

Discussion of the Composition of Jaboticaba in Different Processes

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Abstract

This study discusses the composition analysis and comparison of Jaboticaba under different processing conditions for fermented liquid, jam, fruit powder, and fruit vinegar. The differences and comparison of the contents of total polyphenols, anthocyanins, resveratrol, superoxide dismutase, small molecular peptides and ellagic acid in their products were analyzed. After analysis and comparison of results, the content of total polyphenols was found to be the highest 44.82 ± 0.89 (mg/g) after being fermented directly from fresh fruit. The fresh fruit was dried at low temperature and ground into powder, with a maximum ellagic acid content of 12.799 ± 0.12 (mg/g). Fresh fruit was then made into jam, with the highest anthocyanin content of 57.39 ± 1.20 (mg/g). The fruit vinegar was produced by fermentation. Except for the content of small molecule peptides, which was similar to the content of fermentation broth and fruit powder, they were 20.63 ± 1.61 , 23.84 ± 2.51 and 20.52 ± 1.21 (mg/g), and the rest of the composition was not as good as other samples. In the fresh fruit fermentation broth samples, resveratrol was produced and detected, and its content was 2.14 (mg/L), while it was not detected in other samples, and a superoxide dismutase (SOD)-like enzyme was detected in the fresh fruit fermentation broth. The highest activity was 49002.5 units/mL. Jaboticaba is prepared using a fermentation broth processing method with fresh fruit, and its total polyphenol content is higher than that of fruit powder, jam, and fruit vinegar. The commercial value of Jaboticaba and the establishment of composition content data are relatively improved, which is more promising for the future.

Keywords

Jaboticaba, Total Polyphenols, Anthocyanins, Resveratrol, Small Molecule

1. Introduction

Jaboticaba, the scientific name of which is *Myrciaria Cauliflora*, originates from Brazil. In Taiwan region, it is called vine grape, and Taiwan region's current species are mostly distributed in the central and southern regions and sporadically cultivated. The fruit is spherical, measuring 2 to 3 centimeters in diameter, and resembles a grape in shape. As the fruit ripens, it transitions from a light green hue to shades of purple and dark purple, and the skin becomes brighter. The pulp of the fruit is juicy and sweet, with a slightly sour taste. The sugar content of the fruit ranges from 13 to 17 Brix⁰. It also contains numerous minerals (2.8% - 3.8% DW) and fiber (18% - 19%). It has 45.7 calories per 100 grams, with 87.1% water, 12.58 grams of carbohydrates, 0.11 grams of protein, and trace amounts of tryptophan (1 mg) and lysine (7 mg). Additionally, the fruit contains vitamin B1 (0.02 mg), vitamin B2 (0.02 mg), vitamin C (22.7 mg), calcium (6.3 mg), phosphorus (9.2 mg), and iron (0.49 mg), as well as 1 to 4 brown seeds. The peel is rich in phenolic compounds such as Pyranocyanin B, Quercetin, Isoquercitrin, Quercimeritrin, Quercitrin, Tannins, Gallic acid, and Ellagic acid Anthocyanins and Flavonoids [1] [2].

According to the relevant fruit quality benchmark reference table announced by the Taiwan Agricultural and Food Agency, the sugar content of generally fresh sugar cane is about 20 Brix⁰, and that of sugar cane is about 24 Brix⁰. Ministry of agriculture. The sugar content of brix⁰, pineapple, lichee, pitaya and other fruits is above 13 Brix⁰ [3].

The peel of the Jaboticaba fruit contains depside phenolic substances with a special structure, called Jaboticabin. Depside phenolic substances have antibacterial, anti-HIV, and anti-inflammatory activities; Jaboticaba has been proven to exhibit inhibitory effects on the activity of cancer cells in the intestines, lungs, and leukocytes proliferation [4]. Its peel can increase high-fat diet HDL cholesterol and improve insulin resistance, reduce saturated fatty acids in serum, increase antioxidant defense in plasma, and avoid lipid peroxidation in the liver [1] [5].

The human body constantly produces free radicals. During the process of respiration and metabolism, about 2% to 3% of oxygen is converted into superoxide free radicals. Free radical scavenging, interrupts the chain reaction of lipid oxidation and prevents the progress of oxidation reaction [6] [7]. It can make up for the body's lack of resistance to oxidative damage, help achieve the purpose of anti-oxidation, and delay aging and increase lifespan [8].

Thus far, many studies have focused on the development of plant antioxidant potential. These studies have found that antioxidant capacity is positively correlated with the content of polyphenols in plants [9]. Related reports have also in-

indicated that the antioxidant and free radical scavenging ability of anthocyanins in the human body is 50 times that of vitamin E and 20 times that of vitamin C and that its antioxidant ability can maintain normal cell connections, strengthen the elasticity of microvessels, improve the flow of capillaries and veins, stabilize phospholipids on endothelial cells, prevent arterial and venous cells from being damaged by free radicals, increase the synthesis of colloids and mucopolysaccharides, and prevent excessive aggregation of aggregates attached to the surface of platelets to maintain the arterial wall. It can prevent cardiovascular disease, delay cell aging, slow down diabetes, and improve vision and have anti-cancer functions.

Resveratrol (3,5,4-trihydroxystilbene) is a member of the polyphenol family. Plants produce resveratrol as an antitoxin in response to fungal infections, ultraviolet radiation, and pathological conditions. Dark berries such as grapes, grape seeds, grape skins, and peanuts are particularly rich in resveratrol, with red grapes and red wine being the most abundant sources [10] [11].

Resveratrol has the physiological function of inhibiting cancer cell migration and metastasis. It is also an antioxidant and demonstrates anti-melanoma effects. Additionally, it reduces fatty liver. and can combat obesity [12] [13] [14] [15].

In traditional food processing, processing methods such as high-temperature sterilization, drying, pasteurization (HTST), ultra-high temperature instant sterilization, cooking, pickling, or the use of food additives are often used to sterilize or inhibit the growth of microorganisms and prolong food preservation. The period of time may give processed foods different flavors and shapes; however, thermal processing may also cause adverse changes in the color, aroma, flavor, texture, nutrition, and function of food raw materials. In order to enable food to maintain better quality, nutrition and functionality, the global food industry is actively exploring different processing technologies and innovating from traditional “thermal processing” to “non-thermal processing”.

The scope of non-thermal processing technology is quite broad, including refrigeration and refrigeration, radiation irradiation technology, pulsed strong light irradiation, low-temperature plasma, ozone, and high-pressure processing (HPP) technology that has attracted widespread attention in recent years. However, the flavor of food using high-pressure processing technology is not destroyed by high temperatures, so it is closer to fresh raw materials. Applying it to juice products that have high requirements for fresh flavor, or prepared foods with a special texture, will have better quality evaluations on the consumer side [16]. According to different processes and product requirements, food can be heated through the process to improve the food’s preservation and digestibility at a lower cost. However, the disadvantage of heating is that it can easily lead to the loss of nutrients [17].

Tree grapes are mainly used as fruits in South America. Due to their high sugar content, they can be processed into jams, fruit juices, dried fruits, fruit vi-

negar, or made into fermented liquid nutrients. Since the current cost of high-pressure processing is still much higher than that of thermal processing technology, even though many experiments have proven that high-pressure processing technology has good effects on sterilization or flavor improvement, the progress in commercial application is still relatively slow; in reducing the cost of high-pressure processing, the first priority is to develop cheaper equipment or increase the batch processing capacity of the equipment [16]. Fermenting food can not only make the product more nutritious but also improve its flavor and texture, prolonging its preservation and removing toxic substances from the raw materials [18]. After approximately 3 to 4 days of harvesting fresh Jaboticaba, the fermentation occurs during the day, so it is often used to make juices, sauces, jellies and ciders [19]. which has great market potential, and is not only used in food but also in industry and landscape gardening [20].

2. Materials and Methods

The raw materials for this analysis are obtained from four products of Jaboticaba: fermentation liquid, fruit vinegar, jam, and fruit powder planted and produced by Jaboticaba Biotechnology Company.

At the first stage (starter) of the pretreatment of the raw material for the fermentation broth, the Jaboticaba was washed using RO water, after which it was crushed. For strain activation, dry commercial yeast was added to 100 mL of water at a temperature of 35°C - 40°C. It was stirred to dissolve and adjusted for sugar content to 24 - 26 Brix⁰, with 0.03% activated yeast added. It was then fermented at room temperature in a semi-sealed room. This was performed for a period of 14 days. At the second stage, the individual fermentation broth of the first phase was coarsely filtered, and an equal weight of Jaboticaba was added to it, with its sugar content being adjusted to 65 Brix⁰. The fermentation continued for half a year. After the fermentation broth was matured, the total polyphenols, anthocyanins, Analysis of superoxide dismutase activity, small molecule peptides, ellagic acid, and resveratrol.

Fruit vinegar adjusts the alcohol content of yeast fermentation liquid to 5% - 7% (v/v), and 1% acetic acid bacteria liquid is added for fermentation. The fermentation period at room temperature is 180 days. After the fruit vinegar is fermented and matured, it is related to the detection and analysis of fermentation liquid component content value.

The Jaboticaba must be cleaned, washed with RO reverse osmosis water, then ground into the puree, filled in a vacuum, and sealed. Finally, it must be sterilized at a high temperature of 121°C. For the preparation of fruit powder, the fresh fruit was first dried in the sun for three days and then at a low temperature of 48°C for 72 hours, after which it was ground into a powder at room temperature. The particle size was set so as to pass through a 40-mash aperture filter screen, and the number of revolutions was set to 1600 rpm. After completion, analysis and detection of the sample components' contents were carried out.

The composition analyses of the four samples, including total polyphenols,

anthocyanins, resveratrol, highly active SOD-like enzymes (superoxide dismutase), small molecular peptides, ellagic acid, and so on, are described below.

2.1. Analysis of Total Phenolics [21]

The principle is that in the phosphor molybdotungstic acid complex (Folin-Ciocalteu's reagent), phenol can reduce molybdenum, so when there are more phenol rings in the sample, the phenolic group will interact with Folin-Ciocalteu's reagent. More blue-green complexes can be produced after the phenol reagent reaction. Therefore, we also dissolved the standard substance (cyanidin) and an appropriate amount of methanol extract with 80% methanol solution to a certain concentration, respectively took 0.2 mL and added 0.8 mL of 7.5% sodium carbonate solution, and added 1 mL of Folin-Ciocalteu's phenol reagent after mixing it evenly. It was then made to stand for 30 minutes in the dark at room temperature, while a spectrophotometer (UNICAM-2.06V, UK) was used to measure the absorbance at 765 nm and calculate the total phenolic content of the sample from the standard curve of cyanidin. The phenolic content of the sample is expressed in milligrams of cyanidin equivalent (CYA) per 100 grams of extract dry weight (mg/CYA/100 g dry mass).

2.2. Determination of Anthocyanin Content [22]

1 g of the dry powder was added to methanol containing 1% hydrochloric acid for extraction. 0.5 mL of the extract was added to 0.25 mL of 2.4 N hydrochloric acid, mixed uniformly, acidified at 100°C for 40 mins, concentrated with a vacuum concentrator (Thermo SPD111V, USA) after acidification, and then re-dissolved in methanol containing 1% hydrochloric acid. The filtrate (~20 µL) was filtered through a 0.45 µm filter membrane, and it (~20 µL) was used for high-performance liquid chromatography (HPLC) to analyze the type and content of anthocyanins. The photo-diode array detector (PDA) detection system was used to characterize and quantify anthocyanins. The retention time was used as the qualitative basis, the chemical standard as a comparison for UV spectral separation and detection, and the area of the UV light peak as the quantitative anthocyanin content. The mobile phase of HPLC (Waters 2695, Waters 2996, USA) was 69% water: 10% acetic acid: 21% methanol, flow rate 1 mL/min, and the analytical column was ODS column (Inertsil ODS-3 column, 4.6 × 250 mm, 5 µm; Precolumn: Inertsil ODS-3 column, (4.6 × 33 mm, 5 µm). The types and contents of anthocyanins were compared with standard products such as cyanidin, pelargonidin, delphinidin, paeoniflorin, and malva (Extrasynthese, France), and calibration lines were made (0.01, 0.02, 0.04, 0.06, 0.08, 0.1 mg/mL).

Determination of resveratrol: using high-performance liquid chromatography, the analytical column is C-18 reverse phase column (Mightysil, RP-18 GP250-4.6, 5 mm), and the mobile phase is 10 mM phosphoric acid (65%) and acetonitrile (35%). The flow rate was 1.0 mL/min (measured with a UV detector), the wavelength was 310 nm, and the injection volume was 10 µL.

Determination of highly active SOD-like enzymes: According to the method of Shimada, [23] after centrifugation of the sample, use 1 mL of the sample after appropriate dilution, add 5 mL of freshly prepared 0.1 mM DPPH methanol solution, shake and mix evenly, and then let it stand in the dark at room temperature. After 50 minutes of reaction, measure the absorbance at the wavelength of 517 nm. The lower the absorbance, the stronger the scavenging ability. The absorbance was then entered into the formula below to compute the scavenging rate, which is reported as a percentage. The samples were replaced with distilled water in the blank group, and 1 mL of the sample was mixed with 5 mL of methanol solution in the control group. In addition, ascorbic acid is used as the standard substance to make a standard curve, and the relative ascorbic acid concentration of the sample is calculated. The higher the clearance rate, the higher the corresponding ascorbic acid concentration.

Determination of the total concentration of small molecule peptides: Ultrafiltration centrifugation is used to separate small molecules with a molecular weight of less than 10KD in the sample, and the Pierce BCA protein Assay Kit is used to determine the total protein concentration of small molecules in the separation solution.

Termination of ellagic acid: The analytical column was a C-18 reverse phase column (Mightysil, RP-18 GP250-4.6, 5 mm), the mobile phase was 10 Mm phosphoric acid (65%) and methanol (35%), the flow rate was 1.0 ml/min, the wavelength was 254 nm, and the injection volume was 10 L, according to the National Institute of Traditional Chinese Medicine of the Ministry of Health and Welfare, 2019 Chinese Medicine Quality Analysis Method [24].

3. Results and Discussion

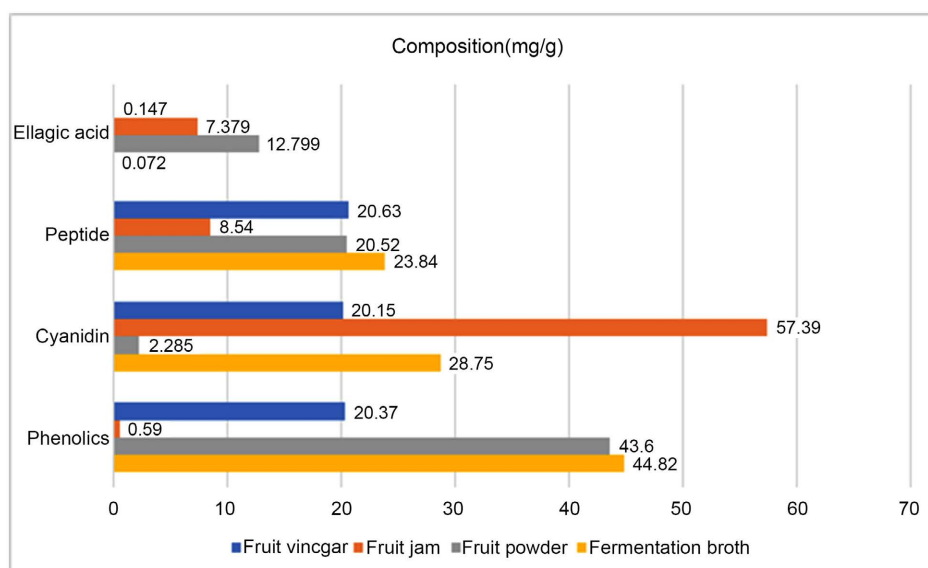
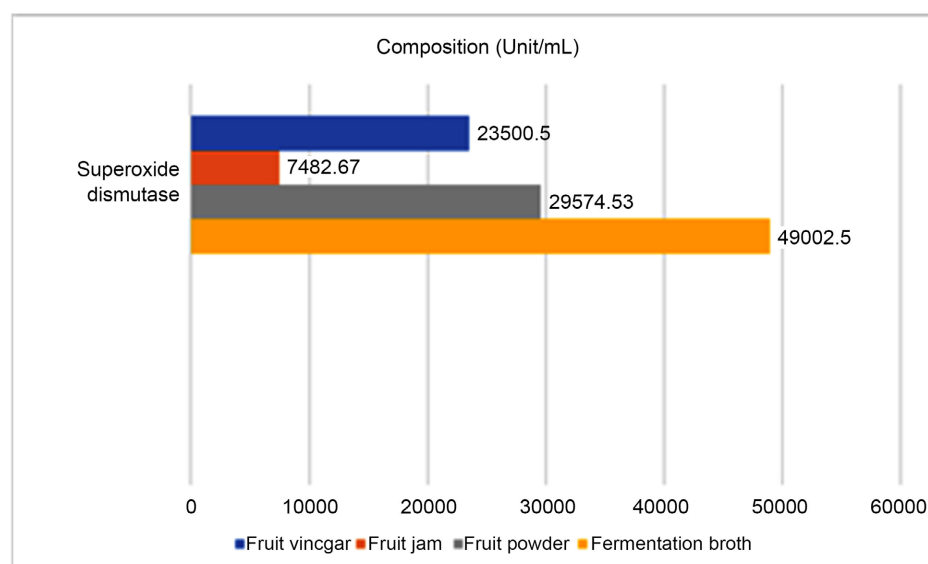
In this study, the contents of total polyphenols, anthocyanins, resveratrol, SOD-like activity, small molecule peptides, and ellagic acid were first detected and analyzed in fresh fruits, as shown in **Table 1**. In addition, Jaboticabin was prepared into experimental samples of fermentation broth, fruit powder, and jam, with the maximum content of total polyphenols in the fermentation broth being 44.82 ± 0.89 (mg/gallic acid) in all samples, as shown in **Table 2** and **Figure 1**. The active content of the SOD-like enzyme was also the highest, at 49002.5 (Unit/mL), as shown in **Figure 2**. Resveratrol was also detected at a content of 2.14 mg/g, as shown in **Figure 3**.

Table 1. Changes in composition and content of Total polyphenols, Anthocyanins, Resveratrol, ellagic acid, small molecule peptides and SOD-like of flesh jaboticaba.

Cultural/Item	Total polyphenols	Anthocyanins	Resveratrol	Ellagic acid	Small molecule peptides	SOD-like
Unit	mg/g	mg/g	mg/L	mg/g	mg/g	unit/mL
Flesh Jaboticaba	44.82 ± 0.89	28.75 ± 0.74	-	9.02 ± 0.2	23 ± 2.51	7482.67
-Not detected						
*Mean ± SE						

Table 2. The content of total polyphenols, anthocyanins, resveratrol, SOD-like activity, small molecule peptides and ellagic acid measured by different processing and preparation of jaboticaba.

Cultural/Item	Total polyphenols	Anthocyanins	Resveratrol	Ellagic acid	Small molecule peptides	SOD-like
Unit	mg/g	mg/g	mg/L	mg/g	mg/g	unit/mL
Jaboticaba fermentation broth	44.82 ± 0.89*	28.75 ± 0.05	2.14	0.072 ± 0.001	23.84 ± 2.51*	49002.5
Jaboticaba powder	43.66 ± 0.16	2.285 ± 0.047	-	12.79 ± 0.12*	20.52 ± 1.21	29574.5
Jaboticaba jam	0.59 ± 0.01	57.39 ± 1.20*	-	7.379 ± 0.21	8.54 ± 0.25	7482.67
Jaboticaba vinegar	20.37 ± 0.29	20.15 ± 0.39	-	0.147 ± 0.001	20.63 ± 1.61	23500.5
-Not detected						
*Means ± SE						

**Figure 1.** Comparison of total polyphenols, anthocyanins, small molecule peptides, and ellagic acid measured by different processing and preparation of Jaboticaba.**Figure 2.** Comparison of the SOD-like activity of the Jaboticaba after different processing.

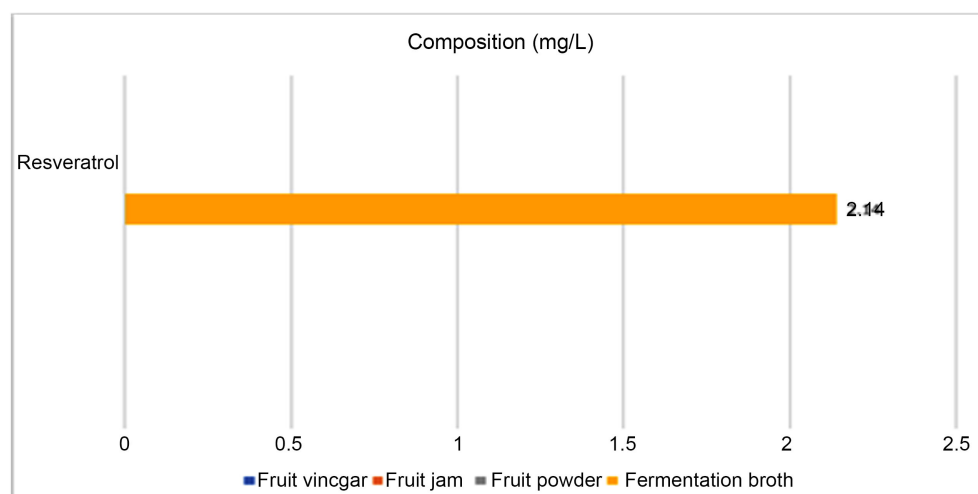


Figure 3. Comparison of the resveratrol content measured in the samples of Jaboticaba after different processing.

In other samples, the content value was not detected. The content of fruit powder in comparison to the total polyphenol content and the fermentation broth was not significantly different, but the content of ellagic acid was the highest of all items at 12.799 ± 0.12 (mg/g), as shown in **Figure 1**, while the content of anthocyanins was the lowest at 2.285 ± 0.047 (mg/g). The jam contained a maximum anthocyanin content of 57.39 ± 1.20 (mg/g), the highest content of the four samples. Compared with the fermentation broth, although the content analysis of fruit vinegar is also through the fermentation process, the content of related components is lower than that of fermentation broth, and the content of small molecule peptides is 20.63 ± 1.61 (mg/g), which is better than that of jam as shown in **Figure 1**.

After Jaboticaba is processed into different products, experimental analysis confirms that different processing and preparation conditions have obvious differences and effects on the content of its components. When fresh Jaboticaba is treated by different processes such as fermentation, grinding, and heating and so on, in its total polyphenols, anthocyanins, resveratrol, SOD-like activity, small molecule peptides and ellagic acid, and so on, the component content is significantly different. The advantage of Jaboticaba in fermentation broth samples is that a relatively complete and high amount of composition can be obtained, and the composition of resveratrol can be produced and detected. Although fruit vinegar is also processed by fermentation, the relative component content is still inferior to the fresh fruit fermentation broth, possibly because of the influence of bacteria or other processing factors, resulting in a decrease in the content; even in the fruit vinegar sample, the content of resveratrol is not detected.

In addition, in terms of heat treatment, the anthocyanins retained by jam are higher than other samples, as shown in **Figure 1**, but after other processing procedures, such as mashing, filling, and sterilization, the content of phenolic compounds and SOD-like activities will be affected and destroyed. In terms of drying and grinding advantages to the sample, after the fresh fruit is dried and ground

into powder, the total polyphenol content and the fermentation broth content are not much different, but the content of ellagic acid is the highest in all samples as shown in **Figure 1**. However, the anthocyanin content is the lowest, so when the Garbo fruit is made into a powder by drying and grinding processing procedures, the sample gets affected by the temperature and grinding particle size, which has an effect on the anthocyanin and SOD-like active content value of the sample.

4. Conclusions

According to different food processing technologies such as fermentation technology, low-temperature grinding, and heating filling, the fresh fruit of Jaboticaba is used to discuss the components of Jiabao fruit that are conducive to commercialization. Analyses of the contents of total polyphenols, anthocyanins, resveratrol, SOD-like activity, small molecule peptides, and ellagic acid in the samples provided the following:

- 1) After fermentation, the total polyphenols, anthocyanins, SOD-like, small molecule peptides, and resveratrol in the four samples can be obtained as a relatively complete composition.
- 2) The content of peptides is not as good as that of the fermentation broth, and it is easily affected by grinding in the process, such as temperature, revolutions, and particle size set by grinding.
- 3) Jaboticaba jam can only retain anthocyanin and ellagic acid, and the remaining components are seriously lost due to thermal damage.
- 4) Jaboticaba is fermented to produce fruit vinegar. Total polyphenols, anthocyanins, SOD-like, small molecule peptides, and ellagic acid can be retained. The content of its related components is not as good as that of fermentation broth, and no white gluten has been detected. The main effect of the veratrole content should be the decrease or loss of components due to the length of microbial fermentation and the utilization of substances.

To investigate the development and manufacture of various Jiabao fruit products using various processing methods, we need to analyze and compare the content of the components in the products, understand the changes in the components of the samples, and select appropriate processing conditions and technologies to produce output. The best health products will be an important basis for the future development of Jaboticaba into healthy food.

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Author Contributions

Ching-Hu Tsai data analysis and writing; Yi-Chun Lin formal analysis; Chih-Ta Liu validation; Yo-Ju Chen methodology.

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Conflicts of Interest

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

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