International Food Research Journal 31(1): 253 - 265 (February 2024)
Journal homepage: *http://www.ifrj.upm.edu.my*

Biotransformation of bitter gourd (*Momordica charantia*) by *Lactobacillus plantarum* and its bioactivities

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Article history

Received:
12 May 2023
Received in revised form:
24 November 2023
Accepted:
12 December 2023

Keywords

biotransformation, fermentation, bitter gourd, Lactobacillus plantarum, antiglycation, anti-aggregation

Abstract

Bitter gourd is renowned for its various bioactivities, including antioxidant, antiglycation, and antiplatelet aggregation. However, the bioactivity and bioavailability of plant extracts could be low. Biotransformation through *Lactobacillus plantarum* fermentation is capable of enhancing these beneficial properties. Therefore, the present work aimed to investigate the physical and pH profiles, total phytochemical contents, bioactive contents, and bioactivities of the juices of unfermented bitter gourd (UBG) and fermented bitter gourd at 48 (FBG-48) and 96 (FBG-96) h. Fermentation gradually altered the physical and pH profile of bitter gourd, while non-significant decrease was seen in the total flavonoid content ($p > 0.05$). The highest total phenolic content was observed in FBG-48, followed by UBG and FBG-96. UBG exhibited the highest total triterpenoid content, followed by FBG-48 and FBG-96. Interestingly, fermentation increased the antioxidant, antiglycation, and anti-aggregation activities of bitter gourd. FBG-48 demonstrated the highest antioxidant and antiglycation activities, with 10.77 and 8.68% higher activity, respectively, compared to UBG and FBG-96. Meanwhile, FBG-96 exhibited the highest antiglycation activity, with 60% increase. These could have been attributed to the biotransformation of tannic acid into syringic acid, and momordicoside Q into kuguacin P, along with the potential release of *p*-coumaric acid and caffeic acid from the cell walls. These findings demonstrated bitter gourd's promising role in type-II diabetes complication treatment, particularly through its protein aggregation inhibition activity, and that fermentation could increase its bioactivities.

DOI
https://doi.org/10.47836/ifrj.31.1.22

Introduction

Type-2 diabetes is known to lead to various degenerative diseases, some of which are considered the deadliest (Papatheodorou *et al*., 2018). This is due to glycation, where excess sugar binds with proteins, producing a radical called the advanced glycation end products (AGEs) (Takahashi, 2015). These radical AGEs can alter the protein structure, and cause aggregation, thus resulting in the damage of tissues, and leading to degenerative diseases, such as Alzheimer's disease (Chen *et al*., 2019). Furthermore, degenerative diseases can also be generated by environmental pollution and food additives, since these factors may alter the protein structure, and cause aggregation (Devi *et al*., 2022). Antioxidants, including natural antioxidants and other natural bioactive compounds are believed to inhibit AGEs and protein aggregation (Song *et al*., 2021; Liu *et al*., 2022). Hence, they may prevent the development of AGEs and protein aggregates.

Bitter gourd (BG), *Momordica charantia*, is a popular vegetable and traditional folk medicine usually found in Asia, South America, and East Africa. Various beneficial effects have been partly attributed to the various bioactive components of bitter gourd, including triterpenoids, flavonoids, polyphenols, alkaloids, and sterols (Jia *et al*., 2017). It has long been believed that bitter gourd has antioxidant and antidiabetic activities (Mazlan *et al*., 2015; Gao *et al*., 2019; Hartajanie *et al*., 2020). Recent studies also showed that bitter gourd possess antiplatelet aggregation (Manjappa *et al*., 2019), anticancer (Yue *et al*., 2019), and antiglycation activities (Aljohi *et al*., 2016). The main bioactive compounds responsible for these bioactivities are

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triterpenoids and phenolics (Mazlan *et al*., 2015; Aljohi *et al*., 2016; Yue *et al*., 2019).

However, the bioactivities and bioavailability of plant extracts tend to be low because some secondary metabolites, such as flavonoids and insoluble-bound phenolics, are found to be bound to cell walls, making extraction difficult using only solvents. Moreover, secondary metabolites are bound to sugar, forming a complex organic compound called glycoside. Biotransformation has the potential to solve this problem as it is the only method that can transform glycosides into aglycones, and release insoluble-bound phenolics without losing their carbon skeleton (Shahidi and Yeo, 2016).

Biotransformation may be defined as the specific structural modification of a definite compound to a distinct product by the use of biological catalysts, including microorganisms and enzymes (Hegazy *et al*., 2015). One common method used in biotransformation is fermentation. Lactic acid bacteria (LAB) are widely used for fruit and vegetable fermentation (Hussain *et al*., 2016). LABs, such as *Lactobacillus plantarum*, can degrade plant cell walls *via* hydrolysis, thus resulting in the release and conversion of complex organic compounds. Furthermore, glycosides can be biotransformed into a simpler bioactive molecule called aglycones, which have higher bioavailability (Zhang *et al*., 2021). In other words, bioactivities have the potential to be increased through biotransformation. One example is from a study by Ibrahim *et al*. (2014) which found that lactic acid-fermented herbal teas exhibited higher phenolic, flavonoid, and antioxidant properties compared to the freshly prepared herbal teas. In addition, *L. plantarum*, apart from *Streptococcus gallolyticus*, is the only lactic acid bacterium that possesses tannase activity (Reverón *et al*., 2017). Therefore, it can degrade hydrolysable tannins into their corresponding aglycones.

Concurrently, Hartayanie *et al*. (2018) found that fermented bitter gourd exhibited 10% higher antioxidant activity compared to unfermented ones. Additionally, Mazlan *et al*. (2015) discovered that the cucurbitane-type triterpenoid called momordicoside was biotransformed into its corresponding aglycones, and other phenolic compounds were also produced in the *L. plantarum*-fermented bitter gourd. This is presumed to be the reason behind the increase in the antidiabetic property of the fermented bitter gourd. To date, no study has reported the anti-aggregation and antiglycation activity of fermented bitter gourd. Therefore, the present work focused on the effect of fermentation on the physical and pH profile, total phytochemical contents, bioactive contents, and bioactivities of bitter gourd.

Materials and methods

Starter preparation

The starter preparation method followed the method conducted by Hartajanie *et al*. (2020) with some modifications. *Lactobacillus plantarum* InaCC B1002 culture was obtained from The Indonesian Institute of Sciences (LIPI, Jakarta, Indonesia). First, MRS broth was dissolved in distilled water, and sterilised at 121°C for 15 min. MRS broth was then inoculated aseptically with 1% *Lactobacillus plantarum* culture. Afterward, the culture was incubated semi-anaerobically at 30°C for 18 h. It was then standardised to 10^8 CFU/mL by measuring its optical density at λ = 600 nm (OD_{600} = 0.15).

Bitter gourd juice fermentation for screening

The fermentation method followed the method conducted by Hartajanie *et al*. (2020) with some modifications. Fresh bitter gourds obtained from Hortimart Agro Center (Semarang, Indonesia) were washed and split to remove the seed inside before being cut into smaller pieces. These pieces were then turned into juice slurry using a vegetable blender. The juice slurry was filtered through a cotton cloth to obtain the juice. Next, the juice, without pasteurisation, was added with 1% *L. plantarum* starter. Afterward, each sample was semi-anaerobically fermented in the dark at room temperature (± 28°C) for 96 h, and the pH was tested every 12 h using a pH meter (Hanna, Mettler-Toledo International Inc., Ohio, USA). The selected conditions for the subsequent bitter gourd fermentation were based on the pH changes throughout the 96 h of fermentation.

Bitter gourd juice fermentation under selected conditions

The fermentation method followed the method conducted by Hartajanie *et al*. (2020) with some modifications. Bitter gourd fermentation was performed based on the selected conditions from the screening procedure. After fermentation, each sample was filtered using Whatman No. 42 filter paper. Some filtrates from each sample were stored in a refrigerator before undergoing the total phenolic test,

total flavonoid test, total triterpenoid test, and LC/MS analysis. The remaining filtrates were filtered with a 0.22 μm microfilter, freeze-dried (Thermo Fisher Scientific Inc., Massachusetts, USA), and stored in a freezer before undergoing bioactivity tests.

Total flavonoid content

The total flavonoid content (TFC) was determined according to Ibrahim *et al*. (2014) with some modifications. Each sample was filtered using 0.22 μm microfilter before the test. Initially, 10% $AlCl_3 \cdot 6H_2O$ in distilled water (w/v), and 1 M CH_3COONa in distilled water were prepared. Afterward, 3 mL of methanol, 200 μL of $AlCl_3 \cdot 6H_2O$, and 200 μL of CH_3COONa were added to 1 mL of each sample. The volume was adjusted to 10 mL using distilled water, and incubated for 30 min at 25°C. The absorbance was measured at 432 nm using a UV-Visible spectrophotometer (Genesys, Thermo Fisher Scientific Inc., Massachusetts, USA). Methanol served as the blank control, and quercetin served as the standard. The blank consisted of all reagents and solvents without the sample solution. The standard quercetin calibration curve was used to determine the content. The results were given as mg quercetin equivalents (QE)/g of dry extract.

Total phenolic content

The total phenolic content (TPC) was determined according to Ibrahim *et al*. (2014) with modifications. Each sample was filtered using a 0.22 μm microfilter before the test. Initially, 10% Folin-Ciocalteu reagent in distilled water (v/v) and 7.5% Na_2CO_3 in distilled water (w/v) were prepared. Afterward, 2.5 mL of distilled water and 2.5 mL of Folin-Ciocalteu reagent were added to 0.5 mL of each sample. After incubation at 25°C for 15 min, 2 mL of Na_2CO_3 was added, followed by incubation at 25°C for 30 min, and the absorbance was measured at 765 nm. Methanol served as the blank control and gallic acid served as the standard. The blank consisted of all reagents and solvents without the sample solution. The standard gallic acid calibration curve was used to determine the content. The results were given as mg gallic acid equivalents (GAE)/g of dry extract.

Total triterpenoid content

The total triterpenoid content (TTC) was determined according to Wei *et al*. (2015) with some modifications. Every sample was filtered using a 0.22 μm microfilter before the test. Initially, 100 μL of samples were evaporated in a water bath. Afterward, 500 μL of 5% (w/v) vanillin-glacial acetic acid solution and 900 μL of sulphuric acid were added, mixed, and incubated at 70°C for 30 min in a water bath. The mixed solution was then cooled, diluted to 5 mL with glacial acetic acid, and the absorbance was measured at 553 nm. Methanol served as the blank control, and ursolic acid served as the standard. The blank consisted of all reagents and solvents without the sample solution. The standard ursolic acid calibration curve was used to determine the content. The results were given as mg ursolic acid equivalents (UAE)/g of dry extract.

LC/MS analysis

The liquid chromatography/mass spectroscopy (LC/MS) analysis was performed according to Lee *et al*. (2021) with some modifications. The samples were filtered using 0.22 μm filters before injection. The LC/MS analysis of triterpenoids was performed on an Advion C18 column (2.1 × 50 mm, 1.8 μm). The mobile phase consisted of solvent A (water + 0.1% formic acid) and solvent B (100% acetonitrile). The gradient solvent system was eluted with 90% A → 100% B (0 - 10 min), 100% B (11 - 16 min), and 90% A (16 - 20 min) for equilibration before the next injection. Mass spectrometer conditions were as follows: ionisation mode, electrospray ionisation (ESI) (+); MS scan range, *m/z* 100 - 1700; capillary temperature, 250°C; source gas temperature, 200°C; and capillary voltage, 180 V. Flavonoid, organic acid, phenolic, and triterpenoid compounds in the samples were tentatively identified based on chemotaxonomy, and by comparing their LC/MS data with published data.

Antioxidant activity test

The antioxidant activity test followed the DPPH assay method conducted by Ibrahim *et al*. (2014) with some modifications. The DPPH solution was prepared by dissolving 4 mg of DPPH in 25 mL of methanol, incubated for 30 min immediately before use, and kept in the dark. Samples and quercetin at various concentrations were prepared. Samples were diluted in a 2% water-methanol mixture, while quercetin was prepared in absolute methanol. From this stock solution, 700 μL of sample was added with 300 μL of DPPH solution. The mixture was incubated at room temperature for 30 min in the dark, and the absorbance was measured against the blank at 516 nm using a UV-Vis

spectrophotometer. The absorbance of DPPH reagent alone served as the negative control, and quercetin served as the positive control.

Antiglycation activity test

The antiglycation activity test followed the method conducted by Liu and Gu (2012) with some modifications. First, BSA (10 mg/mL) and glucose (90 mg/mL) were dissolved separately in phosphate-buffered saline (pH 7.4). Then, 1 mL of the sample was mixed with 1 mL of BSA and 1 mL of glucose solution in a test tube. The blank consisted of 1 mL of phosphate buffer saline with 1 mL of BSA solution and 1 mL of glucose solution. The positive control was 1 mL of AG solution (1 mol/L) mixed with 1 mL of BSA and 1 mL of glucose. The tubes were capped and incubated for 7 d at 37°C in the dark. The fluorescence of the AGEs was measured at excitation and emission wavelengths of 370 and 450 nm, respectively using a fluorescence spectrophotometer (Agilent Technologies Inc., California, USA).

Anti-aggregation activity test

The anti-aggregation activity test followed the turbidimetry method done by Anwar *et al*. (2020) with some modifications. Briefly, 1% aqueous solution of bovine serum albumin (500 µL) was added separately to 100 µL of various concentrations of extract, as well as 100 and 200 µg/mL of quercetin. The mixtures were incubated at 37°C for 20 min, and then heated at 70°C for 45 min. The samples were cooled, and turbidity was measured at 600 nm using a UV-Vis spectrophotometer.

Statistical analysis

All samples were run in triplicate (n = 3). Statistical analysis was performed using Microsoft Excel, and all data were expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) and Tukey's HSD test were performed to determine significance at $p < 0.05$. Pearson's correlation coefficient was used to determine the correlation between variables.

Results and discussion

Screening of fermented products

The liquid part of the juice was initially observed to be white and gradually turned orange, while the sediment part turned from green to pale yellow (Figure 1). This could have been attributed to the biotransformation of the bioactive compounds contained in the juice by *L. plantarum*. The white liquid colour was due to cellulose that has a size of 10 nm. Furthermore, plant cells have sizes ranging between 10 and 100 µm long. Therefore, cellulose and plant cells could still penetrate the cloth, which has a pore size of 250 µm. As stated in the study by Minguez-Mosquera *et al*. (1994), the colour alteration from green to pale yellow was associated with the transformation of chlorophylls into pheophytins and pheophorbides during fermentation of olives,

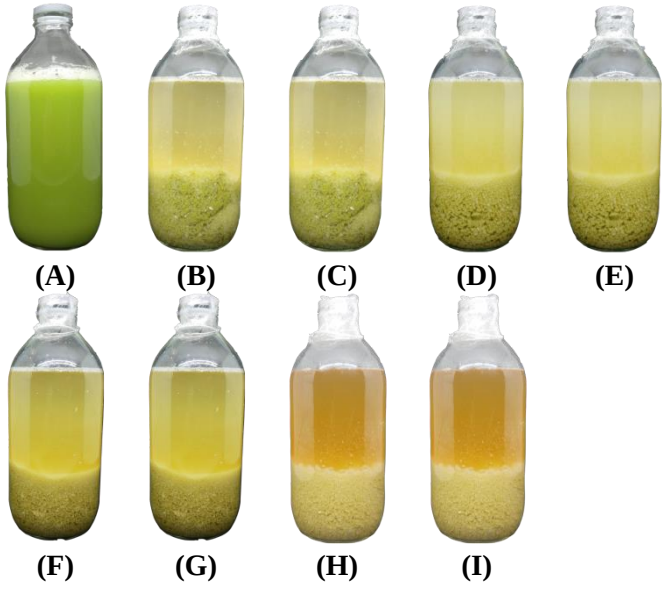

Figure 1. Physical profile of unfermented and fermented bitter gourd juice: UBG **(A)**, FBG-12 **(B)**, FBG-24 **(C)**, FBG-36 **(D)**, FBG-48 **(E)**, FBG-60 **(F)**, FBG-72 **(G)**, FBG-84 **(H)**, and FBG-96 **(I)**.

facilitated by the chlorophyllase enzyme. Meanwhile, the sour smell derived from acids produced by *L. plantarum* through the degradation of macromolecules, such as polysaccharides (Wang *et al*., 2021). Evidence of biotransformation can also be observed in the pH profile of unfermented and fermented BG juices, as shown in Figure 2.

Based on a previous study by Mazlan *et al*. (2015), the pH profile was inversely proportional to the growth phase, suggesting that the change in pH throughout fermentation may reflect the growth phase of *L. plantarum* in the BG juice. The logarithmic phase was observed to commence after 12 h of fermentation, and nearly concluded after 72 h of fermentation (Figure 2). During the logarithmic phase, the number of bacteria increased, characterised by a period of exponential growth, as indicated by the decline in pH (Maier and Pepper, 2015), consistent with the findings by Mazlan *et al*. (2015). The decrease in pH resulted from the decomposition of polysaccharides by *L. plantarum* into organic acids, such as lactic acid, catalysed by lactate dehydrogenase (Wang *et al*., 2021). *L. plantarum* hydrolysed polysaccharides, including other macromolecules and glycosides, to provide energy and nutrients for growth.

The stationary phase commenced after 72 h of fermentation for FBG, and persisted until 96 h of fermentation (Figure 2). During this phase, there was no net bacterial growth present. Growth was simply balanced by an equal number of cells dying. This could be attributed to the scarcity of energy sources or essential nutrients, since they had been utilised (Maier and Pepper, 2015). Nevertheless, a small amount of growth was still present due to the uptake of dead cells as a source of carbon and energy, which can be seen from the slight decrease in pH during the stationary phase.

Therefore, the sample conditions chosen for the subsequent process were UBG as the control, FBG-48 representing the logarithmic phase, and FBG-96 representing the stationary phase.

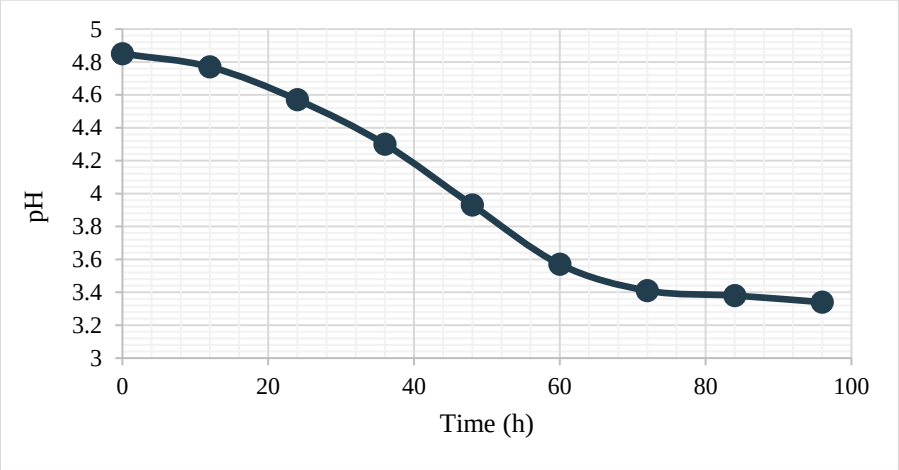

Figure 2. pH profile of fermented bitter gourd juice. Data are mean ± standard deviation of triplicates (n = 3).

Total flavonoid content

The TFC of UBG, FBG-48, and FBG-96 exhibited a decreasing trend from 0.179 ± 0.006, 0.167 ± 0.003, and 0.151 ± 0.009 mg QE/g dry extract, respectively, throughout fermentation, but did not show a significant difference ($p > 0.05$) (Figure 3A). This result aligned with the study by Gao *et al*. (2019). TFC did not increase, possibly due to the absence of enzymes such as ligninase that might aid in the release of cell wall-bound flavonoids (Shahidi and Yeo, 2016). Furthermore, TFC did not decrease as well, suggesting that *L. plantarum* might not be able to transform the flavonoid present in the juices (Karnišová Potocká *et al*., 2021). Nevertheless, these results showed that flavonoids were present in the juices.

Total phenolic content

The TPC of UBG, FBG-48, and FBG-96 showed significant difference ($p < 0.05$) (Figure 3B). The TPC significantly increased after 48 h of fermentation, consistent with the study by Gao *et al*. (2019) who reported an increase in TPC in fermented BG. The TPC of FBG-48 was 27.351 ± 0.222 mg GAE/g dry extract, the highest among the three samples, followed by UBG at 25.018 ± 0.238 and FBG-96 at 21.965 ± 0.287 mg GAE/g dry extract, respectively.

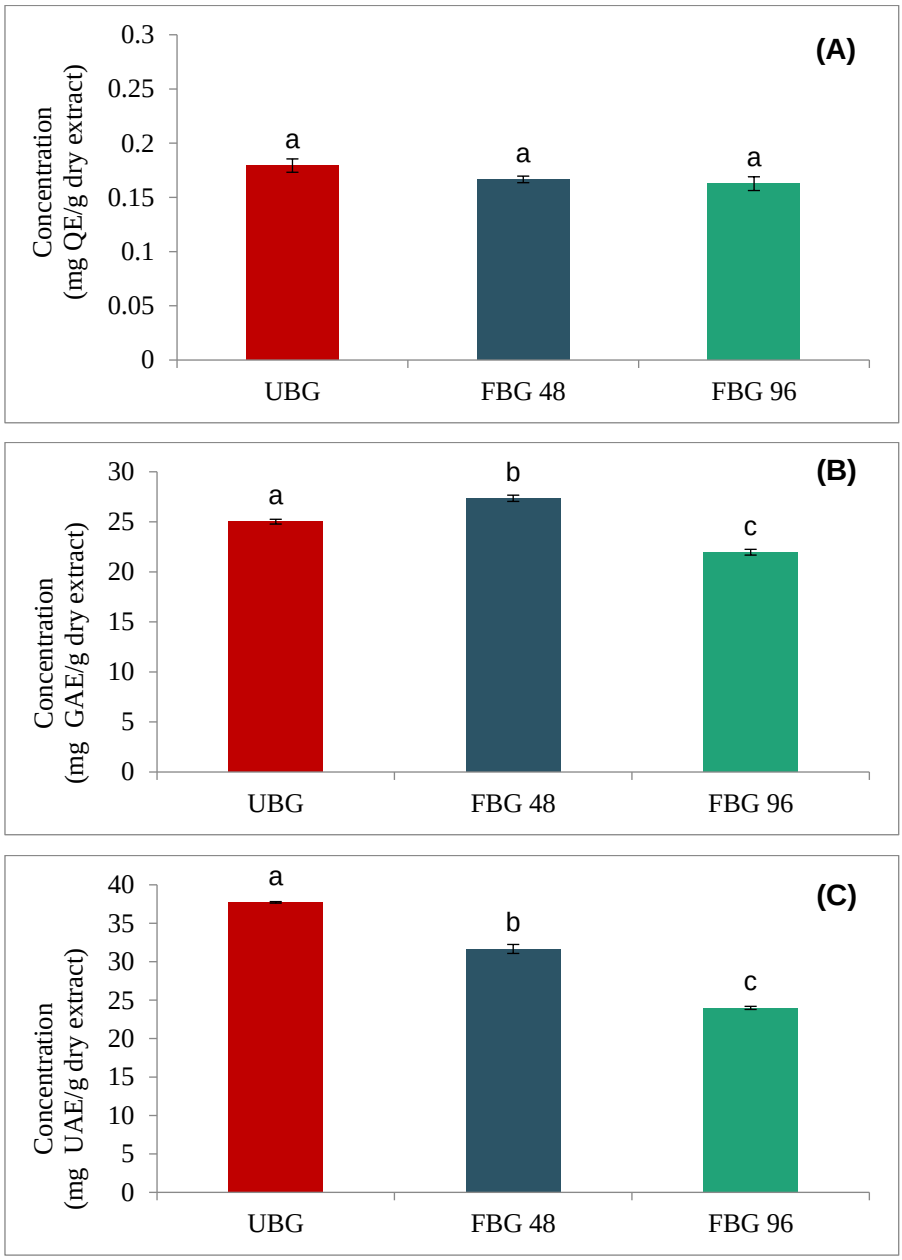

Figure 3. Total phytochemical contents of UBG, FBG-48, and FBG-96. Total flavonoid content **(A)**, total phenolic content **(B)**, and total triterpenoid content **(C)**. Data are mean ± standard deviation of triplicates (n = 3). Different lowercase letters indicate significant differences ($p < 0.05$).

The increase in TPC might have been attributed to the release of corresponding aglycones due to the hydrolysis of glycosides by enzymes excreted by *L. plantarum*, such as glucosidases and esterases (Rodríguez *et al*., 2009; Shahidi and Yeo, 2016). Conversely, the TPC of FBG-96 decreased, which might have been due to the degradation of total soluble phenolics into small molecules by *L. plantarum* (Kaprasob *et al*., 2017). These results showed that phenolics were present in the juices, and fermentation influenced the TPC in them.

Total triterpenoid content

The TTC of UBG, FBG-48, and FBG-96 was significantly different ($p < 0.05$) (Figure 3C). The TTC decreased as the fermentation time increased. This was in accordance with the total saponin content reported by Gao *et al*. (2019). UBG had the highest TTC among the three samples at 37.721 ± 0.098 mg UAE/g dry extract, followed by FBG-48 at 31.659 ± 0.584 and FBG-96 at 23.991 ± 0.203 mg UAE/g dry extract.

The decrease in TTC might have been

attributed to the biotransformation of triterpenoid glycoside into its corresponding aglycone with some structural modification (Mazlan *et al*., 2015). Therefore, the number of triterpenoids that could react with the reagents decreased. This indicated that fermentation influenced the TTC in the juices, and results showed that triterpenoids were present in them.

LC/MS

The flavonoid tentatively identified in UBG was suspected to have a structure similar to that of apigenin-7-(2-O-apiosylglucoside) (Table 1). This flavonoid glucoside was suspected to not be biotransformed as it was still detected in FBG-48 and FBG-96. This result was in accordance with the TFC result, wherein there was no significant difference in flavonoid content among UBG, FBG-48, and FBG-96. This might have been attributed to the inability of *L. plantarum* to biotransform apigenin-7-(2-O-apiosylglucoside) into its aglycone. Apigenin-7-(2-O-apiosylglucoside) can solely be biotransformed into its aglycone by β-apiosidase and β-glucosidase, in which β-apiosidase is only produced by fungi, mostly *Aspergillus* spp. (Karnišová Potocká *et al*., 2021). Furthermore, an organic acid, suspected to have a structure similar to that of jasmonic acid, was found in FBG-48 and FBG-96 (Table 1), consistent with studies by Mazlan *et al*. (2015) and Gao *et al*. (2019). The presence of jasmonic acid might have resulted from the activities of lipases, esterases, and cellulose, which might facilitate the liberation of jasmonic acid from chloroplast membranes.

The phenolics tentatively identified in UBG were suspected to have a similar structure to tannic acid (Table 1). Interestingly, tannic acid was not detected in FBG-48 and FBG-96. This might have been due to the biotransformation of tannic acid by tannase, which product was subsequently methylated by methylase into syringic acid (Rodríguez *et al*., 2009) (Table 1), as it was detected in FBG-48 and FBG-96. Furthermore, caffeic acid and *p*-coumaric acid (Table 1) are classified as insoluble-bound phenolics, which are covalently bound to cell walls. Hence, the presence of caffeic and *p*-coumaric acids in the fermented bitter gourd samples might have been due to the release of the corresponding phenolic acids by cellulases and esterases from the cell walls (Shahidi and Yeo, 2016). The presence of caffeic and *p*-coumaric acids was in accordance with the study done by Gao *et al*. (2019). However, caffeic acid was not detected in FBG-96 (Table 1). This absence might have been due to the degradation of simple phenolic compounds as a possible detoxification mechanism of LAB (Kaprasob *et al*., 2017).

The cucurbitane-type triterpenoid tentatively identified in UBG was suspected to have a structure similar to that of momordicoside Q (Table 1). Moreover, momordicoside Q was still detected in FBG-48 but not in FBG-96, in which its aglycone, with a structure similar to kuguacin P (Table 1), was present. This biotransformation might have been due to the activities of β-glucosidase and alcohol dehydrogenase. β-glucosidase hydrolysed the glycosidic linkage to release the corresponding aglycone, where its OH group at C-3 was then oxidised by alcohol dehydrogenase (Rodríguez *et al*., 2009; Mazlan *et al*., 2015). This aligned with the decrease in TTC due to the absence of OH group at C-3 that could react with the reagents (Hiai *et al*., 1976).

Antioxidant activity

The IC_{50} values of UBG, FBG-48, and FBG-96 were found to be significantly different ($p < 0.05$) and higher than that of quercetin (Table 2). This proved that the antioxidant activity of crude extracts was lower than that of the corresponding pure compound. The antioxidant activity of UBG is similar to the results of Perumal *et al*. (2021), and the overall antioxidant activities of the three samples were considered low. FBG-48 had the highest antioxidant activity, followed by UBG and FBG-96. There was 10.77% increase in the antioxidant activity of FBG-48 compared to UBG, which was similar to the study by Hartayanie *et al*. (2018). This antioxidant activity result showed a very strong correlation with the TPC result ($r = -0.987$) of the three samples, indicating that there was a positive correlation between TPC and the antioxidant activity of UBG, FBG-48, and FBG-96.

The increase in the antioxidant activity of FBG-48 might have been due to the biotransformation of phenolics during fermentation. It can be seen from the LC/MS profile of UBG and FBGs (Table 1) that caffeic, *p*-coumaric, and syringic acids were found in FBG-48, but were not detected in UBG. In contrast, caffeic acid was not detected in FBG-96, whereas *p*-coumaric and syringic acids were still present (Table 1). Therefore, the decrease in the antioxidant activity of FBG-96 might have been influenced by the degradation of phenolic compounds by *L. plantarum* (Kaprasob *et al*., 2017). In addition,

Table 1. LC/MS profile of suspected bioactive compounds in unfermented and fermented bitter gourds.

Bioactive compound	***m/z***		**Sample peak area**		
	Theoretical	**Experimental**	**UBG**	**FBG-48**	**FBG-96**
Apigenin 7-(2-O-apiosylglucoside)	564.49	563	0.002 ± 0.1 (× 10^4)^a	0.003 ± 0.4 (× 10^4)^a	0.002 ± 0.4 (× 10^4)^a
Momordicoside Q	653	656	1.8 ± 0.5 (× 10^4)^a	1.2 ± 1.6 (× 10^4)^b	n.d.
Tannic acid	1701.2	1699.8	6.5 ± 2.3 (× 10^4)^a	n.d.	n.d.
Jasmonic acid	210.27	211	n.d.	0.002 ± 0.4 (× 10^4)^a	0.001 ± 0.7 (× 10^4)^a
Syringic acid	198.17	200	n.d.	2.9 ± 1.9 (× 10^4)^a	2.0 ± 2.2 (× 10^4)^b
Kuguacin P	428.6	426.6	n.d.	5.1 ± 1.6 (× 10^4)^a	2.2 ± 0.6 (× 10^4)^b
p-Coumaric acid	164.158	164.1	n.d.	7.7 ± 0.4 (× 10^4)^a	4.6 ± 1.1 (× 10^4)^b
Caffeic acid	180.16	181.6	n.d.	2.7 ± 1.1 (× 10^4)^a	n.d.

n.d.: not detected. Data are mean ± standard deviation of triplicates (n = 3). Different lowercase letters in the same row indicate significant differences ($p < 0.05$).

Table 2. Bioactivities of UBG, FBGs, quercetin, and aminoguanidine.

Samples	Antioxidant activity	Antiglycation activity		Anti-aggregation activity	
	DPPH IC_{50} (μg/mL)	**Concentration**	**%inhibition**	**Concentration**	**%inhibition**
Quercetin	3.632 ± 0.154^{a}	-	-	50 μg/mL	75.704 ± 0.434^{a}
AG	-	1 mol/L	87.408 ± 0.434^{a}	-	-
UBG	1472.511 ± 0.013^{b}	0.1×10^{5} μg/mL	55.740 ± 0.932^{b}	500 μg/mL	41.432 ± 0.332^{b}
FBG-48	1313.962 ± 0.033^{c}	0.1×10^{5} μg/mL	60.577 ± 0.359^{c}	500 μg/mL	61.502 ± 0.996^{c}
FBG-96	1858.545 ± 0.003^{d}	0.1×10^{5} μg/mL	58.171 ± 0.378^{d}	500 μg/mL	65.728 ± 1.992^{d}

Data are mean ± standard deviation of triplicates ($n = 3$). Different lowercase letters in the same column indicate significant differences ($p < 0.05$).

the antioxidant activity of UBG might have been due to the presence of tannic acid (Table 1). These results showed that fermentation affected the antioxidant activity in the BG juices, which was in agreement with previous research by Hartayanie *et al*. (2018) and Gao *et al*. (2019).

Antiglycation activity

The antiglycation activities of UBG, FBG-48, and FBG-96 were significantly different ($p < 0.05$). The antiglycation activity of aminoguanidine was observed to be the highest. Nevertheless, the antiglycation activity of FBG-48 and FBG-96 increased (Table 2), with FBG-48 exhibiting 8.68% increase in antiglycation activity compared to UBG. FBG-48 had the highest glycation inhibition activity, followed by FBG-96 and UBG. This might have been due to the presence of syringic acid in FBG-48 and FBG-96 (Table 1), which is believed to have the ability to protect the protein structure on its lysine (Lys93, Lys232, and Lys261) binding site (Bhattacherjee and Datta, 2015). Furthermore, caffeic acid was also present in FBG-48 (Table 1), which might have contributed to the increase of antiglycation activity through radical scavenging and the protection of protein structure. Cao *et al*. (2019) reported that caffeic acid binds to Arg185 and Tyr137 of BSA, stabilising the protein's structure and preventing glycation. However, caffeic acid was not detected in FBG-96.

In addition, the antiglycation activity of UBG might have been due to the presence of tannic acid (Table 1) which can trap active dicarbonyl compounds, may hinder protein cross-linking mainly in the final stage of AGEs formation, and protect the protein structure through hydrogen bond and hydrophobic interaction (Huang *et al*., 2019). Furthermore, the presence of momordicoside Q (Table 1) may play a role in the antiglycation activity of UBG and FBG-48, as did its cucurbitane-type triterpenoid glycoside counterpart called charantin in the study by Aljohi *et al*. (2016). Concurrently, the biotransformation of momordicoside Q into kuguacin P might have contributed to the increased antiglycation activity of FBG-48 and FBG-96. These results showed that fermentation influenced the antiglycation activity in the BG juices.

Anti-aggregation activity

The anti-aggregation activities of UBG, FBG-48, and FBG-96 were significantly different ($p < 0.05$), with quercetin exhibiting the highest activity (Table 2). This result strongly correlated with the TTC result ($r = -0.909$) of the three samples. The anti-aggregation activity of FBG-48 and FBG-96 at 500 μg/mL, was 49.7 and 60% higher than that of UBG, respectively (Table 2). Furthermore, FBG-96 exhibited the highest anti-aggregation activity, followed by FBG-48 and UBG. Moreover, the presence of caffeic acid (Table 1) might have contributed to the increase in the anti-aggregation activity of FBG-48. Precupas *et al*. (2017) found that caffeic acid could bind to the protein (Tyr149, Arg198, Lys204, and Cys467) through hydrogen bonding, electrostatic interaction, and hydrophobic interaction, resulting in the stabilisation of the protein structure upon heating.

However, caffeic acid was not detected in FBG-96. The anti-aggregation activity of FBG-96 might have been due to the biotransformation of momordicoside Q to kuguacin P, which has a structure similar to that of its cucurbitane-type triterpenoid counterpart, momordicoside I. Momordicoside I was reported to bind with non-polar residues near the core C-terminal region of human islet amyloid polypeptide (hIAPP) through hydrophobic, electrostatic, and hydrogen bonding, thereby impeding hIAPP aggregation (Zheng *et al*., 2022).

The anti-aggregation activity of UBG might have been due to the presence of tannic acid and momordicoside Q. Tannic acid has been reported to inhibit β-amyloid peptide (Aβ) fibrillation in a dose-dependent manner (Ono *et al*., 2004). Meanwhile, saponins such as ginsenoside K have been reported to decrease Aβ plaque deposition, and increase the heat stability of BSA (Zong *et al*., 2019). Thus, momordicoside Q, categorised as a saponin, may have a similar ability.

In contrast, the protein aggregation inhibition activity of the three samples at 5,000 μg/mL was negative, with the lowest activity exhibited by UBG, followed by FBG-48 and FBG-96, respectively. This indicated that ligands in high concentrations might have caused protein aggregation at high temperatures, and that both ligand concentration and temperature affected the protein structure. These results were in accordance with the study by Precupas *et al*. (2017), which found that BSA in high concentrations of caffeic acid at temperatures above 334 K experienced denaturation, whereas in lower concentrations, there was structural stabilisation under the same thermal

conditions. Furthermore, tannic acid, which was also detected in UBG (Table 1), was reported to alter protein conformation, such as in concanavalin A, resulting in the formation of aggregates (Khan *et al*., 2018). These results showed that fermentation influenced the anti-aggregation activity in the BG juices.

Conclusion

In general, fermentation was observed to influence the physical profile, chemical profile, and bioactivities of bitter gourd. Fermentation reduced the pH, and altered the colour of the liquid and sediment of the bitter gourd juice. Subsequently, fermentation increased TPC and decreased TTC. However, fermentation did not affect TFC. Nonetheless, fermentation was suspected to have caused a phenolic acid and triterpenoid glycoside biotransformation into their corresponding aglycones. Furthermore, fermentation increased the antioxidant, antiglycation, and anti-aggregation activities of bitter gourd juice. These results showed that bitter gourd juice could have a promising role in type-II diabetes complication treatment through its protein aggregation inhibition activity, and that fermentation could enhance its bioactivities. However, future studies using LC/MS-MS and an *in-vivo* approach may be considered to further determine the biotransformation of bitter gourd juice by *L. plantarum*, as well as its bioactivities regarding the increase of bioavailability of the fermented products.

Acknowledgement

The present work was financially supported by the funding source other than the state budget of the Faculty of Science and Mathematics, Diponegoro University 2022 (grant no.: 1263A/UN7.5.8/PP/2022).

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