Effect of Chemical Treatment on the Phenolic Content in Pineapple Leaf Fiber (PALF)

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Abstract: Chemical pretreated pineapple leaf fiber (PALF) can be most widely used in many industries.Since no information on the bioactive compounds in PALF is currentlyavailable, the aim of this study was to assess the effect of chemical treatment on the phenolic content of PALF extracts. The phenolic contentsand antioxidant properties of PALF extracts were determined as free radical-scavenging ability of DPPH andusing the ABTS radical cation decolorization assay..The important variables in chemical treatment process including temperature, time and acid/alkali concentration were investigated. According to the results, the optimal pickling condition was 10 g/L H_2SO_4 at 55℃ for 40 minutes and the optimal alkali boiling condition was 5 g/L NaOH at 50℃ for 60 minutes. Under the optimal condition, the free phenolic content of PALF was up to 0.2855 mgGAE/gDW.

Introduction

Pineapple is one of the mostimportant tropical fruit which grows in the tropics and sub-tropics, particularly in leading pineapple producing nations such as Malaysia, Thailand, India, Philippines, Australia, Mexico, Kenya, South Africa, China and so on[1,2]. Besidesnutritional uses, its medicinal properties have been widely studied[3]. Pineapple leaves which are the byproduct of pineapple cultivationalso have many potential functional activities. In recent years, with the development of science technology, previous research on the extract of pineapple leaves revealed phenolic components and their valuable pharmacological activities, including anti-oxidative, anti-diabetic and antidyslipidemic activities[4].

The pineapple's total world production almost approach 20 million tons in 2010. Such a large amount of production and worldwide distribution will make considerable pineapple leaves. How to properly deal with the pineapple leaves has been a knotty problem for years. At present, pineapple leaves are mainly used in some ways such as feeds, paper production, and fiber. Most of the rest almost were fired, which lead to great waste and environmental pollution. Recently, due to the concerns of sustainable development, more and more pineapple leaves as an alternative fiber resource has been highlighted[5]. Up till now,many researchers have selecting nano-crystalline cellulose(NCC) from pineapple leaf fiber(PALF) as reinforced material, which have broadapplicationprospect in the field of food, medicine, papermaking chemistry and green

packaging materials[6-9]. But by now, there is almost no study concerned the active substance such as phenols of PALF.

In the study, the contents of total phenolsandflavonoids in PALF and their antioxidant activity were determined. Further,the effect of chemical treatmenton the free phenolic content inPALF was investigated. It is the first study focusing on the phenolscompounds and its antioxidant activity in PALF.

Materials and Methods

Materials. PALFused in this studywas obtained from Agricultural Machinery Reserch Institute, Chinese Academy of Tropical Agricultural Sciences.Folin-Ciocalteu reagent, 2,2-azino-bis (3-ethylbenzthiazoline -6-sulphonic acid) (ABTS+) and 1,1'-diphenyl-2-picrylhydrazyl (DPPH) were bought from Sigma Chemical Co.(St. Louis, MO, USA). Gallic acid, rutin were bought from Yuanye Biotechnology. TotalantioxidantcapacityAssayKit were bought from The Institute of jiancheng biology engineering, Nanjing. All other chemicals and reagents were of analytical grade.

Sample Preparation and Particle Size Measurement. PALF was grinded coarsely, then the powders were screened through 40-mesh sized sieve. The particle size distribution of PALF particles was determined by a laser diffraction instrument (Mastersizer 2000, UK).

Determination of Phenols and Flavonoids Content. Five grams ofPALF powder were homogenized with 115 mL of 59% ethanol, Phenolic compounds were extractedat 71 ℃ for 118 min under stirring. The mixture was centrifuged and the supernatant liquid was filtered, concentration. Phenols of PALF powder extracts were determined by Folin-Ciocalteu reagent[10]. All samples were determined in triplicate. The filter residue was further processed to determine bound phenolic using a modified method[11]. 25 mL of 16% NaOH in deionizedwater was added to each filter residue sample with constant shaking for 1h. And added a certain amount of 14.6% HCl to neutralize pH 6.8-7.2. Then extractingneutral liquor with acetoacetic ester, evaporating by a water bath at 45 ℃ and constant volume to 10 ml. Extraction was repeated three times. The results were expressed as gallic acid equivalents in mg/g dry matter (dw).

The total free flavonoids content of the PALF powder extract was estimated using a slight modified method[12]. Ethyl alcohol (30%) was added into the Solutions to 5 mL. $NaNO_2$ (0.3 mL, 5%) were added and letstand for 10minutes. $Al(NO_3)_3$ (0.3 mL, 10%) were added and let stand for 10minutes. Then added NaOH (4 mL, 4%), and the solution was set to 10 mL, kept for15 min.The absorbance measured at 510 nm. Measurements were repeated three times. The results were expressed as rutin equivalents in mg/g dry matter (dw).

Evaluation of the Antioxidant Capacity. The extract of PALF was prepared in above. Absorbance measurements were read on a UV-1780 spectrophotometer (Shimadzu, Japan).

Evaluation of DPPH Scavenging Assay. Scavenging DPPH radical capacity of PALF powder extract was carried out according to the modified method of Monica L et al[13]. The standardcurve was drawnwith gallic acid. The DPPH solution in anhydrous ethanol was adjusted absorbance 0.60±0.02 at 515 nm. 100 μL sample extracting solution was mixed with 2 mL DPPH solution. The mixture was shaken well, allowed to stand for 10 min in the dark, and then the absorbance at 515 nm was determined(A_i). The blank absorbance(A_0) consisted of 100 μLdeionizedwater to replace sample. Measurements were repeated three times. The capability to scavenge DPPH radical was calculated using the following formula:

$$\text{Scavenging rate}(\%) = \frac{A_0 - A_i}{A_0} \times 100\%. \quad (1)$$

Evaluation of ABTS Radical Cation Inhibition. Scavenging ABTS radical capacity of PALF powder extract was carried out according to the modified method of Re R et al[14]. The standardcurve was drawnwith gallic acid. Reacting 7 mM ABTS solution with 2.45 mM potassium persulfate to prepare ABTS radical cation and was set to 25 mL by deionizedwater, then allowing the mixture to stand in the dark at room temperature for 12~16 h. The solution was diluted with deionizedwater to an absorbance of 0.90±0.02 at 734 nm. 100μL sample extracting solution was mixed with 2 mL ABTS solution. The mixture was shaken well, allowed to stand for 10 min in the dark, and then the absorbance at 734 nm was determined(A_i). The blank absorbance(A_0) consisted of 100 μLdeionizedwater to replace sample. Measurements were repeated three times. The capability to scavenge ABTS radical was calculated using the following formula:

$$\text{Scavenging rate}(\%) = \frac{A_0 - A_i}{A_0} \times 100\%. \quad (2)$$

Acid-alkali Treatment. Weighed 40.0 g PALF samples accurately. Based on chemical degumming process including pickling, water washing, alkali boiling, water washing, acid washing and water washing[15], mainly evaluated the effect of pickling and alkali boiling treatment on the free phenolic content in PALF. The condition of acid washing was at room temperature for 10 minute with 2 g/L H_2SO_4 which was definite. The condition of alkali boiling was at 50 ℃ for 60 minutes with 5 g/L NaOH. Under the conditions, determine the chromaticity and the content of free phenols in PALF after treatment, to optimize the time, temperature, concentration of the pickling. Then, under the optimum conditions of pickling, optimize the condition of alkali boiling.

Statistical Analysis. All results were worked out standard deviation of three replicates, and were evaluated by one-way ANOVA. Statistical analysis of the data was done using the Origin and excel software. Means for differences were considered statistically significant at $P < 0.05$.

Results and Discussion

Particle Size Distribution. Particle size distribution of PALF sample was shown in Fig. 1, which mainly concentrated in the range from 100 to 400 μm. The particle size d(0.5), surface weighted mean, volume weighted mean and surface area were 116.385 um, 34.699 um, 168.898 um, 0.173 m^2/g, respectively.

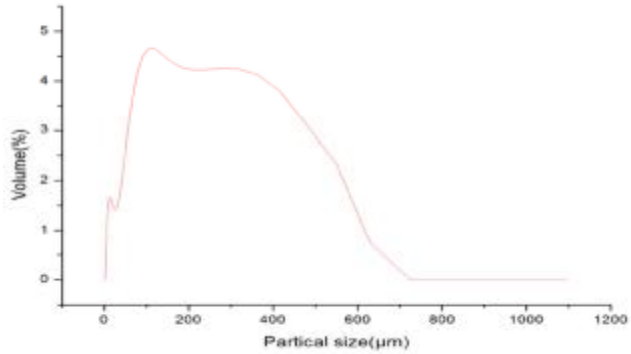

Fig. 1 Particle size distribution of PALF

Active Phenolic Compounds in PALF and Its Antioxidant Activity. Result for bioactive compounds content was presented in Fig.2. The content of free phenolicextracts from PALF is the largest at 1.7524 mgGAE/gDW. However, the content of bound phenolic extracts reduced by 64.80% at 0.6169 mgGAE/gDW, which had significant difference with the free phenolic. Comparing with the phenolic, flavonoids was the lowest content inPALF at 0.2756 mgRE/gDW, which lower than one second of bound phenolic extracts'. On the whole, the content of total phenolic including free and bound phenolic in PALF was still well appreciable. Therefore, PALF had a lot of application in reinforced composite materials, especially in degradation of Membrane

material. Correspondingly, as shown in Fig.2, the antioxidant capacities of free phenolic from PALF valued the DPPH and ABTS radical scavenging capacity was 0.1690 and 0.1289 mgGAE/gDW, respectively. All in all, PALF will widely used in the cosmetics, food and medical industry has enormous potential.

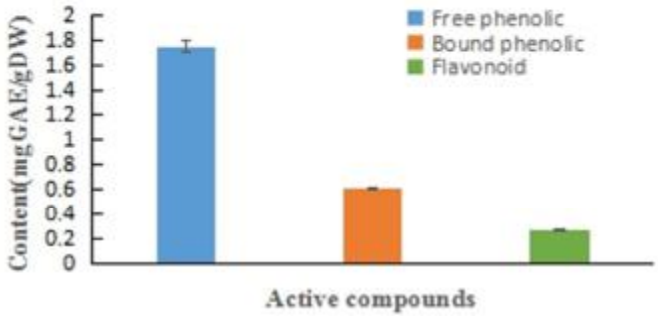

Fig.2 Thefree phenolic, bound phenolic and free flavonoids content of PALF

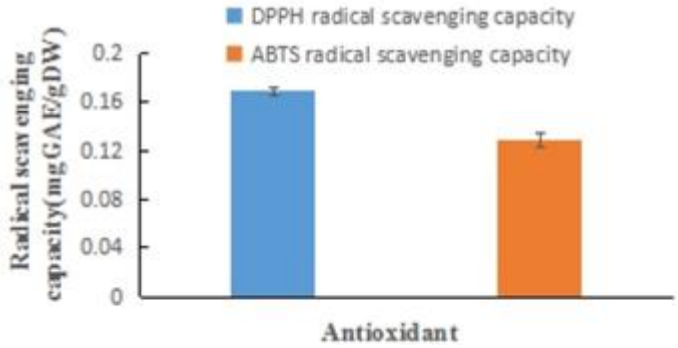

Fig.3 Antioxidant capacities of PALF extracts

Effect of Chemical Treatment on Free Phenolic Content in PALF. As shown in table 1, with the increase of pickling time, the mass loss rate always increased and the free phenolic content from PALF increased first and then decreased. Moreover, the highest content of free phenolic was 0.2809mgGAE/gDW with the pickling time at 40 minutes. As for pickling temperature, the changing trend of free phenolic content was the same as the former and further definite the most suitable temperature was 55℃. Since the temperature exceeded 55℃, the cellulose began to hydrolyze and the structure of polyphenol compounds were damaged. Similarly, free phenolic content also increased first and then decreased as the pickling concentration increased. This may be due to the low acid concentration, the pineapple leaf fiber structure was loose, thus releasing more free phenols. However, when acid concentration increased, the polyphenol compounds was destructed. So the optimal pickling condition was 10 g/L H_2SO_4 at 55℃ for 40 minutes.

Different alkali boiling treatment used resulted in significant decreases in content of free phenolic from PALF. An increase in alkali concentration was observed in PALF samples, which attributed to the degradation and dissolution of pectin, hemicellulose and lignin, thus resulting in decreased content of free phenolic. While the alkali concentration exceeded 15 g/L, the free phenolic content had not much change. Comparing with alkali concentration, the other tow factors including time and temperature had no extensive impact on phenolic content. It decreased as the alkali time and temperature increased. Because of the alkali time and temperature extend, Hydrolysis of non-cellulose components of PALF fuller and partial phenols was destructed. When alkali temperature was at 80℃, the Content of phenolic had no significant difference with at 50℃, which may be related to PALF's preparation. Finally, based on the optimal pickling condition, the

optimal alkali boiling condition was 5 g/L NaOH at 50℃ for 60 minutes. On this condition, content of free phenolic from PALF was up to 0.2855 mgGAE/gDW.

Table 1 Effect of chemical treatment on the free phenolic content of PALF extracts

Pickling			Alkali boiling			Mass loss rate/[%]	Free phenolic content/[mgGAE/gDW]
Time/ [min]	T /[℃]	Concentration /[g/L]	Concentration /[g/L]	Time /[min]	T /[℃]		
20	55	5	5	60	50	4.96	0.2473±0.0061
40	55	5	5	60	50	12.56	0.2809±0.0052
60	55	5	5	60	50	13.85	0.2614±0.0059
80	55	5	5	60	50	16.83	0.2537±0.0019
40	35	5	5	60	50	11.7	0.2718±0.0088
40	75	5	5	60	50	14.525	0.2454±0.0053
40	95	5	5	60	50	15.575	0.1987±0.0032
40	55	10	5	60	50	13.90	0.2855±0.0081
40	55	15	5	60	50	13.55	0.2265±0.0059
40	55	20	5	60	50	13.63	0.2334±0.0035
40	55	10	15	60	50	18.78	0.1733 ±0.0115
40	55	10	25	60	50	21.55	0.1834 ±0.0085
40	55	10	35	60	50	21.45	0.1505 ±0.0087
40	55	10	5	120	50	15.43	0.2504 ±0.0081
40	55	10	5	180	50	15.78	0.2310 ±0.0049
40	55	10	5	240	50	17.05	0.1954 ±0.0040
40	55	10	5	60	60	15.45	0.2085 ±0.0035
40	55	10	5	60	70	15.78	0.2060 ±0.0020
40	55	10	5	60	80	16.33	0.2751 ±0.0048

The effect of different acid-alkali treatment on content of free phenols from PALF were significantly different ($P < 0.05$).

Conclusion

There are bioactive compoundsin ethanol extracts of PALF. The free phenoliccontent is the largest at 1.7524 mgGAE/gDW, while the bound phenolic and flavonoids content was much lower inPALF. And the extracts have exhibited antioxidant potential.Further more, results of the study showed that chemical treatment on PALF significantly affect the free phenolic content. The maximum value of the free phenolic content was achieved when samples were subjected to under the optimal condition(10 g/L H_2SO_4, 55℃, 40 minutes; 5 g/L NaOH, 50℃, 60 minutes). It means that bioactive compounds are in chemical treated PALF, and it can be used for developing functional material in biomedical, cosmetic and food industry.

Acknowledgments

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