Effect of Steam Explosion Modification and in Vitro Simulated Digestion on Antioxidant Capacity of Dietary Fiber of Pineapple Peel

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Abstract. Pineapple peel is an important by-product in the processing of pineapple, which is rich in dietary fiber. In this paper, the dietary fiber of pineapple peel was modified by steam explosion method, and its effect on the morphology of dietary fiber was investigated. The dietary fiber was simulated in vitro to investigate its effect on the antioxidant activity of dietary fiber. The results showed that the steam explosion treatment can break the bulk volume of dietary fiber and increase the surface area. The in vitro simulated digestion, especially simulated gastric digestion, improved the FRAP antioxidant capacity of pineapple peel dietary fiber, and the antioxidant activity of dietary fiber modified by steam explosion was higher under the same conditions.

1. Introduction
Pineapple [Ananas comosus (L.) Merrill], a perennial herb of Bromeliaceae, Ananas Merr., is the third largest tropical fruit crop after banana and citrus fruits. Global pineapple harvest area accounts for about 35% of the global tropical fruit Harvest Area [1]. According to statistics, the world's total output of pineapples continues to grow, reaching 26.9 million tons in 2016 [2]. Pineapple peel, the by-product in the processing of pineapple, accounts for 40% to 50% of the total weight of the whole fruit [3]. Without utilized effectively, pineapple peel not only caused waste of resources, but also polluted the ecological environment of the processing area.

Pineapple peel is rich in sugars, proteins, pectins, vitamins, minerals, dietary fiber and pigment substances. Furthermore, content of crude protein and ash in peel is higher than that in pulp. Cellulose is the main component of pineapple peel, accounting for about 66.29% of dry weight [4]. The development and utilization of pineapple peel has been widely concerned, but few studies have been able to take advantage of the cellulose in pineapple peel. In the aspect of being a metal ion adsorbent and preparing a hydrogel, organic solvents are used in the cellulose modification process [5], which causes secondary pollution to the environment. Therefore, how to realize the high-value utilization of pineapple pomace resources through green methods is the main direction of future research.
The currently accepted definition of DF was proposed by the American Association of Cereal Chemists (AACC): “Dietary fiber is the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fiber includes polysaccharides, oligosaccharides, lignin, and associated plant substances. Dietary fibers promote beneficial physiological effects including laxation, and/or blood cholesterol attenuation, and/or blood glucose attenuation. [6]” According to the solubility, DF can be divided into two categories, Soluble Dietary Fiber (SDF) and Insoluble Dietary Fiber (IDF). The research showed that SDF and IDF play different roles, among which the latter acts as filler in human body, mainly plays a physical role, while the former can participate in metabolic reaction in human body and more make physiological effect. High quality DF has good processing characteristics, physiological activity and health care function, and the SDF content should not be less than 10%. However, although the proportion of total dietary fiber in many plants is very high, the content of SDF is relatively low, about 3% - 4% only the total dietary fiber. Using a simple and effective method to modify the by-products in the raw materials, so that the SDF content increases, is the focus of DF research field. The steam explosion (SE) technology utilizes superheated steam to treat the raw materials with high temperature and high pressure, so that the raw material voids are filled with steam and swelled by the superheated liquid, and then the volume of the material is expanded to an explosion by instantaneous decompression, so that the cell wall is broken. Because of its low pollution level and low price, SE technology has only the participation of water in the treatment process, and there is basically no chemical reagent addition. In recent years, it has been widely used in food. Li et al. [7] treated okara by SE at different pressures and holding times, and the result showed that when the SE strength was 1.5 MPa for 30 s, SDF content increased significantly from 1.34% to 36.28%. Scanning electron microscopy (SEM) demonstrated that the loose sheet structure of okara surface was destroyed into small fragments after SE treatment and the internal nucleated structure was disintegrated into small particles at high SE strength. In recent years, steam blasting technology has shown higher potential in practical applications, and is a hot spot in the future research of dietary fiber modification.

In 1998, Spanish scholars first proposed the concept of “antioxidant dietary fiber”, which was defined as a combination of a large number of natural antioxidants in dietary fiber matrix [8]. Natural antioxidants mainly refer to polyphenols, including some flavonoids and polymerized tannins [9]. The modified dietary fiber, after in vitro simulated digestion, can reflect its antioxidant capacity by measuring ferric ion reducing antioxidant power (FRAP). In recent years, in vitro simulated digestive models have been used as substitutes for human or animal experiments because of their simplicity, cheapness and renewable advantages, and are increasingly being used to study complex physiological changes of some foods or drugs in the human gut. Most in vitro simulated digestive models are divided into two steps, namely, gastric digestion model and intestinal digestion model [10]. In the process of simulated gastric digestion, pepsin is generally used to simulate gastric juice, and the pH value is adjusted to 1 - 2 with concentrated hydrochloric acid to simulate the acidic environment in the stomach. In the simulated bowel digestion process, the pH is generally adjusted to between 6.5 and 7.5 with NaHCO₃, and then the mixed extract of pancreatic fluid-bile salt is added to the gastric digestion product [11].

2. Materials and methods

2.1. Materials
Raw Dietary fiber (R-DF)
Normal saline (NS)
Hydrochloric acid (1 M, 0.01 M)
Sodium bicarbonate solution (1 M, 0.1 M)
Simulated gastric fluid (SGF): 0.2 g pepsin dissolved in 5 mL 0.01 M HCl
Simulated intestinal fluid (SIF): 2 g trypsin and 12 g bile dissolved in 500 mL 0.1 M NaHCO₃
2.2. Steam explosion treatment
The R-DF was modified by a steam blasting machine. The experimental parameters were: pressure 1.5 MPa, pressure maintenance time 30 s. After processing, the sample is dried and stored in a ziplock bag.

2.3. Morphological determination of pineapple peel dietary fiber before and after modification
A small amount of sample was fixed on a conductive tape observation stage, and subjected to gold spray treatment by an ion sputtering method, and placed under a scanning electron microscope.

2.4. Gastric and intestinal digestion simulation
The in vitro simulated digestion model is based on the method of Miller et al. [12] with a slight improvement.

Gastric digestion simulation: 1) The Erlenmeyer flask is wrapped in tin foil to protect it from light. Add 20 g of RM and 200 mL of NS and mix well with a glass rod. The pH of the suspension was adjusted to 2.0 with 1M HCl and then 2.5 mL of SGF was added. 2) The blank control group replaced the SGF with deionized water. Three parallel experiments were set in all groups. 3) The flask was filled with nitrogen to avoid air oxidation. It was digested in a constant temperature water bath shaker at 37°C. A certain amount of sample was taken in the centrifuge tube at 0, 0.5, 1, 2 and 3 h, and centrifuged at 8000 r/min for 15 min at 4°C. 4) Take the supernatant and store in a refrigerator at -20°C for later use.

Intestinal digestion simulation: 1) Repeat step 1) of gastric digestion simulation. 2) The Erlenmeyer flask was filled with nitrogen and digested in a 37°C constant temperature water bath shaker for 2 h. 3) To the suspension was added 1M NaHCO₃ to adjust the pH to 6.9, then 5 mL of SIF was added, and the control group replaced the SIF with 0.1M NaHCO₃ without trypsin and bile. 4) After purging with nitrogen, it was digested in a 37°C constant temperature water bath shaker. A certain amount of sample was taken in a 50 mL centrifuge tube at 0.5, 1, 2, 3, and 4 h, and centrifuged at 8000 r/min for 15 min at 4°C. 5) Take the supernatant and store in a refrigerator at -20°C for later use.

2.5. Determination of antioxidant capacity
The FRAP value of sample was determined by referring to the method of Thaipong et al. [13] with a slight modification.

Preparation of FRAP working solution: Mix 10 mmol/L TPTZ solution, 20 mmol/L FeCl and 300 mol/L sodium acetate buffer with pH 3.6 in a volume ratio of 1:1:10, shake well and incubate in a 37°C water bath for later use.

0.6 mL sample and 5.4 mL FRAP working solution was mixed and store in a dark environment for 30 min, and measure the absorbance at 593 nm with UV-Vis spectrophotometer. A standard curve was prepared using ferrous sulfate solution as a standard. The FRAP antioxidant value of the sample was calculated as μmol FeE/g for the FRAP antioxidant capacity in the sample.
3. Results and discussion

3.1. Morphology of dietary fiber of pineapple peel before and after modification

Scanning electron micrographs of pineapple pomace dietary fiber are shown in figure 1. It can be seen that the pineapple pomace dietary fiber had a regular surface, and the structure was complete, compact and massive. After the sample was treated by steam explosion, the bulk structure was broken, the bulk volume was significantly reduced, the surface wrinkles were increased, and there are many voids, and the relative surface area was increased.

Figure 1. Scanning electron micrograph of dietary fiber of pineapple peel (a and b are scanning electron micrographs of unmodified pineapple peel DF, c and d are scanning electron micrographs of pineapple peel DF modified by steam explosion).
3.2. Effect of in vitro simulated digestion on antioxidant capacity of dietary fiber of pineapple peel

The changes of FRAP antioxidant capacity of pineapple peel DF before and after SE modification by in vitro simulated digestion are shown in figure 2 and figure 3. It can be seen from figure 2 that the FRAP of R-DF with simulated gastric digestion group increased significantly (p<0.05) within 0.5 h, and became stable after 0.5 h, and was significantly higher than that of the blank control group (p<0.05); The FRAP value of R-DF was up to 1594.68 ± 52.20 μmol FeE/g, which was about 1.65 times that of simulated gastric digestion at 0 h. The FRAP of the modified DF with simulated gastric digestive group was significantly increased within 0.5 h (p<0.05), and reached the maximum at 0.5 h, which was 3374.43 ± 52.33 μmol FeE/g, which was approximately 2.12 times that of R-DF. It showed a decreasing trend after 0.5 h, but was significantly higher than the FRAP of blank control group.

Figure 2. FRAP changes in simulated gastric digestion of A) Raw DF, and B) Modified DF.

The changes of FRAP antioxidant capacity of pineapple peel DF before and after SE modification by in vitro simulated digestion are shown in figure 2 and figure 3. It can be seen from figure 2 that the FRAP of R-DF with simulated gastric digestion group increased significantly (p<0.05) within 0.5 h, and became stable after 0.5 h, and was significantly higher than that of the blank control group (p<0.05); The FRAP value of R-DF was up to 1594.68 ± 52.20 μmol FeE/g, which was about 1.65 times that of simulated gastric digestion at 0 h. The FRAP of the modified DF with simulated gastric digestive group was significantly increased within 0.5 h (p<0.05), and reached the maximum at 0.5 h, which was 3374.43 ± 52.33 μmol FeE/g, which was approximately 2.12 times that of R-DF. It showed a decreasing trend after 0.5 h, but was significantly higher than the FRAP of blank control group.
It can be seen from figure 3 that the FRAP of R-DF with simulated intestinal digestion group increased significantly (p<0.05) within 0.5 h, stabilized after 0.5 h (p>0.05), and the maximum value was 2048.13 ± 61.45 μmol FeE/g. And it was about 2.37 times that of simulated gastric digestion at 0 h, about 1.19 times that of simulated intestinal digestion at 0 h, and about 1.10 times the maximum FRAP of the blank control group (1856.56±58.95 μmol FeE/g); The blank control group showed a trend of increasing first (0 h-1 h) and decreasing (after 1 h). The FRAP of the modified DF with simulated intestinal digestion group increased significantly (p <0.05) within 0.5 h, and decreased significantly after 0.5 h (p<0.05), the highest was 3635.36 ± 45.36 μmol FeE/g. It was about 2.18 times that of simulated gastric digestion at 0 h, about 1.07 times that of simulated intestinal digestion at 0 h, and about 1.64 times that of the R-DF simulated intestinal digestion group. The blank control group showed a significant decrease trend during the whole simulated intestinal digestion process (p<0.05).

Based on the results of figures 2 and 3, compared with the control group, the antioxidant capacity of DF was significantly improved after the two digestion stages of the simulated stomach and intestine. At the same time, comparing the FRAP of simulated digestion before and after modification, it can be seen that the antioxidant activity of DF can be significantly improved by steam explosion.

Figure 3. FRAP changes in simulated intestinal digestion of A) Raw DF, and B) Modified DF.
modification.

4. Conclusion
In this paper, the DF prepared from pineapple peel was used to investigate the effect of physical modification method —— SE on the morphology of pineapple peel DF, and the effect of in vitro simulated digestion on the antioxidant activity of DF before and after modification. The results of morphological analysis showed that the SE treatment can break the bulk volume of DF and increase the surface area, which may increase the content of SDF in total dietary fiber. The results of antioxidant capacity test showed that the in vitro simulated digestion, especially the simulated gastric digestion process, improved the FRAP antioxidant capacity of pineapple peel DF. And the antioxidant activity of DF modified by SE was higher under the same conditions. According to the comprehensive evaluation, SE treatment significantly improved the quality of pineapple peel DF.

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