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Development of a Fermented Bitter Gourd (*Momordica charantia*)–Grape Beverage Using Optimized Conditions

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Abstract: Bitter gourd beverages are well acclaimed for their health benefits, which have propelled their consumption. The beverages are prepared through a fermentation process, which is one of the oldest means of preserving and enhancing the flavour of many foods. Optimized conditions for the fermentation of a bitter gourd–grape beverage were investigated in our previous study. In the present study, a statistical comparison (one-way analysis of variance (ANOVA), Tukey's honestly significant difference (HSD) test and an independent *t*-test)) of grape juice, bitter gourd juice and the fermented bitter gourd–grape beverage (with and without enzymes) was carried out to find significant differences among the products. Alcohol was found to be consistent for the four products with $p > 0.05$, whereas significant differences ($p \leq 0.05$) in the pH, antioxidant activity (ferric reducing antioxidant assay (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS)), total titratable acidity (TTA), total soluble solids (TSS), total flavonoid content (TFC) and total phenolic content (TPC) were observed. The fermented bitter gourd–grape beverage (FBGGB) with enzymes had the highest antidiabetic potential content (27.07). The data obtained demonstrate that fermentation indeed enhances the biochemical function of vegetables (in this case, bitter gourd) and could thus be considered for the commercial processing of bitter gourd.

Keywords: fermentation; antidiabetic potential; antioxidant activity; optimum conditions; enzyme

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1. Introduction

Fruits and vegetables constitute a significant part of the food basket. They are relatively good sources of energy and rich sources of phytochemicals and nutrients, such as carbohydrates, protein, carotene, ascorbic acid, calcium, iron and trace elements [1]. In recent times, consumers have become more conscious about their health, such that they increasingly look for beneficial natural products [2].

In the vegetable category, bitter gourd (*Momordica charantia*), which is also known as balsam pear, karela and bitter melon, is among the highly popular vegetables in the Asian and Indian communities, and it belongs to the *Cucurbitaceae* family. In Latin, the term *Momordica* translates as "to bite", which describes the spiky ends of the leaves of this vegetable. It is considered one of the main vegetables of the world and has great trade and industry value [3].

Bitter gourd has high nutritional value and is popular among the gourds, but it may not be liked by many people due to its bitter taste and flavour [4]. All components of the plant, including the leaves, stems, fruits and seeds, are widely utilized in folk medication. Bitter gourd consumption is steadily increasing, not only because of its nutritional value but also because of its medicinal benefits [5,6]. Bitter gourd has pharmacological importance due to the reported presence of advantageous phytochemicals that have immune-enhancing, antioxidant, antidiabetic and antimutagenic properties [7].

Beneficial plant chemicals with protective properties are known as phytochemicals. Plants produce phytochemicals to protect themselves, but recent studies have shown that they have the ability to also protect humans from diseases [8]. They are linked to protection against and/or treatment of chronic conditions, such as cardiovascular disease, cancer, increased blood pressure, diabetes and other chronic conditions [9]. Bitter gourd contains vicine, an alkaloid glycoside, which is found in the seeds, and its benefit is its hypoglycaemic activity [10]; charantin, which is found in the vegetable and its benefits come from a non-nitrogenous substance with hypoglycaemic properties [11]; momordicosides A and B, which are found in the seeds, and their benefit is the inhibition of tumour growth due to triterpene glycosides [12]; polypeptide-p, which is found in the seeds and fruits, and its health benefit is its hypoglycaemic peptide, called plant insulin [7]; and carotenoids, which are found in the seeds and fruit, and possess antioxidative activity that decreases the incidence of cancer as well as cardiovascular illnesses [13]. Efird et al. [14] conducted a study that showed that blood sugar levels were lowered after fresh bitter gourd juice was administered to diabetic rabbits. The minimum dose required for causing a significant impact on blood glucose ($p \leq 0.05$) was 5 mL.

Furthermore, bitter gourd has an array of beneficial bioactive compounds, such as antioxidants, vitamins, dietary fibre and minerals [15]. Several studies have demonstrated that bitter gourd, in addition to its antioxidant properties, has excellent free radical scavenging properties [16–18]. The antioxidant activity of phenolic compounds varies. According to several researchers, total phenolics have a significant correlation with antioxidant activity [18].

It has been observed that peristalsis of food through the bowel until it is excreted from the body is stimulated by bitter gourd, helping to relieve indigestion and constipation [13]. Studies have indicated that dried powdered bitter gourd in honey has significant and dose-dependent antiulcerogenetic effects in rats [19]. Joseph and Jini [7] also indicated that bitter gourd juice was very effective in treating blood problems, such as blood boils and itching from toxaemia. Bitter gourd extracts possess immune-boosting properties and its isolated compounds have a various impacts on the immune system. However, its immunostimulant action has been credited to its ability to enhance the production of numerous compounds such as MRK-29, MAP-30, momorcharin and lectin. Moreover, the plant possesses anti-inflammatory activity, which inhibits proinflammatory cytokines, namely interferon (IFN)-c and natural killer cell production. Bitter gourd juice assists in building immunity and increases resistance against contamination by infections in the body [20].

Studies and experiments have been carried out on the possible pharmaceutical properties of bitter gourd [21], while work is currently ongoing on different bitter gourd preservation methods, as well as its toxic effects [22]. Wine is a product of the fermentation of grape juice, and enzymes are among the key components of alcohol production. The grape itself generates many of these enzymes, as well as microorganisms present during winemaking and the microflora present on the grape [23]. Most enzymes used in winemaking preparations come from the pectinase family, though glucanases, xylanases and proteases are also commonly used to enhance the clarification and the processing of beverages [24].

Fermentation in the beverage industry results in the conversion of sugars into ethanol, antibacterial compounds and acidulants, and it increases protein and carbohydrate digestibility, resulting in increased nutritional value and flavour [13]. Fermented vegetables have better functionality than unfermented ones [25].

The physical characteristics and chemical composition of minimally processed vegetables have short shelf lives, since they are subject to rapid microbial spoilage, which would alter them in not always desirable ways [26]. In contrast, fermented beverages with pre- and probiotic properties have become increasingly popular over the past few decades because they provide several health benefits [27]. Fermentation of bitter gourd juice could enhance the nutritional consistency and adjust the taste of the juice, creating a matured drink with a better taste profile [28].

In this study, bitter gourd was fermented using a starter culture concentration of 4.07 v/v. The present study was a comparison of the physicochemical properties of bitter gourd juice, grape juice and two fermented bitter gourd–grape beverages (with and without enzymes). Furthermore, an investigation of the antioxidant activity and anti-diabetic potential of these products was carried out.

2. Materials and Methods

2.1. Materials and Raw Material Processing

Fresh bitter gourds (*Momordica charantia*) were collected from Mpumalanga province in South Africa. To remove adhering foreign materials, fresh bitter gourd (*Momordica charantia*) was sorted and thoroughly washed in running water. It was then cut at both ends and blended (Milex, Sandton, South Africa). The grapes were washed and blended likewise, and the two slurries were mixed at 35% and 65%, respectively. Yeasts (Anchor Yeast, Lallemand, Cape Town) were transported in a polystyrene cooler box (with dry packs). *Saccharomyces cerevisiae* yeast in conjunction with a non-*Saccharomyces* yeast *Metschnikowia pulcherimma* was weighed at a 1:1 ratio under sterile working conditions, and the yeast powder was then dissolved in 100 mL of distilled water and incubated at 28 °C for 15 min. The bitter gourd juice and grape juice mixture was inoculated, with the starter culture (4.07 mL) acting as a reference strain.

2.2. Production of the Bitter Gourd–Grape Beverage

The optimum conditions used for the processing/production of the beverage were obtained from a previous optimization study carried out by means of response surface methodology (RSM) software. The fermentation conditions were a fermentation temperature of 28.60 °C, a fermentation time of 52.60 h and a starter culture concentration of 4.07 v/v. The enzyme used with the bitter gourd–grape beverage was pectin esterase enzyme (50 mL) (Sigma-Aldrich, Modderfontein, South Africa) at 0.2%.

2.3. Determination of the Physiochemical and Microbial Properties

2.3.1. Alcohol (Degree Plato °P)

The alcohol content of the beverage was determined using a digital refractometer (Hanna Instruments (PTY) Ltd., Johannesburg, South Africa). A clean pipette was used to place 1 mL of the fermented beverage in the sample well. The Plato readings were recorded afterward.

2.3.2. pH

The pH meter was first calibrated with standard buffers of pH 4 and 7. The pH measurement was performed as indicated by Tomovska et al. [29], for which 10 mL of the fermented samples was transferred into sterile containers, and the pH value was measured with a pH meter (Hanna Instruments (PTY) Ltd., Johannesburg, South Africa).

2.3.3. Total Titratable Acidity (TTA) (Lactic Acid %)

The approved method of the American Association of Cereal Chemists (AACC) 02–31.01 [30] was used to determine the total titratable acidity. This entailed dissolving 10 g of the sample in 100 mL of distilled water. Thereafter, the solution was mixed well, and 0.5 mL of 1% phenolphthalein indicator was added. The prepared solution was titrated with 0.1 N sodium hydroxide (NaOH) (Sigma-Aldrich, Modderfontein, South Africa), while stirring continuously until a faint pink colour was observed.

2.3.4. Total Soluble Solids (TSS) (g/100 g)

The total soluble solids of the samples were determined using a digital refractometer (Hanna Instruments (PTY) (Johannesburg, South Africa). Prior to analysis of the TSS content of the sample, the refractometer was calibrated with distilled water. A clean pipette

was used to place 1 mL of the sample in the sample well; thereafter, the readings of the samples were recorded accordingly.

2.3.5. Total Phenolic Content (TPC) (mg GAE/g)

The Folin–Ciocalteu (FC) method was used to determine the total phenolic content (TPC) as reported by Kupina et al. [31] with slight modifications. Extracts of 1 mL were pre-incubated with 1.5 mL of Folin–Ciocalteu (FC) reagent for 15 min at room temperature (25 °C), followed by the addition of 2 mL sodium carbonate (Na_2Co_3) (7.5%). The extracts were shaken and incubated for 30 min in the dark. Gallic acid was used as a standard, and the absorbance was read at 750 nm.

2.3.6. Total Flavonoid Content (TFC) (mg QE/g)

The total flavonoid content (TFC) was determined by using the aluminium chloride method as reported by Aryal et al. [32], with slight modifications. Extracts of 0.5 mL (1 mg/mL) were mixed with 0.5 mL of $NaNO_2$ (2.5%) and incubated for 5 min; thereafter, aluminium trichloride ($AlCl_3$) (10%) and sodium hydroxide (NaOH) (2%) were added. Quercetin was used as a standard, and the absorbance was read at 450 nm.

2.3.7. Antioxidant Activity

The (2,2-Diphenyl-1-picrylhydrazyl) DPPH Assay (μM TE/mL)

The (DPPH) assay was performed according to the method reported by Kedare and Singh [33], with some modifications. A DPPH solution (0.4 mM) in methanol was stirred for 20 min until all particles were dissolved, and the absorbance of the solution was adjusted to 1.1 at 490 nm. Thereafter, 40 µL of the extracts was mixed with 160 µL of the DPPH solution and incubated for 10 min in the dark at room temperature (25 °C). The reduction in the DPPH radical was measured by monitoring the decrease in absorption at 490 nm, and the radical scavenging was calculated.

Ferric Reducing Antioxidant Power (FRAP) Assay (μM Fe (II) E/mL)

The FRAP ability of the extracts was assessed following the method reported by Chaves et al. [34]. FRAP reagent was freshly prepared from 2.5 mL of 10 mM 2,4,6-Tris (2-pyridyl)-1,3,5-triazine (TPTZ) solution in 40 mM HCl, 2.5 mL of 20 mM $FeCl_3$ and 25 mL of 0.3 M acetate buffer (pH 3.6). Aliquots (40 µL) of the studied extracts were added. In parallel, a solution containing 0.24 µL of ultrapure distilled water and 1.8 mL of the FRAP reagent was prepared as a negative control, and the absorbance was measured at 595 nm.

The 2,2-Azinobis (3-Ethyl-Benzothiazone-6-Sulfonic Acid) (ABTS) Assay (μM TE/mL)

The ABTS test was performed using the method reported by Martysiak-Żurowska and Wenta [35], with slight modifications. The ABTS+ solution was obtained by mixing ABTS+ (7 mM) at pH 7.4 with potassium sulphate (2.5 mM). The solution was stored in the dark at room temperature (25 °C) for 12 h and then used within 16 h. Samples (20 µL) of each diluted aqueous extract were mixed with 180 µL of the ABTS+ solution. The samples were left for 5 min at room temperature, followed by measuring the absorbance at 750 nm with potassium sulphate (2.5 mM).

2.3.8. Antidiabetic Potential

For the antidiabetic potential test, 3,5 dinitrosalicylic acid (Sigma-Aldrich, Modderfontein, South Africa) was used. The 3,5-dinitrosalicylic acid (DNSA) method reported by Gonçalves et al. [36] was used, with some modifications. An analysis of the ability of samples to reduce sugars was conducted in order to determine their antidiabetic potential. The reaction mixture included 5 g of 3,5-dinitrosalicylic acid in 250 mL of distilled water at 80 °C. When this solution reached room temperature, 100 mL of NaOH and 150 g of potassium sodium was added, and the volume was completed with distilled water to 500 mL. Thereafter, 500 µL of DNSA acid reagent and 500 µL of the sample were added

to the test tubes. The test tubes were immersed in the boiling water bath (100 °C) for 5 min and then cooled in cold water, while 5 mL of distilled water was added to each test tube, resulting in the final reaction mixture. The addition of water while the tubes were immersed in cold water was performed to immediately stop the reaction. When the test tubes reached room temperature, 300 µL of the resulting reaction mixture of each test tube was transferred to the well of a microtiter plate, and the absorbance was measured at 540 nm.

2.3.9. Microbial Load Determination (CFU/mL)

The pour plate technique for microbial load determination was adopted from Sebastia et al. [37], using plate count agar (PCA) and De Man, Rogosa and Sharpe (MRS) agar. For determination of the total number of live aerobic bacteria in a sample, a bacteriological substrate (PCA) was used, which was not a selective medium. To favour the growth of *Lactobacilli*, which is prominent in fermented foods and beverages, MRS was used as a selective culture medium. Exactly 1 mL of the beverage was pipetted into petri dishes; thereafter, PCA (Sigma-Aldrich, Modderfontein, South Africa) was added into these dishes and the dishes were rotated gently to ensure uniform mixing of the sample with agar. The petri dishes were incubated at 30 °C for 3 days (72 h) and the number of colonies was counted. For microbial enumeration with MRS media, the MRS agar (Sigma-Aldrich, Modderfontein, South Africa) was prepared and poured into sterile petri dishes. The beverage sample was weighed into the diluent and left for 30 min, shaking periodically, then 0.2 mL of the prepared beverage sample was pipetted onto the MRS agar and spread. The petri dishes were incubated at 30 ± 1 °C and examined after 3 days (72 h).

2.4. Statistical Analysis

Descriptive statistics with values of the mean, standard deviations (SD) and maximum/minimum values were used to carry out an exploratory analysis of the different products and their contents. Independent sample *t*-tests carried out in SPSS version 27 (IBM Corp, Armonk, NY, USA) were used to determine if there were any significant differences between two sample groups. If a comparison was made for more than two groups, a one-way analysis of variance (ANOVA) was used to determine whether there were any statistically significant differences between the means of the independent (unrelated) groups. Tukey's honest significant difference (HSD) test was used to examine the source of the significantly different groups further.

3. Results and Discussion

3.1. Effects on Antioxidant Activity

The mean, standard deviation, minimum and maximum values of the ferric reducing antioxidant assay (FRAP) antioxidant activity for the four products are presented in Table 1 below. A one-way ANOVA (Table S1) showed that the FRAP antioxidant activity among the four products had a significant difference ($p \leq 0.05$ level). The mean score was obtained by using post hoc comparisons via Tukey's HSD test; grape juice (340.90 ± 0.1) was significantly different from the bitter gourd juice (M = 280.73), FBGGB with enzymes (M = 371.57) and FBGGB without enzymes (M = 571.03). It was also observed that FBGGB without enzymes had the highest FRAP content, and bitter gourd juice had the lowest.

Table 2 below presents the mean, standard deviation, minimum and maximum 2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS) antioxidant activity values (in µmol/L Fe(II)/g) for the four products. Grape juice had the highest mean ABTS content, followed by FBGGB without enzyme, and bitter gourd juice had the lowest mean value. A one-way ANOVA (Table S2) was conducted to compare the ABTS antioxidant activity in the products. The results revealed that the ABTS antioxidant activity of the four products had significant differences ($p \leq 0.05$ level). Post hoc comparisons using Tukey's HSD test indicated that the mean score for the grape juice (M = 234.17) was significantly different

from that of the bitter gourd juice (M = 12.47), FBGGB with enzymes (M = 27.77) and FBGGB without enzymes (M = 40.30).

Table 1. Descriptive statistics of FRAP antioxidant activity in the four products.

	Mean	Std. Deviation	Minimum	Maximum
Grape juice	340.90	0.10	340.80	341.00
Bitter gourd juice	280.73	0.12	280.60	280.80
Fermented bitter gourd–grape beverage (FBGGB) with enzymes	371.57	0.12	371.50	371.70
Fermented bitter gourd–grape beverage (FBGGB) without enzymes	571.03	0.058	571.00	571.10

Table 2. Descriptive statistics of ABTS antioxidant activity in the four products.

	Mean	Std. Deviation	Minimum	Maximum
Grape juice	234.17	0.06	234.10	234.20
Bitter gourd juice	12.47	0.06	12.40	12.50
Fermented bitter gourd–grape beverage (FBGGB) with enzymes	27.77	0.06	27.70	27.80
Fermented bitter gourd–grape beverage (FBGGB) without enzymes	40.30	0.17	40.10	40.40

Table 3 below shows that bitter gourd juice had the highest 2,2-diphenyl-1-picrylhydrazyl (DPPH) antioxidant activity (419.47), followed by FBGGB without enzymes (81.07) and FBGGB with enzymes (51.07), and grape juice had the lowest content (35.77). Analysis of variance (ANOVA) was carried out to analyse the DPPH antioxidant activity in the products. There was a significant difference in the amount of DPPH antioxidant activity in the four products. The results obtained from using Tukey's HSD test showed that there was a significant difference in the amount of DPPH antioxidant activity in each product.

Table 3. Descriptive statistics of DPPH antioxidant activity in the four products.

	Mean	Std. Deviation	Minimum	Maximum
Grape juice	35.77	0.25	35.50	36.00
Bitter gourd juice	419.47	0.12	419.40	419.60
Fermented bitter gourd–grape beverage (FBGGB) with enzymes	51.07	0.057	51.00	51.10
Fermented bitter gourd–grape beverage (FBGGB) without enzymes	81.07	0.057	81.00	81.10

Grapes are a rich source of the antioxidant flavonoids catechin, epicatechin, quercetin and anthocyanins [38,39]. Similar to our findings, Cosme et al. [40] reported that grapes as well as their derivatives such as grape juice were some of the richest sources of phenolic compounds among the fruits and exert strong antioxidant capacity.

Aljohi et al. [41] showed that bitter gourd (*Momordica charantia*) had antioxidant effects (in that they inhibited free radical activity). Polyphenolic compounds might be responsible for the free radical scavenging activities associated with bitter gourd. A report has suggested a relationship between the free radical scavenging activity of polyphenolic substances and their chemical structures [42].

Low-molecular-weight antioxidant compounds, such as polyphenols, flavonoids and flavonols, have been identified in bitter gourd [43,44]. Similar to the results of a study conducted by Krishnendu and Nandini [45], the DPPH antioxidant activity of bitter gourd was the highest among other antioxidant activity methods used. Rathor and Pandey [46] reported that the ABTS inhibition activity was found to be lower than DPPH inhibition activity and FRAP inhibition activity under the same conditions.

3.2. Effects on Antidiabetic Potential

FBGGB with enzymes had the highest antidiabetic potential (27.07), followed by FBGGB without enzymes (25.07), as shown in Table 4 below. One-way ANOVA showed that there was a significant difference in the level of antidiabetic potential in the three products. The results obtained from using Tukey's HSD test showed that there was a significant difference in the level of antidiabetic potential in each product. These results suggest that the levels of antidiabetic potential in the three products are different, with FBGGB with enzymes having the highest content and bitter gourd juice having the lowest.

Table 4. Descriptive statistics of antidiabetic potential in the three products.

	Mean	Std. Deviation	Minimum	Maximum
Bitter gourd juice	18.03	0.06	18.00	18.10
Fermented bitter gourd–grape beverage (FBGGB) with enzymes	27.07	0.12	27.00	27.20
Fermented bitter gourd–grape beverage (FBGGB) without enzymes	25.07	0.12	25.00	25.20

One of the best studied medicinal plants with antidiabetic effects is bitter gourd [7], a plant with known hypoglycaemic properties in both animals and humans [47]. Several studies have shown that bitter gourd significantly lowered blood glucose concentrations in diabetic patients [48] and in diabetic rats [7].

3.3. Relationship between Antioxidant Activity and Antidiabetic Potential

Pearson's correlation coefficient (SPSS version 27, IBM Corporation, New York, NY, USA) was used to investigate if there is a relationship between antioxidant activity and antidiabetic potential. There was a positive correlation between the results (Table 5) of antidiabetic potential and ABTS antioxidant activity ($r = 0.78$, $p = 0.013$) and a negative correlation between antidiabetic potential and DPPH antioxidant activity ($r = -0.99$, $p < 0.005$). This suggests that as antidiabetic potential increased, ABTS antioxidant activity also increased and DPPH antioxidant activity reduced. However, there was no significant relationship between antidiabetic potential and FRAP antioxidant activity.

3.4. Comparison among Fermented Bitter Gourd–Grape Beverage (FBGGB) with and without Enzymes, Grape Juice and Bitter Gourd Juice

An independent *t*-test (SPSS v27) was used to compare grape juice and FBGGB with enzymes (Table S3). The *t*-test results showed that only alcohol was not significantly different between the two samples. However, the amount of TTA, antioxidant activity FRAP and antidiabetic potential were significantly different when comparing grape juice and FBGGB with enzymes. A comparison of grape juice and FBGGB without enzymes produced similar results, with only alcohol not being significantly different (Table S4).

The content of alcohol, pH, TTA, antioxidant activity and antidiabetic potential in bitter gourd juice when compared with FBGGB with and without enzyme (Tables S5 and S6), were significantly different. This was in agreement with another study by Devaki and Premavalli [28], which found that total phenols and antioxidant activity were positively affected by fermentation. The study conducted concluded that the bitter gourd beverage

after fermentation possessed nutritional and functional strength as well as being sensorily acceptable. Using a nine-point hedonic scale, an overall hedonic score of 6.7 was given by diabetic panellists and 6.2 was given by nondiabetic panellists.

Table 5. Correlations of antioxidant activity and antidiabetic potential.

		Antioxidants—FRAP	Antioxidants—ABTS	Antioxidants—DPPH	Antidiabetic Potential
Antioxidants—FRAP	Pearson's Correlation	1.00	−0.16	−0.50	0.58
	Sig. (2-tailed)		0.61	0.09	0.10
	N	12.00	12.00	12.00	9.00
Antioxidants—ABTS	Pearson's Correlation	−0.16	1.00	−0.49	0.78 *
	Sig. (2-tailed)	0.61		11.00	0.01
	N	12.00	12.00	12.00	9.00
Antioxidants—DPPH	Pearson's Correlation	−0.50	−0.49	1.00	−0.99 *
	Sig. (2-tailed)	0.99	0.11		0
	N	12.00	12.00	12.00	9.00
Antidiabetic potential	Pearson's Correlation	0.58	0.78 *	−0.99 *	1.00
	Sig. (2-tailed)	0.10	0.01	0	
	N	9.00	9.00	9.00	9.00

* Significant at $p \leq 0.05$.

3.5. Effects of Enzymes on Fermentation

An independent *t*-test was used to investigate the effects of enzymes. The alcohol content was not significantly different ($p \geq 0.05$); therefore, equal variance was assumed for the *t*-test. The results (Table 6) shows that the alcohol content in fermented BGW with and without enzymes was not significantly different ($p \geq 0.05$). However, the amount of TTA, antioxidant activity (FRAP) and antidiabetic potential were significantly different ($p \leq 0.05$) when comparing these two beverages. Equal variances were not assumed. Antioxidant activity, pH, TFC and TPC were all found to be significantly different. In summary, the results suggest that enzymes had an effect on the fermentation.

Table 6. Independent *t*-test of FBGGB with enzymes.

	t	Df	*p*	Significant/Not Significant
Alcohol	1.79	4.00	0.15	S
TTA	41.00	4.00	<0.005	N
Antioxidant activity—FRAP	−2676.13	4.00	<0.005	N
Antidiabetic potential	−21.21	4.00	<0.005	N

3.6. Effect on Alcohol Content

The mean, standard deviation and maximum values of alcohol content in the products are presented in Table S7. The mean values shows that bitter gourd juice had less alcohol content than the rest of the products. An ANOVA test was carried out to find if there was a difference in the alcohol content of the different products ($F(3,8) = 1339.07$, $p < 0.005$) (Table S8). A post hoc test using Tukey's HSD test (Table S9) of comparisons between product (i) and product (j) was carried out, and the mean difference and *p*-values (sig.) are given. The results obtained showed that there was a significant difference ($p \leq 0.05$) between the alcohol content of grape juice (M = 3.67) and bitter gourd juice (M = 0.1), and between that of grape juice and FBGGB with enzymes (M = 3.87). However, there was no significant difference ($p = 0.373$) between the alcohol content of grape juice (M = 3.67) and FBGGB without enzymes (M = 3.73). The results suggest that enzymes had an effect on reducing the alcohol content in the FBGGB.

3.7. Effect of Fermentation on pH, TTA, TSS, TFC and TPC

The effect of fermentation on other physiochemical characteristics, namely pH, TTA, TSS, TFC and TPC, are presented in Table 7 below. The table contains the mean, standard deviation and minimum/maximum values. Grape juice had the lowest (3.40) pH content, closely followed by FBGGB with enzymes (3.90), while bitter gourd juice (5.20) had the highest pH content. Grape juice had the highest value (10.13) for TTA, followed by FBGGB with enzymes (7.67). Bitter gourd juice had the lowest TSS content at (0.53) and the others were much closer to each other. FBGGB with and without enzymes had a similar TFC content and there was no significant difference between the two. However, there was a significant difference when grape juice (67.73) was compared with bitter gourd juice (318.40). In all products with grape juice, there was a significant difference in the TPC content (215.67) compared with bitter gourd juice (59.83), FBGGB with enzymes (341.07) and FBGGB without enzymes (411.07). Yeast was not detected in either FBGGB with or without enzymes.

Table 7. Effects on other physiochemical characteristics.

		Mean	Std. Deviation	Minimum	Maximum
pH	Grape juice	3.40	0	3.40	3.40
	Bitter gourd juice	5.20	0	5.20	5.20
	Fermented bitter gourd–grape beverage (FBGGB) with enzymes	3.90	0	3.90	3.90
	Fermented bitter gourd–grape beverage (FBGGB) without enzymes	4.80	0	4.80	4.80
TTA	Grape juice	10.13	0.06	10.10	10.20
	Bitter gourd juice	3.43	0.06	3.40	3.50
	Fermented bitter gourd–grape beverage (FBGGB) with enzymes	7.67	0.06	7.60	7.70
	Fermented bitter gourd–grape beverage (FBGGB) without enzymes	6.30	0	6.30	6.30
TSS	Grape juice	4.20	0.06	4.10	4.20
	Bitter gourd juice	0.53	0.06	0.50	0.60
	Fermented bitter gourd–grape beverage (FBGGB) with enzymes	4.40	0.06	4.30	4.40
	Fermented bitter gourd–grape beverage (FBGGB) without enzymes	3.83	0.06	3.80	3.90
TFC	Grape juice	67.73	0.06	67.70	67.80
	Bitter gourd juice	318.40	0	318.40	318.40
	Fermented bitter gourd–grape beverage (FBGGB) with enzymes	46.53	0.06	46.50	46.60
	Fermented bitter gourd–grape beverage (FBGGB) without enzymes	46.53	0.06	46.50	46.60

Table 7. *Cont.*

		Mean	Std. Deviation	Minimum	Maximum
TPC	Grape juice	215.67	0.12	215.60	215.80
	Bitter gourd juice	59.83	0.12	59.70	59.90
	Fermented bitter gourd–grape beverage (FBGGB) with enzymes	341.07	0.12	341.00	341.20
	Fermented bitter gourd–grape beverage (FBGGB) without enzymes	411.077	0.12	411.00	411.20

3.8. Microbial Analysis Results

The mean, standard deviation and minimum/maximum values of the lactic acid bacteria (LAB) count and total aerobic mesophiles are shown below in Table 8. Grape juice had the highest LAB count (4.70), followed by bitter gourd juice (3.83), and both FBGGBs had equally low values of 2.03. For the total aerobic mesophiles, grape juice had the highest value (8.87) and FBGGB with enzymes had the lowest at 1.07. One-way ANOVA showed that there was a significant difference in the LAB count and total aerobic mesophiles for all the products.

Table 8. Descriptive statistics of the microbial analysis.

		Mean	Std. Deviation	Minimum	Maximum
Microbial analysis—LAB count	Grape juice	4.70	0	4.70	4.70
	Bitter gourd juice	3.83	0.06	3.80	3.90
	Fermented bitter gourd–grape beverage (FBGGB) with enzymes	2.03	0.06	2.00	2.10
	Fermented bitter gourd–grape beverage (FBGGB) without enzymes	2.03	0.06	2.00	2.10
Microbial analysis—total aerobic mesophiles	Grape juice	8.87	0.15	8.70	9.00
	Bitter gourd juice	6.50	0	6.50	6.50
	Fermented bitter gourd–grape beverage (FBGGB) with enzymes	1.07	0.12	1.00	1.20
	Fermented bitter gourd–grape beverage (FBGGB)without enzymes	3.83	0.06	3.80	3.90

In the microbial analysis results, it can be seen that grape juice had the highest value for lactic acid bacteria (LAB) count as well as total aerobic mesophiles. Aneja et al. [49] reported that a pH less than 4.5 in fruit juices was the main barrier for most microorganisms. Barros et al. [50] conducted a study and stated that the pH did not differ between the tests, staying between 3.32 and 3.39.

The occurrence and distribution of microorganisms may be influenced by the pH levels. Acidophiles, neutrophiles and alkaliphiles are described as microbes that live over a range of 3–4 pH units, their growth being dependent on their optimal pH [51]. Flavonoids are responsible for antimicrobial activity of plants such as bitter gourd due to their ability to complex with extracellular and soluble proteins of bacterial cell walls [52]. FBGGB with and without enzymes showed similar results to those reported by Silva et al. [15].

4. Conclusions

The fermented bitter gourd–grape beverage (FBGGB) with and without enzymes was found to have better physiochemical content when compared with grape juice and bitter gourd juice. The amount of FRAP antioxidant activity was significantly different in all products, with FBGGB without enzymes having the highest content (M = 571.03). DPPH antioxidant activity was found to be significantly different as well, and bitter gourd juice had the highest content (M = 419.47), followed by FBGGB without enzymes (M = 81.07). FBGGB with enzymes was also found to have the highest antidiabetic potential (M = 27.07). Therefore, it can be concluded that FBGGB without enzymes performed better in terms of antioxidant activity content and antidiabetic potential. An analysis of antidiabetic potential and antioxidant activity showed a positive correlation of antidiabetic potential with ABTS antioxidant activity ABTS (r = 0.78) and a negative correlation with DPPH antioxidant activity (r = −0.99). Therefore, an increase in antidiabetic potential resulted in an increase in ABTS activity and a decrease in DPPH antioxidant activity and no significant change in FRAP antioxidant activity. It was also found that the physiochemical characteristics (pH, TTA, TSS, TFC and TPC) were significantly different in all the products.

FBGGB with and without enzymes was found to have better physiochemical content when compared with grape juice and bitter gourd juice, and the physicochemical and microbial characteristics of FBGGB with the enzymes were rated as better when compared with FBGGB without the enzymes. FBGGB has shown to retain the vegetable's nutritional properties following fermentation, which indicates the potential to be a promising beverage in the industry once further investigations have been conducted.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/fermentation8090439/s1. Table S1: One way ANOVA of FRAP Antioxidant activity in the four products. Table S2: One way ANOVA of ABTS Antioxidant activity in the four products. Table S3: Comparison of grape juice and FBGGB with enzyme. Table S4: Comparison of grape juice and FBGGB without enzyme. Table S5: Comparison of bitter gourd juice and FBGGB with enzyme. Table S6: Comparison of bitter gourd juice and FBGGB without enzyme. Table S7: Alcohol content in the products. Table S8: ANOVA of Alcohol content. Table S9: Tukey HSD test of Alcohol content in the products.

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Abbreviations

AACC	American Association for Clinical Chemistry
ABTS	2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid)
ANOVA	Analysis of variance

DNSA	3,5-dinitrosalicylic acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
FBGGB	Fermented bitter gourd–grape beverage
FC	Folin–Ciocalteu
FRAP	Ferric reducing antioxidant assay
HSD	Honest significant difference
MRS	de Man, Rogosa and Sharpe
NaOH	Sodium hydroxide
PCA	Plate count agar
RSM	Response surface methodology
SD	Standard deviation
TFC	Total flavonoid content
TPC	Total phenolic content
TSS	Total soluble solids
TTA	Total titratable acidity

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