Effects of Superfine Grinding on the Structure, Bioactive Components and Antioxidant Activity of Pineapple Leaf Fiber

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Abstract. To investigate the influence of particle size of pineapple leaf fiber (PALF) powders on their structure, bioactive components and antioxidant activities, different sized PALF powders were prepared using superfine grinding. Particle sizes and surface morphology of PALF powders were examined with laser diffraction instrument and scanning electron microscopy (SEM) respectively. Meanwhile, the contents of polyphenols and flavonoids in PALF powders were determined by UV method, and the antioxidant capacity was investigated. During the superfine grinding process, as the time prolonged, the particle size of PALF powders sharply decreased and the surface area increased. When the grinding time reached to 60 min, PALF superfine powder having a median diameter of 18.209 µm was obtained. Scanning electron micrograph displayed that the shape and particle size of superfine powders were uniform. After superfine pulverization, the bioactive components of PALF can be well preserved. The total polyphenols content of PALF superfine powder was 2.347 mg GAE/g DW and the total flavonoids content was 0.227 mg RE/g DW. Therefore, the antioxidant capacity of PALF superfine powder remains high level, including DPPH radical scavenging rate (0.167 mg GAE/g DW), ABTS radical scavenging rate (0.105 mg GAE/g DW) and total antioxidant activity (67.379 unit/g DW). The results demonstrated that PALF superfine powder has good potential to be used in functional food, cosmetics and biomedical materials.

1. Introduction
Pineapple is one of the most popular and delicious perennial fruit. With the rapid development of pineapple cultivation, a large number of pineapple leaves are disposed as a waste after fruit harvesting annually. Recently, due to the concerns of sustainable development, more and more pineapple leaves as an alternative fiber resource has become increasingly attractive[1]. Many attempts has been made to develop the potential applications of this agricultural residues [2].
Pineapple leaf fiber (PALF) is a natural and environmentally friendly fiber extracted from the pineapple leaves, which presents superior mechanical properties[3]. It also possesses some better characteristics such as vascular bundle system and hygroscopicity compared to other natural fibers [4]. In recent years some researchers have isolated nanofibers from PALF, expanding their applications in the field of textile, food, drug delivery, medicinal implants and green packaging materials [5].
Superfine grinding technology is a physical processing approaches to make superfine powder with superior properties [6-7]. It has been adopted in food material industries and biotechnology companies nowadays, but far from prevalent [7]. Many scholars have reported the effects of this technology on
the physicochemical properties of fruit, vegetable and grain powders, such as pear pomace powders [6], sugar beet pulp powders [7], red grape pomace powders [8], ginger powders [9] and red rice powders [10]. However, to our knowledge there is no report of PALF superfine powders. This study aims to evaluate the influences of superfine grinding on the surface structure, polyphenol content and antioxidant activities of PALF. We hope this research could provide a useful basis for green utilization of PALF.

2. Materials and Methods

2.1. Materials and Regents

Pineapple (Ananas comosus cv. Cayenne) leaf fibers were supplied by Agricultural Machinery Institute, Chinese Academy of Tropical Agricultural Sciences. Gallic acid (≥98%), rutin (≥98%), 1,1-diphenyl-2-picryl-hydrazyl (DPPH), Folin-Ciocalteu reagent and 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were bought from Merck Co., Ltd. Total antioxidant capacity (T-AOC) assay kit were bought from Institute of Jiancheng Biology Engineering, Nanjing.

2.2. Preparation of PALF Powders

Dried PALF was treated with a two-roll mill, and then screened through 40-mesh sieve to obtain coarse powder. The superfine powders were prepared by a vibrating micronizer (WZJ-12B1, China) for 15, 30 and 60 min, respectively. Finally, four sizes of PALF powder samples were obtained, numbered 0, 1, 2 and 3.

2.3. Particle Size Determination

The particle size determination of PALF powders were carried out on Mastersizer 2000 laser particle analyzer manufactured by Malvern, UK. All samples were measured three times.

2.4. Surface Morphology Analysis

The surface morphology of PALF particles was analyzed by using a scanning electron microscope (Hitachi S-4800, Japan) with 0.5-30.0 kV accelerated voltage. All samples were coated with gold of 20 nm before analysis.

2.5. Polyphenols and Flavonoids Content Analysis

The free phenolic, bound phenolic and flavonoid fractions in PALF powder were extracted and determined by our previously reported methods [11]. All samples were extracted three times. The free and bound phenolic content of PALF powder extracts were determined at 760 nm using Folin-Ciocalteu reagent in a UV–visable spectrophotometer (Shimadzu UV-1780, Japan). Total flavonoid content in PALF powder extracts were measured at 510 nm.

2.6. Antioxidant Capacity Analysis

Extracts of PALF powders were prepared as described in section 2.5 and stored at 4 °C for further use. All samples were measured for three times. The activities of PALF powder extracts to scavenge DPPH and ABTS free radicals were measured by the method of Wang et al [11]. T-AOCs of different sized PALF powder extracts were investigated using a commercial assay kit. Measurements were performed according to the procedure of the assay kit.

3. Results and Discussion

3.1. Particle size of PALF Powders

Table 1 shows the influence of superfine grinding on PALF particle size. The D0.5 value represents the median diameter of the particles and can be used to compare the size of different PALF powder samples. Comparing to the coarse powder (0), the diameter of the PALF powder treated by superfine grinding for 15 min (1) was significantly reduced. Then the particle size decreased gradually with
longer treating times. The D₀.5 of the sample obtained after 60 min of superfine grinding was only 18.209 µm.

According to table 1, as the particle size decreased, the specific surface area increased from 0.173 to 0.474 m²/g. It was clear that micronization treatment effectively increased the specific surface area of PALF powder by breaking down it into smaller particles. The superfine powders would successfully achieved homogeneity when they are mixed with other powder additives [12].

| Samples | Particle size D₀.5 (µm) | Surface-weighted mean diameter (µm) | Volume-weighted mean diameter (µm) | Specific surface area (m²/g) |
|---------|-------------------------|-------------------------------------|-----------------------------------|-----------------------------|
| 0       | 116.385±0.172           | 34.699±0.006                        | 168.898±0.092                     | 0.173±0.0001                |
| 1       | 41.510±0.081            | 18.883±0.002                        | 56.963±0.036                      | 0.318±0.0004                |
| 2       | 29.630±0.066            | 17.409±0.004                        | 38.329±0.042                      | 0.345±0.0004                |
| 3       | 18.209±0.003            | 12.647±0.004                        | 22.435±0.022                      | 0.474±0.0005                |

Table 1. Micromeritic parameters of different size PALF particles

a Each data in the table is expressed as the mean ± standard deviation of three replicates.

3.2. Surface Morphology of PALF Powders

The surface structural changes of PALF powders after superfine grinding treatment were shown in figure 1. The PALF coarse powder retained with original fiber structure and irregular shape. The size of fine powder (treated for 15, 30 min) obviously reduced, but not uniform, still existing partial long fibers. And the size of superfine powder (treated for 60 min) was small and uniform, almost not existing long fiber, which has better structural property than other superfine grinding samples.

Figure 1. Morphology of different sizes of PALF powders: Coarse powder (a), superfine grinding for 15 (b), 30 (c) and 60 min (d).

3.3. Polyphenols and Flavonoids Content in PALF Powders

It has been reported that the grinding-fractionation process has a great effect on chemical composition separation of different particle size fractions [13]. In our previous studies [11], PALF was found to contain polyphenols and flavonoids. Therefore, the influence of size variation of PALF powder on these bioactive components was investigated. As shown in figure 2, the free phenolic content of PALF powder decreased from 1.752 to 1.344 mg GAE/g DW after 15 min of superfine grinding, and then gradually increased to 1.615 mg GAE/g DW as the grinding time increased from 15 to 60 min. While in the same process, the bound phenolic content of PALF powder increased slightly as the size reduction. For all superfine grinding treated PALF powders, the free phenolic content increased with decreasing particle size. This might be due to the finer the powders and the larger the specific surface area, making it easier to release the soluble free phenolic component [14]. The variation trend of total phenolic content was consistent with that of free phenolic content. Total phenolic content of PALF coarse powder and superfine grinding 60 min powder could reach 2.369 and 2.347 mg GAE/g DW, respectively, the difference being not significant. It implied that the bioactive polyphenols of PALF could be preserved well after treated by superfine grinding.

In addition, the total flavonoids content decreased with the size reduction of PALF powders, but increased slightly when the superfine grinding time reached to 60 min. Interestingly, it was not consistent with the changing trend of total polyphenols. The results indicated that the total flavonoids content in PALF powder was more sensitive to temperature and O₂ than total polyphenols. During the
process of superfine grinding, the oxidation of total flavonoids might be accelerated by the increasing temperature and the contact area with O₂ along with the treating time.

Figure 2. The free phenolic, bound phenolic and total flavonoids content of different particle-sized PALF powders.

3.4. Antioxidant Activity of PALF Powders
The DPPH and ABTS radical models were the basis of antioxidant assay. According to figure 3, the polyphenols in PALF coarse powder have higher values both of DPPH and ABTS radical scavenging capacity than superfine grinding treated powder, reaching to 0.169 and 0.129 mg GAE/g DW, respectively. For all samples treated by superfine grinding, the DPPH and ABTS scavenging activity increased with the size reduction of PALF powder, and these capacities of ultrafine powder obtained at 60 min of superfine grinding were 0.167 and 0.105 mg GAE/g DW, respectively. The changing trend of these two free radical scavenging capacities were nearly identical, and was in agreement with the total polyphenols content.

Furthermore, the T-AOCs assay of different sized PALF powders were carried out, and the change of T-AOC was also in accord with the total polyphenols content. It might be suggested that the antioxidant activity of PALF powder extracts has a great correlation with the total polyphenols content.

Figure 3. Antioxidant activities of different sized PALF powders extracts

4. Conclusion
Superfine grinding was employed in this work to prepare finer PALF powders. After 60 min of treatment, the particle size of PALF powders drastically decreased from 116.385 to 18.209 µm. According to our results, the content of free and total phenolic increased along with the particle size reduction in the superfine grinding process, as well as DPPH free radical scavenging activity and T-AOC. Compared with the coarse powder, the content of total flavonoids in treated PALF powder decreased slightly, but the superfine PALF powder also had some characteristics keeping high level, including free and total phenolic content, DPPH free radical scavenging activity and T-AOC. In view of these structural advantages and bioactive components, superfine PALF powder can be used in functional food, cosmetics, medicines and biomaterials.
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