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Evaluation of a sugarcane juice beverage fermented by *Ganoderma lucidum*: nutritional and antioxidant activity

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Abstract

A novel sugarcane juice beverage was successfully produced by liquid fermentation of *G. lucidum* and homogenate process with selecting the day 10 fermentation broth as the raw materials. The beverage was evaluated for its sensory, nutritional and antioxidant activities. The results showed that the beverage by the *G.lucidum* fermentation was rich in polysaccharides, triterpenes, proteins, and flavonoids, which were all significantly higher than sugarcane juice, especially polysaccharides and triterpenoids. And the beverage could retain the sensory quality of sugarcane juice and could therefore be favored by the consumers. The in vitro antioxidant activity showed that the beverage had high scavenging and reducing abilities. DPPH and $ABTS^{\bullet+}$ radical scavenging rate of 1 mL fermented beverage were similar to 0.04 mg/mL VC, while the hydroxyl radical scavenging rate and reducing power of 1 mL of fermented beverage were similar to 1.68 mg/mL and 0.19 mg/mL VC, respectively. Taken together, the sugarcane juice fermented beverage was a natural flavor substances with high antioxidant activity and non-toxicity.

Keywords: sugarcane juice; *Ganoderma lucidum*; fermentation; nutritional composition; antioxidant.

Practical Application: This study provided an efficient and environmentally friendly method for sugarcane juice processing through *G. lucidum* fermentation to produce a new type of sugarcane juice fermented beverages with high original health functions and flavor substances.

1 Introduction

Sugarcane (*Saccharum officinarum* L.) is an important energy matrix in tropical and subtropical crop, which has been cultivated globally for hundreds of years (Ali et al., 2019; Lajolo et al., 2021; Souza et al., 2019). Sugarcane juice, based on the high sucrose content, is the main raw material for the sugar industry and widely used for the production of sugar, ethanol, and hydrogen production (Cifuentes et al., 2021; Cruz et al., 2021; Saetear et al., 2021). However, these traditional processing methods may lead to a complete loss of the original functional activity of sugarcane. Therefore, developing a new method for processing sugarcane juice is an urgent way to restructure and upgrade the sugarcane industry.

Ganoderma lucidum (*G. lucidum*), commonly known as Lingzhi in China, is a traditional edible medicinal fungus that has been widely used for the food and pharmaceutical industries (Lin, 2019; Meng et al., 2022). As we known, *G. lucidum* is abundant in a variety of bioactive compounds, mainly polysaccharides and triterpenoids, which are known as physiologically active substances (Xiang et al., 2017; Zhang, 2017). Generally, the polysaccharides and triterpenoids are isolated from fruiting bodies, cultured mycelium and cultured broth. However, it takes several months to harvest fruiting bodies using solid cultures and the quality of fruiting bodies is also severely affected by cultivation management. In contrast, submerged fermentation of mycelium is an effective alternative approach that can obtain more bioactive components than the harvesting of fruiting bodies with a shorter culture period, a consistent product and seasonal independence (Ke & Lee, 2019; Tang et al., 2022; Vilela et al., 2021; Zhang et al., 2019).

In our previous study, we have demonstrated that sugarcane juice is a suitable culture medium for *G. lucidum* and first time reported the process of sugarcane juice fermentation with *G. lucidum* (Wang et al., 2018). It is very important that the sugarcane juice provides a source of nitrogen, carbon, inorganic salt, and other factors for *G. lucidum* growth, and the functional ingredients such as polysaccharides, protein, and other function compounds are produced during the fermentation. However, determination of the nutrient composition of this beverage has not yet been reported. Therefore, it triggered us to assess the nutrient composition and evaluate the bioactivity of this novel beverage. In this study, the beverage was obtained by using the sugarcane juice fermentation with *G. lucidum* and homogenate process, and the determination of the nutrient composition and the antioxidant activity were assessed. This study on the natural product with high antioxidant activity and less cytotoxicity would provide an efficient processing method for sugarcane juice by *G. lucidum* fermentation, which may greatly improve the original health functions and flavor substances of sugarcane juice.

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2 Materials and methods

2.1 Materials

Standard minerals (iron and zinc), sugars (sucrose, fructose, and glucose), Amino acids (aspartic acid, threonine, serine, glutamate, proline, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine, and arginine), oleanolic acid, ascorbic acid, rutin, ABTS, 1,1-diphenyl-2-picrylhydrazyl (DPPH), thiazolyl blue tetrazolium bromide (MTT), dimethylsulfoxide (DMSO) and other reagents were obtained from Aladdin (Shanghai. China). All reagents and chemicals were of analytical grade unless stated otherwise.

2.2 Substrates

The strain of *G. lucidum* was stored in the author's laboratory. Seed culture medium consisted of the following components: potato extract powder 3 g/L, peptone 2 g/L, glucose 20 g/L, KH_2PO_4 1 g/L, and $MgSO_4 \cdot 7H_2O$ 0.5 g/L, Vitamin B_1 0.05 g/L. Sugarcane juice fermentation medium were from the following processes: sugarcanes were harvested from the base of Institute of Biological and Medical Engineering, Guangdong Academy of Sciences. The sugarcane juice was collected after washing and squeezing. The total soluble solids of the sugarcane juice was diluted to 5 °Brix and used as fermentation medium, followed by financial sterilization at 121 °C for 20 min. The 10% (v/v) of *G. lucidum* seed culture medium was inoculated into the sugarcane juice fermentation medium and incubated at 28 °C and 150 rpm for 10 days, and fermentation samples were taken for further analysis.

2.3 Beverage production

The *G. lucidum* fermentation products, containing fermentation fluid and mycelium, were fragmented by a high-pressure homogenizer, then filtered and collected the filtrate. The optimum beverage formula was obtained by orthogonal experiment (Karunanayaka & Tang, 2018), in which fermentation products for 70%, citric acid for 1.0 g/L, xanthan gum for 0.2 g/L, stevia glycoside 0.1 g/L.

2.4 Determination of total soluble solids and sugars

Total soluble solids (TSS) (°Brix) were measured with a Digtial Hand-held "Pocket" Refractometer (H315476). The reducing sugars were determined by the 3,5-dinitrosalicylic acid reagent assay (Khatri & Chhetri, 2020). The sucrose content was determined by a high-performance liquid chromatography system (Perkin Elmer Altus A-10) on a Carbomix H-NP 10:8% (7.8 × 300 mm i.d., 10 μm) column with a mobile phase of 2.5 mmol/L H_2SO_4 with the flow rate of 0.6 mL/min and temperature of 35 °C.

2.5 Determination of polysaccharide content

The phenol-sulphuric acid assay was used to determine the total polysaccharides content of the samples (Chen et al., 2019). 5 mL samples were extracted with 20 mL anhydrous ethanol (1:4 v/v) at 4 °C overnight. The precipitate was collected after centrifugation at 8000 r/min for 10 min. The precipitate was washed 3 times with 80% anhydrous ethanol and then re-dissolved by adding 5 mL of distilled water to obtain a crude polysaccharide samples.

2.6 Beverage sensory evaluation

Fifteen experienced members participated in sensory scoring and were provided with water to rinse their mouths between tastings. Parameters are evaluated on a scale from one to ten in terms of appearance, color, taste, smell and other impressions. Each sensory score was 1 to 10. 10 point: the beverages is uniform and stable, no stratification or precipitation, yellow or light yellow in color, moderately sweet and bitter without irritation, unique aroma of sugarcane and *G. lucidum,* and no abnormal odor.

2.7 Determination of protein and free amino acids

The protein content was determined according to the method as described previously by Bradford with some modifications (Kielkopf et al., 2020). First, 100 μL sample (different concentrations of standard protein and fermentation samples) was added into a 10 mL volumetric tube, then 3 mL of G250 liquid was transferred into the solution and mixed thoroughly. The absorbance of the mixture was measured at 595 nm with a UV/Vis spectrophotometer (Shimadzu UV-1750). The amino acids were analyzed according to the method of Chinese national standards (GB 5009.124-2016 - National Health and Family Planning Commission, 2016). The content was determined by an amino acid analyzer with a column of strongly acidic ion exchange resin and detection audio-video length: 570 nm and 440 nm.

2.8 Assay of antioxidant activity of beverages

DPPH radical scavenging activity was measured using a modified method (Kandi & Charles, 2019). DPPH radicals were prepared by dissolving in ethanol (4 mmol/L). Mix 1 mL of DPPH solutions, 1 mL of each sample (take 0.2 mL, 0.4 mL, 0.6 mL, 0.8 mL, 1 mL samples respectively, and replenish water to 1 mL) and 2 mL of 60% ethanol, then incubated at 25 °C for 30 min in the dark. Finally, the absorbance was measured at 517 nm. Ascorbic acid (VC) was used as the calibration standard. The scavenging activity SA_{DPPH} was calculated according to the following Equation 1:

$$SA = (1 - \frac{A_1 - A_2}{A_0}) X 100 \quad (1)$$

Where SA is the scavenging activity of the tested samples (%), A_1 is the absorbance of reactant solution, A_2 is the absorbance sample solution, A_0 is the absorbance of the blank solution.

Hydroxyl radical scavenging activity has experimented with the method of Herraiz & Galisteo (2015) with a minor amendment. In brief, 1 mL of sample solutions of different concentrations (take 0.2 mL, 0.4 mL, 0.6 mL, 0.8 mL, 1 mL samples respectively, and replenish water to 1 mL), 2 mL of $FeSO_4$ solution (5 mmol/L), 2 mL of H_2O_2 solution (5 mmol/L), 2 mL of distilled water and 2 mL of salicylic acid ethanol solution (5 mmol/L) were mixed thoroughly, then incubated at 37 °C for 30 min. The absorbance

was measured at 510 nm. VC was used as the calibration standard. The hydroxyl radical scavenging activity SA_{OH} was calculated according to the Equation 1.

$ABTS^{\bullet+}$ radical scavenging activity was measured by a modified method (Wojtunik-Kulesza et al., 2018). The ABTS reaction solution was prepared by the following steps. First, the same volume of 7.4 mmol/L ABTS and 2.6 mmol/L $K_2S_2O_8$ solution were mixed and reacted in dark for 12~16 h. Then, the solution was diluted with 0.2 mol/L pH 7.4 phosphate buffer to make the absorbance at 734 nm (0.70 ± 0.02) to obtain the ABTS solution, and 1.0 mL of each sample solution (take 0.2 mL, 0.4 mL, 0.6 mL, 0.8 mL, 1 mL samples respectively, and replenish water to 1.0 mL) and VC was mixed with 6 mL ABTS solution. VC was used as the calibration standard. The SA_{ABTS} calculation is according to the Equation 1.

The reducing power was measured by $K_3Fe(CN)_6$ (Jones et al., 2020). 1 mL of different concentrations of sample solution, 1 mL of 1% $K_3Fe(CN)_6$ solution and 1 mL phosphate buffer solution (0.2 mol/L pH 6.6) were homogeneously mixed and reacted at 50 °C for 20 min. Add 2 mL of 10% trichloroacetic acid to the mixture, then centrifuge at 3000 r/min for 10 min. Add 2 mL of distilled water and 0.4 mL of 0.3% ferric chloride to 2 mL of supernatant, mixed well and react at 50 °C for 10 min. The absorbance of samples was determined at 700 nm. VC was used as the calibration standard. The reduction force is calculated according to Equation 2.

$$Reducing\ power = A_1 - A_2 \quad (2)$$

Where A_1 is the absorbance of reactant solution, A_2 is replaced with the ferric chloride solution with distilled water.

2.9 Cytotoxicity assay

Human normal liver cell line (L02) was cultured with RPMI 1640 medium (Containing 10% fetal bovine serum, 100 μg/mL penicillin, and 100 μg/mL streptomycin). Cells were seeded at a density of 8000 cells per well into 96-well culture plates and incubated at the condition of 37 °C and 5% CO_2 for 24 h. Then the samples with different concentrations were added and incubation was continued for 72 hours. After that, 10 μL (5 g/L) MTT solution were added and continued to culture for 4 hours. At last, the supernatant was discarded and 200 μL DMSO was added to each hole. The absorbance value of the solution at 490 nm wavelength was measured by the ELISA (FLUOstar OPTIMA, BMG, Germany). Cell viability = $(OD_a/OD_b) \times 100\%$, OD_a, and OD_b were the absorbance values of the experimental groups and control groups, respectively.

2.10 Microbiological limits

Microbiological Limits conform to the provisions of national standards for food safety of GB7101-2022 in China (National Health and Family Planning Commission, 2022).

2.11 Statistical analysis

The results were expressed as the mean ± standard deviation (SD) in triplicates. Statistical analysis was performed using SPSS version 19.0 software (SPSS Inc., Chicago, IL, USA) and analyzed by one-way ANOVA analysis of variance with the LSD and S-N-K. The differences between the control and tested groups were analyzed by Student's t-test. Differences with $^*p < 0.05$ or $^{**}p < 0.01$ were considered statistically significant.

3 Results and discussion

3.1 Analysis of G. lucidum fermentation products on day 10

We have demonstrated for the first time that sugarcane juice is a suitable culture medium for *G. lucidum*, which can be fermented to enhance the nutritional value and health benefits of sugarcane juice. Sugarcane juice was rich in nutrients, which can provide nutrients for the growth of *G. lucidum*. Meanwhile, sucrose in sugarcane juice could promote the metabolism of extracellular polysaccharides and facilitate the accumulation of functional ingredients in the beverage. Our results showed that the total soluble solids, sucrose and reducing sugars in the fermentation broth decreased with the extension of cultivation time. The content of polysaccharides kept rising with the extension of cultivation time, however, it did not increase significantly after the day10. The pH value was gradually decreased below 4.0 during days 3-8, and after day 8, the pH increased more rapidly. According to the changes of residual sugar, polysaccharide, and pH value, the day10 fermentation broth was selected as the raw materials for beverage production. At this time, the pH was about 4.56 ± 0.06, the biomass was 5.63 ± 0.34 g/L, its polysaccharide content was higher about 1.59 ± 0.09 mg/mL, and the reducing sugars and sucrose were 0.34 ± 0.01 g/L and 1.62 ± 0.01 g/L, respectively. In this work, we'd like to assess and compare the nutrient composition and the antioxidant activity of sugarcane juice without any treatment and sugarcane juice beverage fermented with *G. lucidum*. The process of preparation of a sugarcane juice beverage fermented by *G. lucidum* was shown in Figure 1.

3.2 Sensory evaluation of beverage

Sensory analysis is an important tool for the characterization of the food products. The use of sensory analysis helps to understand the quality characteristics that influence consumer choices (Cais-Sokolińska et al., 2021; Los et al., 2021). In this study, 15 trained sensory evaluators were selected to analyze the appearance, color, taste, and other impressions of the sugarcane juice fermented with *G.lucidum* and without any processing using a ten-point system. Figure 2 shows the sensory evaluation scores of each group of juices over a 10-day storage period. According to the formula for sensory evaluation of beverages, the mean scores of participants for the fermented beverage by *G. lucidum* and sugarcane juice without any treatment were 71.15 ± 2.94 and 71.14 ± 2.72, respectively, which were similar score and not significantly different. Compared to the sugarcane juice group, higher scores were observed for the appearance, color, and smell in the group of fermented beverage by *G. lucidum*. It is worth mentioning the appearance and color attributes of the fermented beverage, which both scored higher than 8.0, significantly higher than the untreated-sugarcane juice. However, fermently processed group showed a higher sense of bitterness compared to the untreated-group. More than half of the participants rated

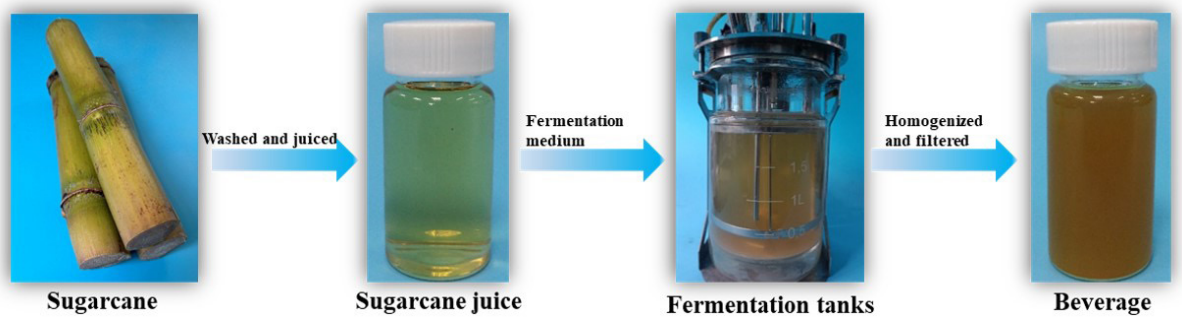

Figure 1. Process illustration of preparation of a sugarcane juice beverage fermented by *Ganoderma lucidum*.

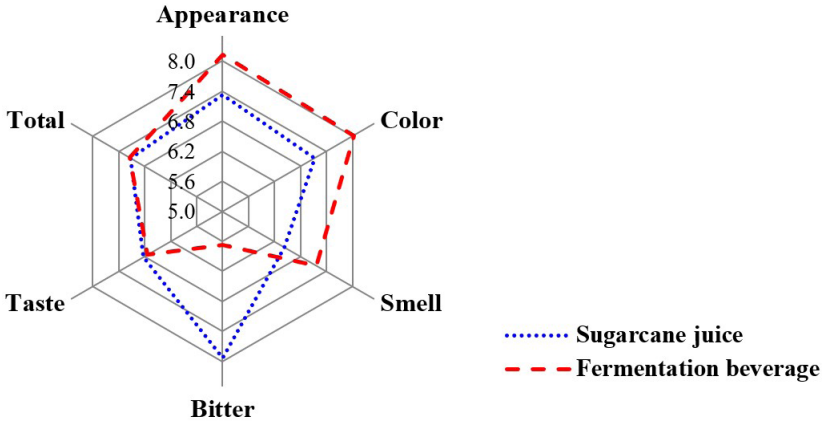

Figure 2. Sensory analysis of sugarcane juice and fermentation beverage.

Table 1. Nutrients and functional components in 5 °Brix sugarcane juice and fermentation beverage.

	Sugarcane juice	Fermentation beverage		Sugarcane juice	Fermentation beverage
Polysaccharide (g/L)	0.62 ± 0.10 b	2.35 ± 0.10 a	Reducing sugar (g/100 mL)	3.52 ± 0.10 a	0.41 ± 0.02 b
Triterpenoids (mg/100 mL)	11.15 ± 0.90 b	55.12 ± 1.74 a	Iron (mg/L)	2.86 ± 0.04 a	1.66 ± 0.05 b
Protein (g/L)	2.74 ± 0.35 b	3.24 ± 0.22 a	Zinc (mg/L)	2.12 ± 0.19 a	1.47 ± 0.06 b
Flavonoids (mg/100 mL)	12.65 ± 0.64 b	21.99 ± 1.47 a	Vitamin E (μg/L)	40.33 ± 3.25 a	29.03 ± 0.61 b
Sucrose (g/100 mL)	13.70 ± 0.63 a	1.77 ± 0.06 b	β-carotene (μg/L)	5.81 ± 0.75 a	3.30 ± 0.10 b

The different letters are significantly different ($p < 0.05$)

the bitterness attributes of fermented beverages higher than 6.0. There are many reasons for the fermented beverage with bitterness, the primary cause being due to the production of triterpenes and other bitter substances during the fermentation process by *G. lucidum*. It was reported that the *G. lucidum* mycelium were rich in active substances including triterpenoids and sterols during liquid fermentation (Feng et al., 2021). In general, triterpenoids are very bitter (Hill & Connolly, 2020). Table 1 shows that the triterpene content of the fermented beverage by *G. lucidum* was 55.12 ± 1.74 mg/100 mL, while that of sugarcane juice was 11.15 ± 0.90 mg/100 mL. This could also explain well why the taste evaluators provided a higher score for bitterness attributes. In addition, the Maillard reaction also causes bitter taste during high-temperature sterilization of sugarcane juice (Chen et al., 2022). Recent studies have shown that bitter beverages can give a sense of bitterness when consumed, leading to an endless aftertaste for bitter flavors, such as coffee, tea, grapefruit juice (Zhong et al., 2019). A slightly bitter sugarcane juice will bring a different taste. Therefore, the processing of the *G. lucidum* fermentation could retain the sensory quality of sugarcane juice and could therefore be favored by the consumers.

3.3 Determination of nutrients and functional components in beverage

Sugarcane juice is a non-alcoholic natural energy beverage, rich in sucrose, fructose, glucose, amino acids, minerals, phenols, flavonoids and other nutrients, and is widely favored by consumers (Rodrigues et al., 2021). As shown in Table 1, sugarcane juice was detected to be abundant in nutrients, such as sucorse,

Table 2. The free amino acids in 5 °Brix sugarcane juice and fermentation beverage (mg/100 mL).

	Sugarcane juice	Fermentation beverage		Sugarcane juice	Fermentation beverage
Aspartic	35.33 ± 0.47 a	15.97 ± 1.53 b	Methionine	2.39 ± 0.2 a	1.67 ± 0.12 b
Threonine	2.15 ± 0.09 b	4.30 ± 0.33 a	Isoleucine	2.90 ± 0.35 a	2.83 ± 0.23 ab
Serine	2.82 ± 0.97 b	4.79 ± 0.35 a	Leucine	0.54 ± 0.02 b	4.70 ± 0.43 a
Glutamic	10.17 ± 0.42 b	17.56 ± 1.03 a	Tyrosine	1.48 ± 0.21 ab	1.62 ± 0.11 a
Proline	2.02 ± 0.16 b	5.76 ± 0.24 a	Phenylalanine	1.50 ± 0.05 b	3.49 ± 0.29 a
Glycine	5.13 ± 0.99 b	7.46 ± 0.36 a	Histidine	1.71 ± 0.29 a	1.72 ±0.16 a
Alanine	4.03 ± 0.14 b	6.01 ± 0.39 a	Lysine	1.14 ± 0.11 b	2.70 ± 0.23 a
Valine	1.37 ± 0.68 b	4.01 ± 0.30 a	Arginine	1.99 ± 0.38 b	3.72 ± 0.32 a

The different letters are significantly different ($p < 0.05$).

reducing sugar et.al, which could provide carbon soures for the mycelia growth of *G.lucidum*. Herein, we'd like to investigate the changes in nutrients and functional components when we use the *G. lucidum* fermentation method to produce a novel sugarcane juice. Previous research have shown that the sucrose, glucose, and fructose in sugarcane juice could be converted into polysaccharides by the *G.lucidum* fermentation, which might greatly improve the nutrition and function of sugarcane juice (Wang et al., 2018). The nutrients and functional components in 5 °Brix sugarcane juice and fermentation beverage were detailed in Table 1. The results showed that the beverage by the *G.lucidum* fermentation was rich in polysaccharides, triterpenes, proteins, and flavonoids, which were all significantly higher than sugarcane juice ($p < 0.05$), especially polysaccharides and triterpenoids, which were 3.79 and 4.94 times higher, respectively. In addtion, the content of protein and flavonoids in fermentation beverages was more than 70% higher than that of sugarcane juice. However, the content of microelements, such as iron, zinc, vitamin E and β-carotene, was inferior to sugarcane juice. From this Table 1, it can be concluded that functional ingredients, such as polysaccharide, protein and other ingredients were produced during fermentation by *G. lucidum*, which greatly improved the sugarcane juice original health care functions, and also improved the flavor of sugarcane juice. A total of 16 hydrolyzed amino acids were recorded in this study (Table 2). The results showed that at a soluble solids of 5 °Brix, the fermented beverage contained more amino acids than sugarcane juice, except aspartic acid, methionine and isoleucine. We classified amino acids according to different types and counted them, as shown in Figure 3. It was clear that the total amino acid (TAA) content of fermented beverages was higher than that of sugarcane juice. It was found that the essential amino acids (EAA), aromatic amino acids (AAA) and branched chain amino acids (BCAA) were higher in fermented beverages than in sugarcane juice. As reported, BCAA can promote the body's protein synthesis and reduce protein decomposition (Xu et al., 2018), the release of insulin and growth hormone, which are very important for the human body. The leucine (one of the BCAA) value of 4.70 ± 0.43 mg/100 mL for fermented beverages was approximately nine times as much as the sugarcane juice value of 0.54 ± 0.02 mg/100 mL (Table 2). In comparison, most of the amino acids values of fermented beverages were much higher than those of sugarcane juice, indicating that the *G.lucidum* fermentation can improve the nutrition of sugarcane juice, which is a suitable method in sugarcane processing.

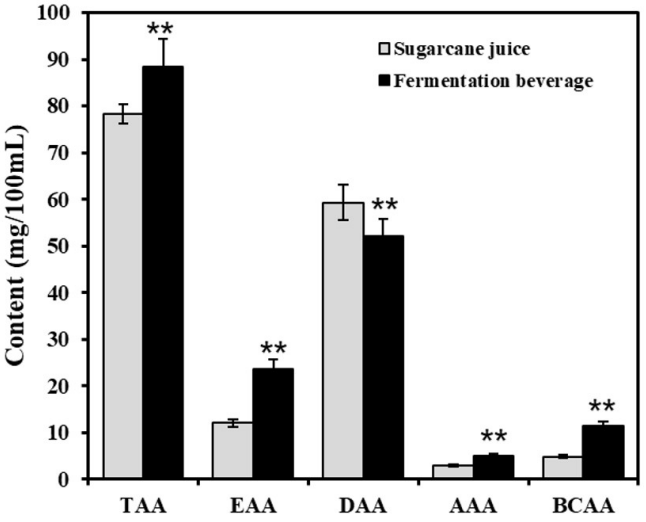

Figure 3. The amino acid content in the sugarcane juice and fermentation beverage. TAA: total amino acid; EAA: essential amino acid; DAA: delicious amino acid; AAA: aromatic amino acid; BCAA: branched chain amino acid. Each value represents the arithmetic mean with the standard deviation (SD) in triplicates, significant differences ($p < 0.01$) are indicated with asterisks (**).

3.4 Antioxidant activity of beverage

The DPPH, Hydroxyl, $ABTS^{\bullet+}$ free radicals, and reduction power has been widely used as the free radical compound to determine the antioxidant capacity for foods and beverages (Bagchi & Kumar, 2016). Sugarcane juice has been reported to have ferric reducing ability and scavenging ability for DPPH, ABTS, and nitric oxide. Fermentation broth of *G. lucidum* also exhibited the same ability to scavenge free radicals (Sarnthima et al., 2017). In this study, the effects of scavenging and reducing abilities by the sugarcane juice and fermented beverage were measured and showed in SI 1. Results indicated that the fermented beverage showed efficient scavenging activity on DPPH, Hydroxyl, $ABTS^{\bullet+}$ free radicals with a concentration-dependent fashion. The scavenging abilities at high conten (1 mL) of fermented beverage were 88.73%, 54.66%, and 98.76%, respectively. which were significantly higher than those of the sugarcane juice with the same content. To more visually represent the antioxidant capacity of sugarcane juice and fermented beverages, we converted their

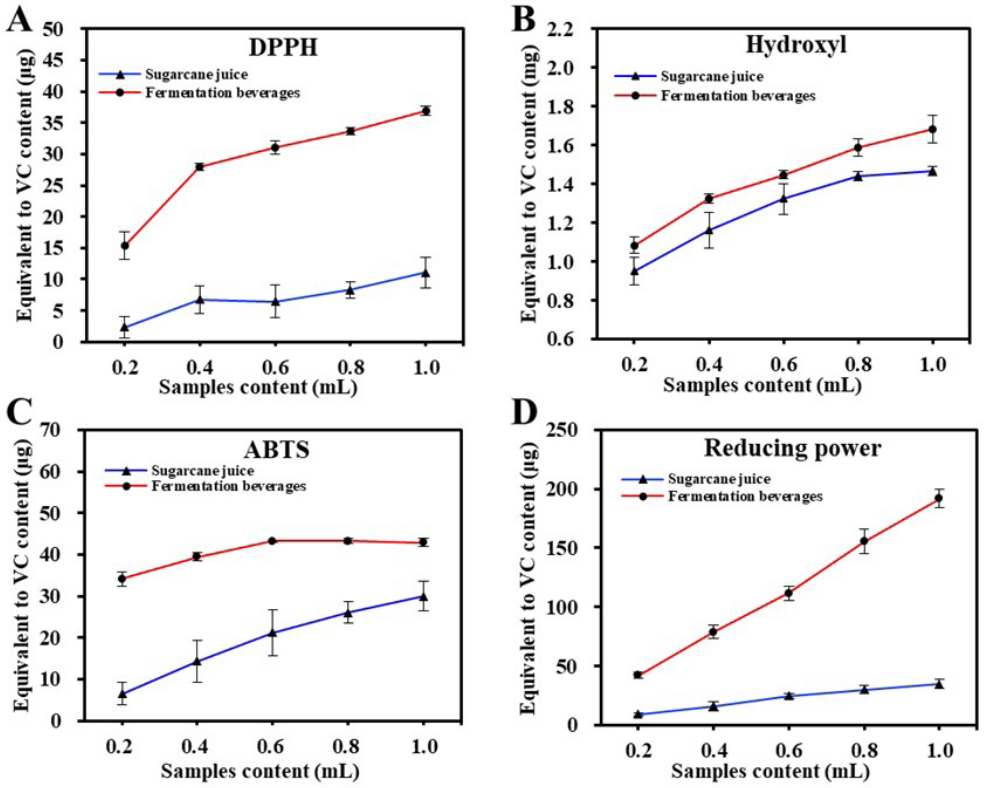

Figure 4. Antioxidant activity of sugarcane juice and fermentation beverages equivalent to VC content. A: the DPPH radical scavenging ability; B: the hydroxyl radical scavenging ability; C: the ABTS$^{\bullet+}$ radical scavenging rate; D: the reducing power ability.

antioxidant capacity to the equivalent amount of VC with the same antioxidant capacity. As shown in Figure 4, the DPPH and ABTS$^{\bullet+}$ radical scavenging rate of 1 mL fermented beverage were similar to 0.04 mg/mL VC, while the hydroxyl radical scavenging rate and reducing power of 1 mL of fermented beverage were similar to 1.68 mg/mL and 0.19 mg/mL VC, respectively. It is worth mentioning that the ABTS$^{\bullet+}$ radical scavenging results suggested that fermented beverage had stronger activity at a lower content compared to the sugarcane juice sample, with a scavenging rate of 80.5% for 0.2 mL, which were 1.7 times higher than sugarcane juice and equivalent to VC content of 0.033 mg/mL (Figure 4C). Figure 4D showed that the reducing power of fermented beverages and sugarcane juice followed the same trend as the free radical assay with 1.10 and 0.71 at 1.0 mL, corresponding to 191.89 μg/mL and 118.62 μg/mL of VC content, respectively. Due to a lot of the functional ingredients, such as triterpenoids, polysaccharides and proteins, produced during the fermentation, the fermented beverage has more active substance, leading to a stronger antioxidant effect. On the basis of the results above, the fermented beverage by *G. lucidum* had higher antioxidant activity and reducing power than sugarcane juice.

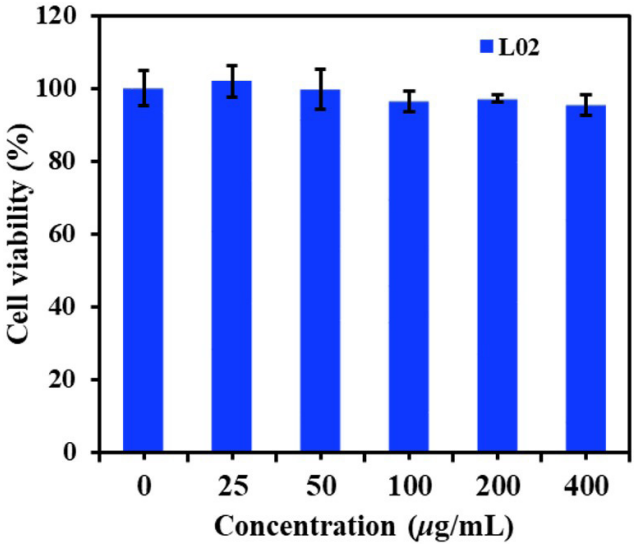

Figure 5. Cytotoxicity assay results of the fermentation beverage on normal hepatocyte cell lines (L02).

3.5 In vitro cytotoxicity assay

In vitro cytotoxicity of the beverage was tested by an MTT assay. The normal hepatocyte cells of L02 were incubated with fermented beverage by *G. lucidum* of different concentrations ranging from 0 μg/mL to 400 μg/mL for 48 h. As shown in Figure 5, The cell viability of the beverage group was not significantly different from that of the control group, and the cell viability of the different concentration groups was above 90%, indicating that the fermented beverage by *G. lucidum* is a safe, green and non-toxic product for human beings.

3.6 Microbiological limits

Microbiological Limits should conform to the provisions of national standards for food safety of GB7101-2022 in China (National Health and Family Planning Commission, 2022). The analyses of fermented sugarcane juice were negative for Coliform, Staphylococcus, and Salmonella. For Aerobic plate count values below 30 colony forming units (CFUs) mL^{-1}, Yeast and myocyte values below 10 colony forming units (CFUs) mL^{-1}. The result complies with microbiological Limits regulations.

4 Conclusion

A novel sugarcane juice beverage was successfully produced by liquid fermentation of *G. lucidum* and homogenate process with selecting the day 10 fermentation broth as the raw materials. Sugarcane juice provided a rich source of nitrogen, carbon, inorganic salts and other factors for the growth of *G. lucidum*, while the functional components such as polysaccharides, proteins and other functional compounds were produced during the fermentation. The results of sensory evaluation of beverage conformed the processing of the *G. lucidum* fermentation could retain the sensory quality of sugarcane juice. The nutrients and functional components in 5 °Brix fermentation beverage was much higher than those of sugarcane juice, indicating that the *G.lucidum* fermentation could improve the nutrition of sugarcane juice. In addition, the fermented beverage samples were found to exhibit significant antioxidant activity without cytotoxicity, for the first time. Due to a lot of the functional ingredients, such as triterpenoids, polysaccharides and proteins, produced during the fermentation, the fermented beverage has more active substance, leading to a stronger antioxidant effect. This study provided an efficient and environmentally friendly method for sugarcane juice processing through *G. lucidum* fermentation to produce a new type of sugarcane juice fermented beverages with high original health functions and flavor substances.

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Supplementary Material

Supplementary material accompanies this paper.

SI 1. Antioxidant activity of sugarcane juice and fermentation beverages. (A) the DPPH radical scavenging ability; (B) the hydroxyl radical scavenging ability; (C) the ABTS•+ radical scavenging rate; (D) the reducing power ability.

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