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Research on Improving Value-Added Processing of Jaboticaba

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Abstract

This research will use food fermentation technology and supercritical extraction technology to enhance the value-added application of the active ingredients of Jaboticaba. The results showed that the bioactive component content of samples after value-added fermentation and extraction was 19.86% to 69.30% higher than that of fresh fruit. In the analysis of antioxidant activity, it was found that the ability to scavenge free radicals was higher than that of fresh fruits. The total antioxidant activity and reducing power were best after fermentation and supercritical extraction, reaching more than 90%. After fermentation, the active ingredients were detected, and the resveratrol content was 3.27 mg/L, the anthocyanin content was 67.71 mg/g, and the tryptophan content was 0.48 gm/Kg, all of which were higher than those in fresh fruits. The results of this research are helpful for the development of natural anti-inflammatory or sleep-aiding products. It is of great significance for the research on anti-inflammatory and sleep-aiding. It can also provide value-added processing conditions to solve the problem of Jiabao fruit during the peak production season. The pressure of excessive production and processing will increase the value-added economy of the industry and make Jaboticaba more valuable for research.

Keywords

Value-Added, Jaboticaba, Supercritical Extraction, Antioxidant, Sleep Aid

1. Introduction

Jaboticaba is rich in phenolic compounds, including depside phenolic substances with a special structure, called Jaboticabin. Jaboticaba has very high economic

benefits under mass production conditions, but the fruit kernels are not easy to preserve. In general, the traditional Jaboticaba processing method, in addition to being eaten directly, can be processed into jam, juice, dried fruit, and fruit vinegar due to its high sugar content. Jaboticaba is rich in phenolic compounds. This study mainly explores the different processing methods of Jaboticaba to increase the value-added of the active ingredients of Jaboticaba, using fermentation technology and supercritical extraction to Conduct effective analysis and purification value-added; a supercritical fluid (SCF) is a substance at a temperature and pressure above its critical point, where distinct liquid and gas phases do not exist, but below the pressure required to compress it into a solid [1]. Including the use of four experimental samples: fresh fruit, fresh fruit through critical extraction, fresh fruit through fermentation treatment, and fermentation through supercritical extraction. Use various chromatography and spectroscopic analysis instruments: UV/VIS, FTIR, GS/LC-MS, NMR to complete the separation and purification of active ingredients and the analysis and comparison of active ingredients. The results of this research are of great significance for its future application in assisting anti-inflammation and improving sleep quality and can provide an academic reference for the possible mechanism of action of natural antioxidants, which will serve as an important basis for subsequent research and development and production of healthy foods.

The flowers and fruits of Jaboticaba grow on the trunk and branches. Because its fruit is similar to grapes, Jaboticaba is commonly known as "tree grape" in the Taiwan region. When ripe, the peel changes from green to red, then to purple. It only takes 4 to 7 days to pick the fruit after maturity. The ripe fruit is purple-black, and the pulp is white translucent crystals [2]. Jaboticaba is an edible, healthy plant whose pulp is rich in acids, sugars, vitamins, dietary fiber, minerals, and polyphenols [3]-[5]. The peel contains a variety of phenolic compounds, including anthocyanins, ellagic acid, and tannins and amino acids [6]-[8], with various biological functions. Activities, such as antioxidant, anti-inflammatory, antibacterial, hypoglycemic and lipid-lowering properties [9]-[13].

Jaboticaba can improve serum antioxidant capacity and hyperglycemic response in healthy adults after ingesting carbohydrate meals [12]. Jaboticaba seed extract has anti-proliferative effects on oral cancer cells [14]. Jaboticaba extract to inhibit oxidative stress and inflammation in mice through streptozotocin-nicotinamide to improve diabetic nephropathy [15]. Jaboticaba extract can promote the fermentation of intestinal microbiota, which is beneficial to human health. As a tropical fruit, Jaboticaba is an agricultural product with potential for development as a bioactive and functional food [16] [17]. Considering the evidence that Jaboticaba by-products comprise a putative food-grade material [7]. The reduction in pH and increased SCFA production during colonic fermentation make the environment unfavorable for pathogen growth [18].

Supercritical fluids have been widely used in various industries so far, mainly using supercritical carbon dioxide to extract organophilic components in natural

plants [18]. Because the critical temperature of supercritical carbon dioxide is not high, the active ingredients of Jaboticaba can be effectively separated without destroying the natural active ingredients. Phenolic compounds extracted from Jaboticaba can be used to treat inflammation. In South American countries such as Brazil, people take Jaboticaba directly to combat certain inflammations [19]. Jaboticabin has been confirmed to have the activity of inhibiting the proliferation of intestinal cancer cells, lung cancer cells and white blood cells, and reducing the production of chemokine interleukin (IL)-8 (47.3% and 70.3%) in SAE cells.), has the potential to treat chronic obstructive pulmonary disease [20]. Therefore, fermented and refined processing of Jiabao fruit can provide good anti-inflammatory research.

2. Materials and Methods

2.1. Jaboticaba

This study used four experimental samples: fresh fruit, fresh fruit subjected to critical extraction, fresh fruit subjected to fermentation treatment, and fresh fruit subjected to supercritical extraction after fermentation, which were purchased from food processing plants in the central Taiwan region.

2.2. Supercritical Fluid Extraction

Referring to the method of Liang 2008 [21], the experimental sample was placed in a 5 L research extraction tank. The extraction conditions were 50˚C, 5000 psi, and adding ethanol co-solvent for supercritical carbon dioxide extraction. The fluid flow rate is 30 kg/hr. And the CO_2 volume is 5 times the sample weight. The collected extracts are divided according to different collection time points: SFE1 (first 60 minutes) samples collected under each separation tank between 0 - 60 minutes of extraction. SFE2 (last 600 min): Samples were collected under each separation tank within 60 - 120 minutes of extraction.

2.3. Analysis of Active Ingredients of Jaboticaba

Use a Gas Chromatography-Mass Spectrophotometer to separate the active ingredients and determine their structure. The analytical instrument used is GC: Agilent, model 7890B-GC, USA. MS: Agilent, model 5977A-MSD, USA. Column: Agilent, model DB-5MS (30 m × 0.25 mm × 0.25 μm). Injector temperature: 250˚C, Detector temperature: 250˚C.

2.4. Analysis of Total Polyphenols

Modified according to the method of Li *et al.* 2020 [22], using 1 mL of anhydrous gallic acid as the standard, producing solutions with concentrations of 0, 10, 20, 30, 40 and 50 μg/mL. And transfer the solution into a 10 mL colorimetric tube, 5 mL distilled water, 1 mL Forinol chromogen and 3 mL 7.5% sodium carbonate solution. After the solution was mixed with a vortex shaker, the reaction was carried out at room temperature in the dark for 2 hours. The absorbance value was

measured at 765 nm and the total polyphenol content was calculated using the regression equation:

$$y = 0.0075x - 0.0039\left(R^2 = 0.9996\right)$$

2.5. Amino Acid Content Analysis

According to the reference CNS (N6221) Method of test for fruit and vegetable juices and drinks-Determination of free amino acids, 2005 [23]. Fruit and vegetable juice beverage test method-determination of free amino acids, column: packing Durrum DC-6A, Biotronik BTC 3118, Biotronik BTC 2710, LKB Ultropac 8 or equivalent. Pipe column material: stainless steel pipe column, inner diameter × length is 3.2 - 9 mm × 140 - 500 mm respectively. Reagents: Tri-lithium citrate tetrahydrate, Lithium chloride, Lithium hydroxide, Phenol, concentrated hydrochloric acid (37%), ethoxyethanol (Ethylene glycol monomethyl ether) or methanol (Methanol), boric acid (Boric acid), polyoxymethylene (Brij 35), 30% aqueous solution, 2,2 Thiodiethanol (2,2 Thiodiethanol), Ninhydrin solution, sodium acetate or Potassium acetate, glacial acetic acid, ethylene glycol or ethoxyethanol, 15% titanium (III) chloride solution (15%), reduced Hydrindantin, Tri-sodium citrate.

2.6. In Vitro Antioxidant Assay

2.6.1. Total Antioxidant Capacity

According to the method of Torel, 1986 [24], take out the Jaboticaba experimental sample, add 0.1 M phosphate buffer (pH 6.0), then add ABTS, H_2O_2, and Peroxide, shake evenly, and react for one hour. Afterwards, the prepared Trolox and Garbo fruit extracts of various concentrations were added, and reacted for 10 minutes, and the absorbance value was measured at 734 nm.

2.6.2. DPPH Free Radical Scavenging Ability

Refer to the method of Liu *et al.*, 2022 [25] modify it. Precisely weigh 7.8864 mg of the DPPH standard and quantify it to 100 mL, add absolute ethanol and a DPPH ethanol solution with a concentration of 0.2 mmol/L. Transfer the solution to a brown bottle and set it aside. Add the sample to the test tube, mix evenly, place it at room temperature, and shield it from light. After reacting for 30 minutes, measure the absorbance value at 517 nm.

$$\text{According to the clearance } \% = \left(1 - As - \frac{Aj}{Ao}\right) * 100\%$$

As: 1 mL DPPH ethanol solution + 2 mL anhydrous ethanol + 1 mL blank solvent.

Aj: 1 mL DPPH ethanol solution + 2 mL anhydrous ethanol + 1 mL sample solution.

Ao: 2 mL anhydrous ethanol + 1 mL sample solution.

2.6.3. Determination of the Ability to Scavenge ABTS Cationic Radicals

ABTS was determined according to the method of Tao *et al.*, 2016; Wang *et al.*,

2022, [26] [27], and the method was modified. 96 mg ABTS was dissolved in 20 mL of deionized water, and then 5 mL of 2.45 mmol/L potassium persulfate solution was added. Place the mixture in a dark room at room temperature overnight (12 - 16 hours) before use. The resulting ABTS + radical solution was diluted 11 times with ethanol, and the absorbance value was measured at 734 nm (±0.02). Then, add 20 μL of the experimental sample appropriately diluted with ethanol into 2 mL of the above ABTS + solution. In addition, 20 μL ethanol was added to 2 mL ABTS solution, and this sample was used as a blank experiment. After being kept at 30˚C for 6 minutes in a dark place, the absorbance value was measured at 734 nm. Results are expressed as ABTS + free radical inhibition percentage:

$$\text{Inhibition}\% = \left(1 - \frac{\text{Asmaple}}{\text{Ablank}}\right) * 100$$

2.7. Statistical Analysis

Each of the above experiments was performed in triplicate. The data obtained from the experiment were statistically analyzed using the SPSS (Statistical Product and Service Solutions version 25) system. Single-factor variation analysis in comparative averages was used, and Duncan's multi-variation analysis method and Pearson correlation analyzes the significant differences and correlations between samples.

3. Results and Discussion

3.1. Analysis and Comparison of Active Ingredient Content of Sample Standards

Table 1 shows the analysis and comparison of the phenolic compounds of four experimental samples of Jaboticaba: fresh fruit, fresh fruit subjected to supercritical extraction, fresh fruit subjected to fermentation treatment, and fermented fruit subjected to supercritical extraction. Experimental analysis results show that under different processing and preparation conditions, the content of the components of Jaboticaba will have significant differences and effects. When fresh has not been processed, such as fermentation or extraction, the total content of Jaboticaba will be significantly different. The component analysis of phenols, anthocyanins, resveratrol, small molecule peptides and ellagic acid, etc., showed significant differences, and their contents were lower than those of other samples. The advantage of processing Jaboticaba through the fermentation process is that more active ingredients can be obtained, and the component resveratrol can be detected. After fresh fruit is fermented and then subjected to supercritical extraction SFE1, the total polyphenols, anthocyanins, ellagic acid, resveratrol and small molecule peptides are higher than those in fresh fruit. However, the content of resveratrol in fresh fruits that have not been fermented and super-critically extracted has not been detected in the ingredients. The main reason is that fresh fruits have not been invaded by bacteria or fungi for fermentation, which may lead to the inability to synthesize resveratrol [28]. The sugar concentration of Jaboticaba

is one of the important factors affecting fermentation. The utilization efficiency of sugar can increase the fermentation rate. The fastest sugars are in the order: fructose > sucrose > glucose. For example, sucrose can be directly absorbed by yeast, and then hydrolyzed into glucose and fructose by intracellular invertase [29].

Table 1. Analysis and comparison of compound content in different Jaboticaba experimental samples.

ITEM	Total phenolics	Anthocyanins	Ellagic acid	Small molecule peptides	Resveratrol
	mg/g	mg/g	mg/g	mg/g	mg/L
Flesh Jaboticaba	44.82 ± 0.32^{b}	28.05 ± 0.05^{b}	7.02 ± 0.20^{b}	22.01 ± 2.15^{b}	ND
Flesh Jaboticaba + SFE1	53.23 ± 0.05^{a}	48.10 ± 0.39^{a}	10.74 ± 0.12^{a}	24.87 ± 1.61^{b}	ND
Flesh Jaboticaba + SFE2	12.68 ± 0.12^{c}	26.25 ± 1.22^{b}	5.96 ± 0.75^{c}	12.68 ± 2.65^{c}	ND
Flesh fermentation	46.82 ± 0.89^{b}	30.75 ± 0.05^{b}	9.72 ± 0.01^{b}	26.84 ± 2.75^{b}	2.14^{a}
Flesh fermentation + SFE1	54.12 ± 0.05^{a}	67.71 ± 0.03^{a}	10.05 ± 0.02^{a}	27.75 ± 0.65^{a}	3.27^{a}
Flesh fermentation + SFE2	14.82 ± 0.89^{c}	17.25 ± 0.05^{c}	5.62 ± 0.01^{c}	13.24 ± 1.35^{c}	1.05^{b}

Different letters indicated significant differences within. ND indicates not detected. $p < 0.05$.

Supercritical fluid extract (SFE), conditions are 5000 psi and 50˚C, SFE1 is the extract collected in the first 60 minutes, SFE2 is the extract collected in the last 60 minutes.

3.2. Analysis of Total Polyphenol Content

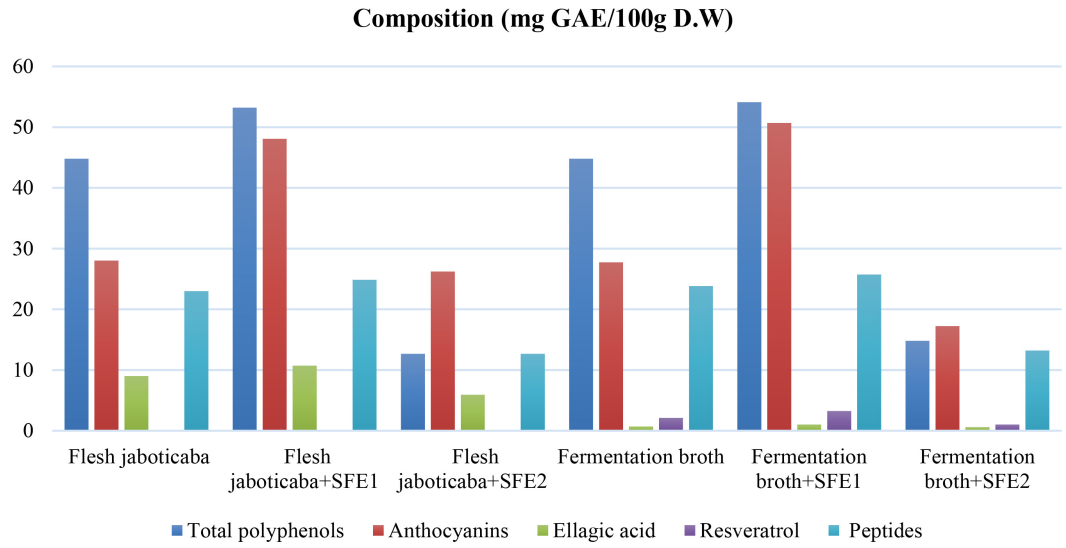

Figure 1. The volatile compounds of Jaboticaba.

This study used four experimental samples of Jaboticaba: fresh fruit, fresh fruit subjected to critical extraction, fresh fruit subjected to fermentation treatment, and fermented fruit subjected to supercritical extraction. The analysis and comparison of total polyphenols is shown in Figure 1. The results show that samples processed through the fermentation process can obtain more phenolic compounds

with functional active ingredients, such as acidic or alkaline sensitive substances, which are one of the important components that improve the antioxidant effect of Jaboticaba, such as total polyphenolic compounds. Phenols, anthocyanins, ellagic acid, resveratrol and small molecule peptides, etc. After fermentation and supercritical treatment, the overall total polyphenol content was significantly increased by 19.86% to 69.30% compared with fresh fruit. The reason may be that supercritical low-temperature extraction is used to retain the active ingredients, and the content is higher than that of fresh fruits. The analyzed content is 54.12 mg GAE/100 g D.W.

3.3. Concentration of Amino Acid (g/Kg) in Jaboticaba

This study used four experimental samples of Jaboticaba: fresh fruit, fresh fruit subjected to critical extraction, fresh fruit subjected to fermentation treatment and fermented supercritical extraction. The target amino acids were detected and analyzed. The results are shown in Table 2. After processing through fermentation procedures, the most abundant amino acid in the product is tryptophan at 0.43 g/Kg, followed by aspic acid at 0.42 g/Kg, both of which are higher than those in fresh fruits. After supercritical fluid extraction, the highest content was found in ascorbic acid at 0.69 g/Kg, followed by tryptophan at 0.48 g/Kg. The results show that Jaboticaba can be improved by processing it in different ways and applying supercritical fluid extraction. The content of certain amino acids in the product reaches 60% - 80%, and they are all higher than those in fresh fruits. During the fermentation reaction, the enzymes contained in Jaboticaba can convert amino acid precursors into target amino acids, such as glycine. The advantage of the fermentation method is that amino acids can be produced in large quantities at low cost using relatively small equipment.

3.4. DPPH free Radical Scavenging Rate

Table 2. Concentration of amino acid (g/Kg) in Jaboticaba.

Amino acid	Flesh jaboticaba	Flesh jaboticaba+SFE1	Fermentation broth	Fermentation broth + SFE2
Tryptophan	0.12 ± 0.05^{c}	0.28 ± 0.16^{b}	0.43 ± 0.00^{b}	0.48 ± 0.01^{a}
Glutamic acid	0.39 ± 0.01^{c}	0.58 ± 0.11^{b}	0.42 ± 0.03^{b}	0.69 ± 0.25^{a}
Arginine	0.16 ± 0.00^{c}	0.20 ± 0.02^{b}	0.35 ± 0.02^{a}	0.41 ± 0.00^{a}
Lysine	0.35 ± 0.22^{b}	0.42 ± 0.01^{c}	0.32 ± 0.03^{c}	0.45 ± 0.02^{a}
leucine	0.15 ± 0.03^{c}	0.18 ± 0.01^{c}	0.18 ± 0.00^{c}	0.19 ± 0.01^{a}
Aspartic acid	0.37 ± 0.32^{b}	0.39 ± 0.00^{a}	0.38 ± 0.01^{c}	0.40 ± 0.01^{a}

Different letters indicated significant differences. $p < 0.05$.

This study used four experimental samples of Jaboticaba: fresh fruit, fresh fruit subjected to critical extraction, fresh fruit subjected to fermentation treatment, and supercritical extraction after fermentation, and the DPPH free radical scavenging rate of Jaboticaba was tested. The results are shown in Figure 2. There is

no significant difference in DPPH free radical scavenging rates between fermentation broth and supercritical extraction experimental samples, both reaching over 91.75%. The DPPH free radical scavenging rate is the highest, reaching 96.03% in the supercritical extraction experimental sample after fermentation. However, the DPPH free radical scavenging capacity of fresh fruits has been analyzed as high as 91.75%. Overall, the DPPH scavenging rates of fresh fruits, fermented and supercritical extraction experimental samples are all over 90%, with little statistical difference, $p < 0.05$.

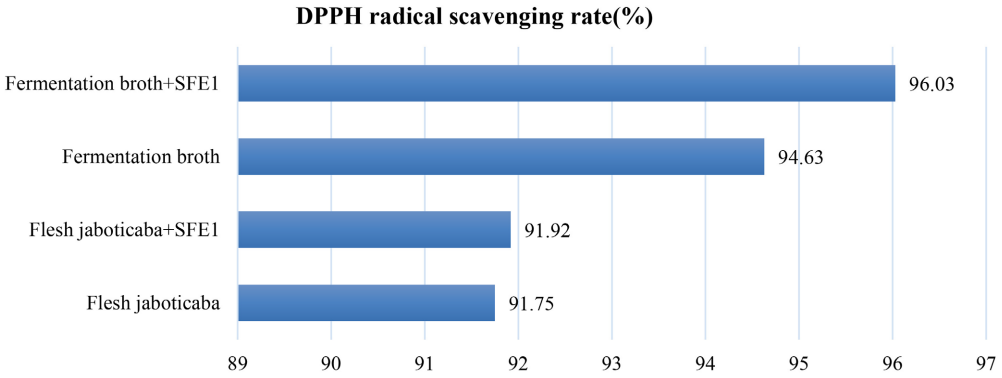

Figure 2. Jaboticaba for DPPH Antioxidant capacity.

3.5. Total Antioxidant Activity (TAA)

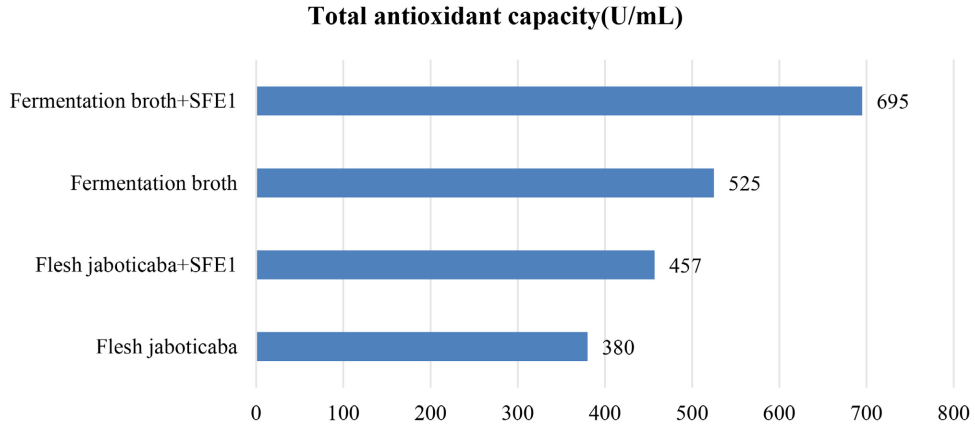

Figure 3. Jaboticaba for total Antioxidant capacity.

The total antioxidant activity was measured based on the ability of the sample to scavenge ABTS (2,2-azinobis-3-ethyl benzothiazoline-6-sulphonic acid) free radicals. There is no significant difference in the ABTS free radical scavenging ability of Jaboticaba whether it is fresh fruit, fermentation broth, or supercritical extraction, as shown in Figure 3. The scavenging capacity of its fermentation broth is 90.38%, while the scavenging capacity of supercritical extraction liquid is 90.80%. Therefore, the difference in ABTS free radical scavenging ability between fermentation broth and supercritical extraction fluid is very small, but it is 54.6% higher than that of fresh fruit.

4. Conclusion

Overall, Jaboticaba contains nutritious polyphenolic compounds and strong antioxidants. However, when fermentation technology and supercritical extraction technology are used to increase the value-added of active ingredients and volatile compounds, their composition and content are significantly different. After fermentation, Jaboticaba has superior flavor and strong antioxidant capacity, making it suitable for development into a healthy drink with raw fermented juice. In addition, Jaboticaba is fermented and then subjected to supercritical extraction. It has a strong antioxidant capacity and is rich in volatile compounds and small molecule amino acids. It is suitable for development into related anti-inflammatory or sleep-aiding health foods. Therefore, the value-added use of fermentation technology and supercritical extraction technology can provide relevant active ingredient data and be used in clinical research and development of anti-inflammatory or sleep-promoting products with high economic benefits.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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