De Vuyst L. The water kefir grain inoculum determines the characteristics of the resulting water kefir fermentation process. J Appl Microbiol. 2017; 122(3): 719-732. doi:10.1111/jam.13370
24. Fels L, Jakob F, Vogel RF, Wefers D. Structural characterization of the exopolysaccharides from water kefir. Carbohydr Polym. 2018; 189: 296-303. doi:10.1016/j.carbpol.2018.02.037
25. Martínez-Torres A, Gutiérrez-Ambrocio S, Heredia-del-Orbe P, Villa-Tanaca L, Hernández-Rodríguez C. Inferring the role of microorganisms in water kefir fermentations. Int J Food Sci Technol. 2017; 52(2): 559-571. doi:10.1111/ijfs.13312
26. Magalhães KT, de Pereira GVM, Dias DR, Schwan RF. Microbial communities and chemical changes during fermentation of sugary Brazilian kefir. World J Microbiol Biotechnol. 2010; 26(7): 1241-1250. doi:10.1007/s11274-009-0294-x
27. Chen CS, Zhang D, Wang YQ, Li PM, Ma FW. Effects of fruit bagging on the contents of phenolic compounds in the peel and flesh of Golden Delicious, Red Delicious, and Royal Gala apples. *Sci Hortic*. 2012; 142: 68-73. doi:10.1016/j.scienta.2012.05.001
28. Qi W, Qi W, Xiong D, Long M. Quercetin : its antioxidant mechanism , antibacterial properties. Molecules. 2022; 27(6545): 1-16.
29. Li N, Shi J, Wang K. Profile and antioxidant activity of phenolic extracts from 10 crabapples (*Malus* wild species). J Agric Food Chem. 2014; 62(3): 574-581. doi:10.1021/jf404542d
30. Hardiansyah A, Ilmi IMB, Marjan AQ, Octaria YC, Saputri KA, Khodijah, Darmuin. Effect of kapok flower honey (*Ceiba pentandra*) addition on antioxidant activity, total flavonoid, total phenolic, and lactose levels in goat's milk

efir. Malaysian J Med Heal Sci. 2024; 20(7): 85-90. doi:10.47836/mjmhs.20.s9.14
31. Du G, Qing Y, Wang H, Wang N, Yue T, Yuan Y. Effects of Tibetan kefir grain fermentation on the physicochemical properties, phenolics, enzyme activity, and antioxidant activity of *Lycium barbarum* (Goji berry) juice. Food Biosci. 2023; 53: 102555. doi:10.1016/J.FBIO.2023.102555
32. Martín-Gómez J, García-Martínez T, Varo MÁ, Mérida J, Serratosa MP. Phenolic compounds, antioxidant activity and color in the fermentation of mixed blueberry and grape juice with different yeasts. LWT. 2021; 146 (111661): 1-7. doi:10.1016/J.LWT.2021.111661
33. Rodríguez LGR, Gasga VMZ, Pescuma M, Nieuwenhove CV, Mozzi F, Burgos JAS. Fruits and fruit by-products as sources of bioactive compounds. Benefits and trends of lactic acid fermentation in the development of novel fruit-based functional beverages. Food Res Int. 2021;140:109854. doi:10.1016/j.foodres.2020.109854
34. Bremus C, Herrmann U, Bringer-Meyer S, Sahm H. The use of microorganisms in l-ascorbic acid production. J Biotechnol. 2006; 124(1): 196-205. doi:10.1016/j.jbiotec.2006.01.010
35. Sabokbar N, Khodaiyan F. Total phenolic content and antioxidant activities of pomegranate juice and whey based novel beverage fermented by kefir grains. J Food Sci Technol. 2015; 53(1): 739-747. doi:10.1007/s13197-015-2029-3
36. Lv QZ, Long JT, Gong ZF, Nong KY, Liang XM, Qin T, Huang W, Yang L. Current state of knowledge on the antioxidant effects and mechanisms of action of polyphenolic compounds. Nat Prod Commun. 2021; 16(7): 1-13. doi:10.1177/1934578X211027745
37. Feng T, Wang J. Oxidative stress tolerance and antioxidant capacity of lactic acid bacteria as probiotic: a systematic review. Gut Microbes. 2020; 12(1): 1801944-1801968. doi:10.1080/19490976.2020.1801944
38. Bourrie BCT, Willing BP, Cotter PD. The microbiota and health promoting characteristics of the fermented beverage kefir. Front Microbiol. 2016; 7: 1-17. doi:10.3389/fmicb.2016.00647
39. Raphaelli CO, Pereira ES, Camargo TM, Vinholes J, Rombaldi CV, Vizzotto M, Nora L. Apple phenolic extracts strongly inhibit α-glucosidase activity. Plant Foods Hum Nutr. 2019; 74(3): 430-435. doi:10.1007/s11130-019-00757-3
40. Renda G, Ozel A, Barut B, Korkmaz B, Soral M, Kandemir U, Liptaj T. Bioassay guided isolation of active compounds from Alchemilla barbatiflora Juz. Rec Nat Prod. 2018; 12(1): 76-85. doi:10.25135/rnp.07.17.07.117
41. Rahman MJ, Camargo AC, Shahidi F. Phenolic and polyphenolic profiles of chia seeds and their *in vitro* biological activities. J Funct Foods. 2017; 35: 622-634. doi:10.1016/J.JFF.2017.06.044
42. Wei M, Chai WM, Yang Q, Wang R, Peng Y. Novel Insights into the inhibitory effect and mechanism of proanthocyanidins from *Pyracantha fortuneana* fruit on α-glucosidase. J Food Sci. 2017; 82(10): 2260-2268. doi:10.1111/1750-3841.13816
43. Tamura T, Ozawa M, Kobayashi S, Watanabe H, Arai S, Mura K. Inhibitory effect of oligomeric polyphenols from peanut-skin on sugar digestion enzymes and glucose transport. Food Sci Technol Res. 2015; 21(1): 111-115. doi:10.3136/fstr.21.111
44. Schmidt JS, Lauridsen MB, Dragsted LO, Nielsen J, Staerk D. Development of a bioassay-coupled HPLC-SPE-ttNMR platform for identification of α-glucosidase inhibitors in apple peel (*Malud domestica* Borkh.). Food Chem. 2012; 135(3): 1692-1699. doi:10.1016/j.foodchem.2012.05.075
45. Proenca C, Freitas M, Ribeiro D, Oliviera EFT, Sousa JLC, Tome SM, Ramos MJ, Silva AMS, Fernandes PA, Fernandes E. α-Glucosidase inhibition by flavonoids: an *in vitro* and *in silico* structure–activity relationship study. J Enzyme Inhib Med Chem. 2017; 32(1): 1216-1228. doi:10.1080/14756366.2017.1368503
46. Kwon YI, Apostolidis E, Shetty K. Anti-diabetes functionality of kefir culture-mediated fermented soymilk supplemented with *Rhodiola* extracts. Food Biotechnol. 2006; 20(1): 13-29. doi:10.1080/08905430500522055
47. Tiss M, Souiy Z, Abdeljelil NB, Njima M, Achour L, Hamden K. Fermented soy milk prepared using kefir grains prevents and ameliorates obesity, type 2 diabetes, hyperlipidemia and liver-kidney toxicities in HFFD-rats. J Funct Foods. 2020; 67:103869. doi:10.1016/j.jff.2020.103869
48. Lestari AR, Batubara I, Wahyudi ST, Ilmiawati A. Phenolic compound in garlic (*Allium sativum*) and black garlic potency as antigout using molecular docking approach. J Kim Sains dan Apl. 2022; 25(7): 253-263. doi:10.14710/jksa.25.7.253-263
49. Wulanawati A, Noviardi H, Ibrohim MSM. Finding a potential bruceine d inhibitor for apoptotic resistance protein pancreatic cancer based on molecular docking. Indones J Chem. 2018;18(3): 566-572. doi:10.22146/ijc.25220
50. Rashidi R, Rezaee R, Shakeri A, Hayes AW, Karimi G. A review of the protective effects of chlorogenic acid against different chemicals. J Food Biochem. 2022; 46(9): 1-19. doi:10.1111/jfbc.14254