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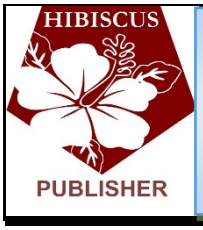

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Potential Antiobesity and Anti-Diabetic Properties of Fermented Kuini (*Mangifera odorata*) Juice

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ABSTRACT

Obesity and diabetes are strongly linked and increase the risk of other critical diseases such as cardiovascular disease, which is highly prevalent today. Currently available synthetic anti-diabetic drugs often cause gastrointestinal side effects, highlighting the need for alternative treatments derived from natural resources, which tend to have fewer side effects and are generally safer. The kuini fruit (*Mangifera* odorata) has been shown to exhibit high antioxidant activity. However, as an underutilized fruit, there is a lack of studies on its potential health benefits, particularly its antiobesity and anti-diabetic properties. Due to the high demand for rich constituents of bioactive compounds, fermentation is considered a viable method to enhance the nutritional value of this fruit. This study evaluates the potential health benefits of fermented kuini (FK) as an antiobesity and anti-diabetic agent through adipogenesis inhibition in vitro using the 3T3-L1 preadipocyte cell line, as well as its inhibition of α-amylase and α-glucosidase activities. A cell viability assay was conducted to assess the toxicity of different sample concentrations. It was found that concentrations below 1.5 mg/mL did not exhibit cytotoxicity, while higher concentrations reduced cell viability. Fermented kuini (FK) showed lower adipogenesis inhibition compared to pasteurized kuini (PK). The highest inhibition of α-amylase activity was observed at 1.0 mg/mL FK, with a percentage inhibition of 41.6%. Similarly, the highest inhibition of α-glucosidase activity was observed at the same concentration, with a percentage inhibition of 39.0%. Based on the data generated, FK demonstrates potential antiobesity and anti-diabetic properties and could serve as an alternative strategy for managing obesity and diabetes.

INTRODUCTION

Obesity is rising at a worrying rate globally. It is a leading health problem in the Noncommunicable Diseases (NCD), which elevates the possibility of diabetes mellitus, insulin resistance, hypertension, and heart disease [3]. This metabolic disorder resulted from the increasing number and size of adipocytes as well as the enlargement of adipose tissue [4]. The National Health and Morbidity Survey Malaysia (NHMS) revealed that the overweight cases among adults in Malaysia were 29.4% in 2011 and 30.0% in 2015, while the occurrence of obesity was 15.1% and 17.7%, respectively [5]. Moreover, Malaysia is reported to have the highest occurrence of obesity and overweight compared to other countries, and shockingly, it is more pervasive in women [6]. It is suggested that in the event of energy imbalance related to weight gain, men tend to gain more muscle tissue than fat tissue compared to women. On the other hand, obesity is strongly linked to type 2 diabetes mellitus. Abdullah et al. stated that body mass index (BMI) affects the probability and severity of type 2 diabetes mellitus, where there is a seven times greater risk of diabetes in overweight people with a threefold risk compared to those with normal weight [7]. Obesity is caused by an imbalance between energy intake and expenditure, leading to the extensive formation and accumulation of adipocytes through the process of adipogenesis. By inhibiting adipogenesis, which is a cell differentiation process from

preadipocytes into mature adipocytes, it is suggested to be an important mechanism for anti-diabetes [8].

The present antiobesity drugs used as a treatment, such as orlistat, lorcaserin and liraglutide, are claimed to generate a number of undesirable side-effects like liver damage, nausea and vomiting [1]. Thus, studies aimed at identifying bioactive compounds from natural resources, including plant-based extracts, to assess their potential in inhibiting adipogenesis are gaining momentum, as it is believed to be a safer alternative. One of the most commonly used in-vitro models to study adipocyte differentiation is the 3T3-L1 cell line, which possesses most of the structural characteristics of adipocytes from animal tissue.

Kuini (*Mangifera* odorata) originates from Anacardiaceae family and shares quite similar characteristics to the ordinary mango (*Mangifera* indica L.). It is a native plant to the tropical Asia region, mainly in Malaysia, Java, and Sumatra. Kuini has fibrous flesh with a strong smell, yet it gives a sweet taste. It can be eaten as raw fruit and blended to form a juice. Previous studies have reported that kuini possesses the highest carotenoid content compared to other Mangifera species, leading to the highest antioxidant activity [9]. To date, there are not many studies being reported on this fruit since it is categorized as an underutilized fruit.

The process of fermentation for beverages has been widely used throughout the world for centuries. Microbial fermentation is usually done by introducing probiotics to the beverages, and among the common bacteria used are the Lactococcus, Lactobacillus, and Streptococcus, while the commonly detected yeasts are Candida and Saccharomyces [10]. Besides its preservation purposes, fermentation also greatly contributes to sensory characteristics enhancement and promotes many health benefits. It is suggested that the addition of probiotics improves the immune system, decreases lactose intolerance, and also decreases cholesterol levels in the blood [11]. Additionally, there have been reports stating that the probiotics added in the beverages cause a significant increase in the bioactive compounds present. This increases the nutrient content as well as the nutritional value of the original product, which consequently further increases its health benefits [12].

Therefore, the present study aimed to investigate the potential anti-obesity properties of fermented kuini (FK) based on its ability to inhibit adipogenesis. Considering that obesity is strongly linked to T2DM, the anti-diabetic properties of FK were also determined by their inhibition towards carbohydrate-hydrolyzing enzymes (α-amylase and α-glucosidase). Therefore, this study seeks to explore the effect of FK juice on the 3T3-L1 cell viability, adipocyte cell development, as well as the anti-diabetic properties of FK against α-amylase and α-glucosidase enzymes.

MATERIALS AND METHODS

Chemicals

The 3T3-L1 fibroblasts used in this study were acquired from the American Type Culture Collection (ATCC) (Virginia, USA). Dulbecco's Modified Eagle Medium (DMEM), trypsin-EDTA, penicillin-streptomycin, dimethyl sulfoxide (DMSO), phosphate-buffered saline (PBS), MTT reagent (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide), and human insulin were obtained from Nacalai Tesque (Kyoto, Japan). Fetal bovine serum (FBS) was obtained from Tico Europe (Netherlands). Other chemicals, including oil red O, α-amylase, α-glucosidase, p-nitrophenyl-α-D-glucopyranoside (PNPG), and 3,5-dinitrosalicylic acid (DNS), were obtained from Sigma-Aldrich (Massachusetts, USA).

Sample material

Approximately 500 mL of both pasteurized and fermented kuini juices were obtained from Dr. Musa Al Bakri Abdul Manan, Research Officer of Malaysian Agricultural Research and Development Institute (MARDI), in sealed bottles for analysis.

Preparation of the sample for analysis

Both fermented and pasteurized kuini juices were incubated in a -20°C freezer overnight prior to freeze-drying (Thermo Electron Corporation, USA) for 10 days to obtain their powders.

Cell growth and maintenance

3T3-L1 fibroblasts were purchased from American Type Culture Collection, USA. In brief, preadipocytes were cultured in Dulbecco's Modified Eagle Medium (DMEM, Gibco®) containing high glucose (4500 mg/L), supplemented with 10% of fetal bovine serum (FBS), 1% of penicillin-streptomycin, and 1% of glutamine, in an atmosphere of 5% CO_2 at 37°C. When reaching 70-80% confluent, cells were sub-cultured into a T75 flask to prevent the culture from becoming fully confluent. Cells were maintained in a T75 flask during routine sub-culturing. Cells with passage numbers between 3 and 10 were used for the experiment.

Determination of cell viability (MTT Assay)

The cell viability assay was carried out using the MTT cytotoxicity method with slight modifications [13]. In short, both extracts were first prepared in 10 mg/mL by dissolving 30 mg of each powder into 3 mL of 1% dimethyl sulphoxide (DMSO) solution as a stock per sample. The extracts were then diluted to various concentrations, namely 0, 0.5, 0.75, 1.0, and 1.5 mg/mL. 3T3-L1 cells were first seeded in a 96-well plate and were grown in high-glucose Dulbecco's Modified Eagle Medium (DMEM) + 10% Fetal Bovine Serum (FBS).

The plate was incubated for 2 days until the cells reached 70 to 80% confluence. After that, the cells were treated with different concentrations of the extracts. The plate was then incubated for 24h before each well containing the cells and sample was washed using 100 µL phosphate-buffered saline (PBS), and the media was replaced with 135 µL DMEM without the addition of 10 % fetal bovine serum. Then, 15 µL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added to each well. Since the MTT solution is light-sensitive, the plate was wrapped with aluminium foil. The plate was further incubated for 6 hours in order to allow the reduction reaction to occur. Then, 150 µL of 1% DMSO solution was added to each well to solubilize the formazan crystals formed. The plate was incubated again for 30 minutes, and the absorbance was measured at 540 nm using the microplate reader (Tecan Group Ltd). The assay was measured in triplicate. The percentage of cell viability was calculated using the formula as stated below:

$$\% = \left(\frac{Abs_{sample} - Abs_{blank}}{Abs_{control} - Abs_{blank}}\right) \times 100$$

Where Abs_{sample} represents the absorbance value of sample reaction which contains the cell and the sample, and $Abs_{control}$ represents the absorbance value of control reaction which contains only the cells (without the presence of sample). Abs_{blank} represents the absorbance value of the blank reaction which contains only 1% DMSO solution.

Cell Differentiation Optimisation

The tissue culture protocol from Johns Hopkins Medicine about cell-line 3T3-L1A was used to differentiate the 3T3-L1 preadipocytes into mature adipocytes. The 3T3-L1 cells were first thawed from liquid nitrogen and cultured in T-25 culture flask by changing the media once in two days. Upon reaching 80-90% cell confluent, the 3T3-L1 cells were seeded into 24 well-plates and cultured with complete growth media containing high glucose-Dulbecco's modified eagle medium and 10% fetal bovine serum. The media was replaced every two days with fresh media until reaching full confluence. The cell was then incubated for another one day to get post-confluent condition before proceeding with differentiation. The differentiation process started at day 0, where the cells in each plate were treated with the differentiation media containing 3-isobutyl-1-methylxanthine (IBMX), dexamethasone (DEX), and insulin, resulting in a total volume of 500 µL in each well. Then, the cells were treated with post-differentiation media containing only insulin until day 8. Meanwhile, the diluted samples in 1% DMSO were injected into the cell at various concentrations ranging from 0, 0.25, 0.50, 0.75 and 1.0 mg/mL from day 0 to day 8. The cells were harvested every two days for observation purposes.

Determination of lipid accumulation via Oil Red O staining

In order to measure the cell differentiation, Oil Red O (ORO) staining was carried out. The differentiated 3T3-L1 cells (Day 8) were first washed with 1X PBS and fixed with 4% formalin and stained with red-colored working solution ORO, which was made in a volume ratio of two ORO to three distilled water, respectively. After that, the stained cells were observed under a microscope to measure the cell differentiation. For quantitative analysis, 100% isopropanol was added to the samples and left to incubate for 15 min to solubilize the Oil Red O stain and read the absorbance at 490 nm.

Alpha-amylase inhibition activity assay

The α-amylase inhibitory activity assay was conducted according to a previously described method, which used the 3,5-dinitrosalicylic acid method with slight modifications [14]. The fermented and pasteurized kuini extracts were prepared in 1 mg/mL by dissolving 5 mg of powder into 5 mL of 10% dimethyl sulfoxide (DMSO). Various concentrations ranging from 0.25, 0.50, 0.75, 1.0, and 1.5 mg/mL were prepared. 200 µL of α-amylase enzyme solution (2 units/ml) dissolved in sodium phosphate buffer, 0.02 mM, containing 0.006 M NaCl, was mixed with 200 µL of the extract in the test tube. The triplicate of positive control (acarbose in 10% DMSO and enzyme), negative control (buffer and enzyme), and blank (test sample without enzyme) were also prepared to determine the inhibition activity. The mixture was then pre-incubated for 15 min at 37°C. One percent starch solution was prepared by dissolving 0.25 g of starch in 25 mL of distilled water at 90°C, and the mixture was continuously stirred throughout the assay.

Next, 200 µL of 1% starch solution as a substrate was added to each of the test tubes and incubated for 10 min to allow the enzymatic reaction. The reaction was terminated by adding 200 µL of DNS reagent, which was prepared by dissolving 12 g of sodium potassium tartrate tetrahydrate in 8 mL of 2 M sodium hydroxide and adding it to 20 mL of 96 mM 3,5-dinitrosalicylic acid solution. The mixture was heated at 90°C in a water bath for 10 min and was then allowed to cool before being diluted with 5 mL of distilled water. Absorbance was read by using a UV-visible spectrophotometer (Labomed, Inc., USA) at 540 nm. The assay was performed in triplicate.

The percentages of inhibition of fermented and pasteurized kuini extracts against α-amylase activity were calculated using the formula as stated below:

$$\% \text{ Inhibition} = \left(\frac{A_{540}^{\text{Control}} - B_{540}^{\text{Extract}}}{A_{540}^{\text{Control}}}\right) \text{x } 100$$

Where A^{Control} represents the absorbance value of control reaction and B^{Extract} represents the absorbance value of sample extract at 540 nm.

Alpha-glucosidase inhibitory activity assay

The α-glucosidase inhibition assay was conducted according to previously described method with a few modifications [15]. The fermented and pasteurized kuini extracts were first prepared in 5 mg/mL by dissolving 50 mg of each powder into 10 mL of 10% DMSO solution as a stock solution. The extracts were then diluted to various concentrations ranging from 0.25 to 1.5 mg/mL. Fifty microliters of α-glucosidase enzyme solution, which was prepared by dissolving 1 mg of α-glucosidase in 50 mL of distilled water, was mixed with 60 µL of sample extract in a 96-well plate. The plate was incubated for 20 min at 37°C. Next, the plate was immediately wrapped with aluminum foil as 50 µL of α-D-glucopyranoside, pNPG was added into each of the well since the pNPG is light-sensitive.

The plate was re-incubated for another 20 min to allow the enzymatic reaction. The reaction was terminated by adding 140 µL of sodium carbonate solution to each well, and the absorbance was measured at 405 nm using the microplate reader (Tecan Group Ltd.). The triplicate of positive control (quercetin in 10% DMSO and enzyme), negative control (buffer and enzyme) and blank (test sample without enzyme) were also prepared to determine the inhibition activity. The assay was measured in triplicate. The percentages of inhibition of fermented and pasteurized kuini extracts against α-glucosidase activity were calculated using the formula as stated below:

$$\% \, Inhibition = \left(\frac{Control_{A405\,nm} Extract_{A405\,nm}}{Control_{A405\,nm}}\right) \times 100$$

Where A^{Control} represents the absorbance value of control reaction and B^{Extract} represents the absorbance value of sample extract at 405 nm.

Statistical analysis

The results were expressed as mean ± SEM of triplicate measurement. Data were analyzed by One-Way ANOVA and the significance of the difference between means was determined by Tukey's multiple comparison test ($p < 0.05$) using GraphPad Prism 8.0 (GraphPad Software, Inc., San Diego, CA).

RESULTS AND DISCUSSIONS

The effect of fermented kuini (FK) and pasteurized kuini (PK) on 3T3-L1 cell viability

A cell viability study was conducted to determine the concentrations that are non-toxic to the cells, which will be used for the subsequent experiments. To determine the effect of FK and PK on 3T3-L1 cell viability, four different concentrations (0.50, 0.75, 1.0, and 1.5 mg/mL) of both FK and PK were assessed. **Fig. 1** demonstrated that the exposure of 3T3-L1 preadipocyte cells to both FK and PK at the highest tested concentration (1.5 mg/mL) resulted in approximately a 20% decrease in cell viability compared to the control (non-treated sample).

All tested concentrations were determined to be non-toxic to the cells, as the cell viability percentages for all tested concentrations exceeded 80%.

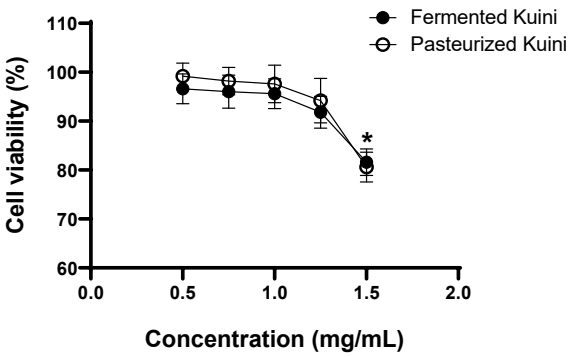


Fig. 1. The effect of FK and PK on 3T3-L1 preadipocytes cell viability. Results are expressed as mean ± SEM (n=3). A one-way ANOVA followed by Tukey´s post-test was performed; * $p<0.05$.

Generally, cell viability percentages of 80% and above are classified as non-toxic, 60%–80% as weakly toxic, 40%–60% as moderately toxic, and 40% and below as strongly toxic. Ismail et al. (2019) report that the fleshy part of the kuini fruit contains high amounts of gallic acid (GA) [9]. Previous research had indicated that gallic acid inhibited cell growth and induced apoptosis in 3T3-L1 preadipocytes, resulting in cell toxicity [16]. This aligns with a prior study that showed gallic acid markedly decreased the viability of 3T3-L1 preadipocyte cells at a concentration of 200 mM. ($p < 0.05$) [16]. However, based on the present study, the reduction in cell viability (20%) is not statistically significant when compared to the control, even at the highest tested concentration (1.5 mg/mL).

Optimization of 3T3-L1 cells differentiation.

Optimisation of 3T3-L1 cell differentiation was carried out to observe the day the preadipocyte cells of 3T3-L1 would differentiate into mature adipocytes for cell harvesting purposes. Shown in **Fig. 2**, the absorbance reading for the cells peaked on day 8. The result obtained through this optimisation corroborated with other studies, which also mentioned that their cells became mature on day 8 [17,18]. Therefore, day 8 was selected as the day to harvest the cells for observation and staining in the cell differentiation assay. **Fig. 2** shows that the absorbance reading for the cells reached its peak on day 8, where the cells are considered fully differentiated at this stage. The results of this optimization corroborated those of other studies, which also demonstrated that 3T3-L1 cells fully differentiated on day 8 [17,18]. Thus, day 8 was chosen for the harvesting of cells to perform the observation and staining procedure of the cell differentiation assay.

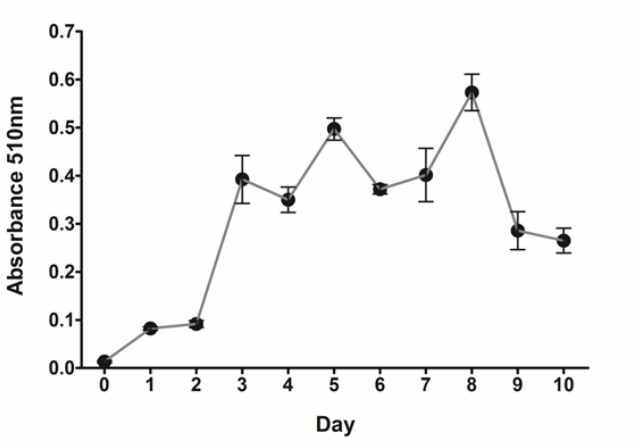


Fig. 2. The optimization of 3T3-L1 cells differentiation. Data represented as means ± standard error of mean (SEM) (n=3).

Day 8 serves as the most effective benchmark for conducting further analysis on intracellular lipid accumulation, as it exhibits the highest level of cell differentiation, with the entire differentiation process having been fully completed. This aligns with earlier research findings, which indicated that the peak intracellular lipid accumulation occurred on day 8, suggesting that the 3T3-L1 cells had attained a fully differentiated state [19,20]. To visualize the differentiated 3T3-L1 cell, Oil Red O staining is used to make the lipid accumulation appear brighter than the background when observed under a microscope**.** The red-colored lipid accumulation on Day 8 indicates that the preadipocytes have been well-differentiated into mature adipocytes, which can be an indicator of obesity.

The effect of different concentrations of FK and PK on 3T3-L1 cells differentiation.

For this study, 3T3-L1 preadipocyte cells were treated with insulin, IBMX, and dexamethasone to induce adipocyte cell differentiation. When the preadipocyte cells were differentiated into mature adipocytes, they would be accumulating lipid within the cells which could be observed through Oil Red O staining, a fat-soluble dye (**Fig. 4**). Lipid accumulation was quantified by measuring the absorbance of eluted Oil Red O. FK and PK were introduced to the culture medium at concentrations of 0.25, 0.5, 0.75, and 1.0 mg/mL to evaluate their efficacy in inhibiting adipogenesis. The effect of FK and PK on the lipid accumulation within matured 3T3-L1 adipocytes on Day 8 is shown in **Fig. 3**, where both FK and PK were found to inhibit 3T3-L1 cell differentiation, with higher inhibition observed when the cells were treated with PK. PK has been shown to inhibit adipogenesis significantly at the concentrations of 0.75 and 1.0 mg/mL. At each individual concentration, the inhibitory potential possessed by PK and FK display significant difference to each other ($p<0.05$).

In contrast, at lower concentrations (0.25 mg/mL – 0.50 mg/mL), the inhibitory potential of both FK and PK is not significantly different from one another ($p>0.05$).

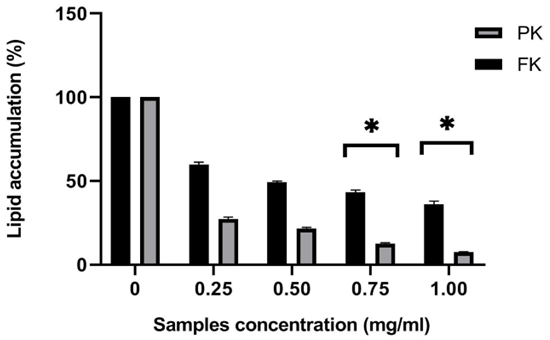


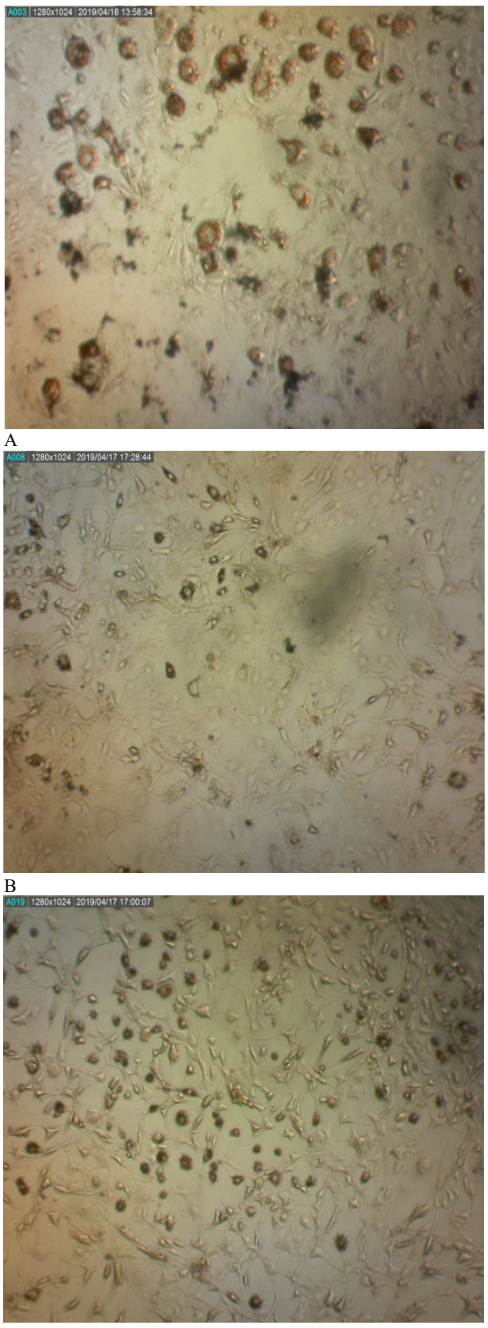
Fig. 3. Effect of FK and PK on the lipid accumulation within matured 3T3-L1 adipocytes on Day 8. Results are expressed as mean ± SEM (n=3). A one-way ANOVA followed by Tukey´s post-test was performed; * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

The results indicate that the significant inhibitory activity of PK on adipogenesis in 3T3-L1 cells, in comparison to FK, may be attributed to the pasteurization method employed. The conventional pasteurization method uses heat, which is the major cause of the reduction of the bioactive compounds, leading to lower inhibition activity. This is supported by previous research done by Patras et al., which reported that heat processing might induce several chemical and physical changes, reducing the content and the bioavailability of some bioactive compounds [21].

As for FK, it exhibits less inhibition activity compared to PK, probably because of the reduction of some bioactive compounds that contribute to the inhibition activity during the fermentation process, which has been reported rarely by previous studies. One has reported that it might be due to the presence of preliminary treatments applied to the juices to be fermented, such as sterilization, filtration, enzymolysis, which are most likely to be the major cause of reduction [22].

A study on lipid accumulation inhibition by cranberry reported that cranberry not only inhibits lipid accumulation but also down-regulates major adipogenesis-related transcription factors, such as PPARγ, C/EBPα, and SREBP1, which are responsible for adipocyte differentiation [23]. The inhibition of these factors is thus deemed crucial for diminishing lipid accumulation and the differentiation of adipocyte cells. A number of bioactive compounds, including resveratrol, anthocyanin, and quercetin, have been reported in the literature for their capacity to reduce the expression of transcription factors associated with adipogenesis [24]. However, no reported data on the comprehensive purification and identification of bioactive compounds from kuini to date.

Lipid accumulation on Day 8 was assessed at a 1.0 mg/mL concentration by staining the samples with Oil Red O, followed by microscopic examination. As illustrated in **Fig.** 4, treatment with FK (**Fig.** 4 (B)) resulted in a greater intensity of red-stained lipid droplets when compared to PK (**Fig.** 4 (C)). This observation indicates that, at a concentration of 1.0 mg/mL, PK exhibits a stronger inhibitory effect on adipogenesis than FK.


A

B

C

Fig. 4. The lipid accumulation observed after Oil Red O staining on Day 8 at 1.0 mg/mL in (A) treated with no sample, (B) treated with pasteurized kuini, (C) treated with fermented kuini (Microscopic observation at 40× magnification).

The potential effect of FK and PK in inhibiting α-amylase activity

The potential effect of FK and PK in inhibiting α-amylase enzyme activity was carried by using an α-amylase inhibition assay. As shown in **Fig. 5**, both samples displayed positive inhibitory activity against α-amylase in a dose-dependent manner, where the highest inhibition was obtained at the concentration of 1.0 mg/mL for both FK and PK. However, at the highest tested concentration (1.5 mg/mL), the inhibitory effect of both FK and PK significantly dropped when compared to 1.0 mg/mL ($p<0.05$).

At the concentration of 1.0 mg/mL, FK exhibits significantly higher inhibitory potential on α-amylase enzyme when compared to PK with the percentage of inhibitions of 43.6% and 35.1%, respectively ($p<0.01$). Based on this present study, FK exhibits higher inhibition towards α-amylase activity.

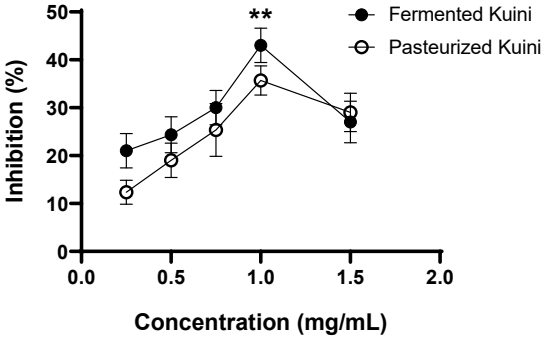


Fig. 5. The effect of FK and PK in inhibiting α-amylase activity. Results are expressed as mean ± SEM (n=3). A one-way ANOVA followed by Tukey´s post-test was performed; *p<0.05, **p<0.01, ***p<0.001.

Phenolic compounds have been shown previously to play a major role in mediating α-amylase inhibition and therefore have the potential to contribute to the management of Type 2 Diabetes Mellitus (T2DM) [25]. Previous studies have demonstrated that carotenoids are notably present and most abundant in *Mangifera odorata* (52.07 ± 2.39 mg BC/100 g in the flesh, 26.52 ± 0.68 mg BC/100 g in seed and 39.11 ± 1.02 mg BC/100 g in peel), compared to another *Mangifera* sp. which are *M. caesia* and *M. quadrifida* [9]. Carotenoid is a type of phytochemical that acts as a pigment, contributing yellow, red, and orange colors to fruits. It has been reported to play a role in the antioxidant activity of the extract [26]. The inhibition of carbohydrate-hydrolyzing enzymes can be linked to antioxidant activity. Previous research has shown that antioxidants are strongly linked to α-amylase and α-glucosidase inhibitors, which makes them useful for managing T2DM [27]. Therefore, the significant concentration of carotenoids found in FK is likely responsible for the pronounced inhibition of α-amylase activity.

However, the inhibition percentage activity of FK drops drastically at the concentration of 1.5 mg/mL. This is probably due to the reduction of some bioactive compounds that are responsible for inhibiting α-amylase activity during the fermentation process. Even though fermentation is a well-known method to enhance the nutritional value and prolong the shelf life of the products, it has also been reported to cause a reduction in bioactive compounds. Wasila et al. (2013) stated that every juice to be fermented is commonly subjected to several preliminary treatments such as enzymolysis, clarification, filtration, and sterilization [22]. These treatments could probably cause the changes in the juice composition and thus alter its contents of bioactive compounds that are capable of inhibiting α-amylase activity.

The potential effect of FK and PK in inhibiting α-glucosidase activity

In addition to α-amylase, α-glucosidase is an important enzyme in carbohydrate metabolism. Inhibiting this enzyme could also be one of the strategies in managing hyperglycemia. As shown in **Fig. 6**, both FK and PK displayed positive inhibitory activity against α-glucosidase in a dose-dependent manner, where the highest inhibition was achieved at the concentration of 1.0 mg/mL.

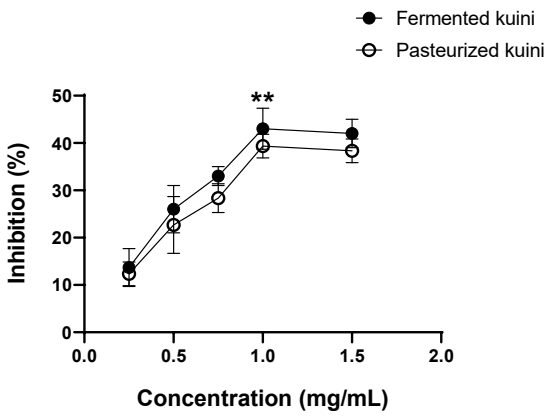


Fig. 6. The effect of FK and PK in inhibiting α-glucosidase activity. Results are expressed as mean ± SEM (n=3). A one-way ANOVA followed by Tukey´s post-test was performed; *p<0.05, **p<0.01, ***p<0.001.

Alpha-glucosidase inhibitors are commonly used as oral anti-diabetic agents for the management of type 2 diabetes mellitus (T2DM), alongside α-amylase inhibitors, which serve as potential targets in the development of lead compounds for diabetes treatment [28]. They function by inhibiting the digestion of complex carbohydrates, which are typically broken down into their simplest form for absorption in the intestine after food ingestion. The inhibition of this enzyme reduces the rate of carbohydrate degradation to glucose, leading to a delayed absorption of glucose. Therefore, blood glucose levels can be regulated to prevent spikes following a meal. However, currently available synthetic enzyme inhibitors, including acarbose and voglibose, are associated with various side effects, such as diarrhea, flatulence, and other gastrointestinal disturbances. This situation necessitates the exploration of alternatives or natural sources that exhibit fewer side effects and are safer for use [29].

The use of FK as the means to treat diabetes could be beneficial based on this present study, where at 1.0 mg/mL, FK displayed the highest significant inhibition on α-glucosidase activity compared to other tested concentrations ($p<0.05$). This is probably due to the presence of bioactive compounds that are responsible for inhibiting this carbohydrate-hydrolyzing enzyme. One of the best candidates is mangiferin. Mangiferin is reported to exhibit α-amylase and α-glucosidase inhibition activity, where it could be found predominantly in *Mangifera* families [30,31]. This is further supported by previous studies, which revealed that mangiferin exhibited both anti-diabetic and hypolipidemic effects. It significantly diminished total cholesterol, triglycerides, low-density lipoprotein (LDL), and very-low-density lipoprotein (VLDL) levels, while simultaneously increasing high-density lipoprotein (HDL) levels in type 2 diabetic rat models. [30]. Given this information, it can be concluded that mangiferin is a promising candidate for presentation in FK, potentially leading to the inhibition of α-glucosidase activity.

CONCLUSIONS

Data from the present study demonstrated that fermented and pasteurized kuini juices were able to inhibit adipogenesis in 3T3-L1 cells. Weak toxicity even at the highest tested concentration (1.5 mg/mL) shows that FK and PK are safe for the cells. Furthermore, both FK and PK demonstrated potential inhibitory effects on carbohydrate-hydrolyzing enzymes, specifically α-amylase and α-glucosidase. This could be due to the presence of bioactive compounds responsible for inhibiting these two

enzymes, such as mangiferin. These findings provide a foundation for exploring the potential anti-obesity and anti-diabetic effects of kuini fruit. This study, however, lacks data on the specific bioactive compounds in the fruit that may influence these observed effects. Therefore, further comprehensive in vitro and in vivo studies are necessary to validate the potential of kuini fruit, including its juice extract, as a promising approach for managing obesity and diabetes.

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