In Vitro Hypoglycemic Potential of Dietary Fiber From Fermented Seaweed

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Abstract. Dietary fiber can regulate metabolic activity of the body by affecting enzyme activity in the intestine. It cuts down the speed of digestion to relieve the release of glucose, so blood sugar can be controlled in the optimal range. In this study, seaweed was used to prepare dietary fiber by microbial transformation of Lactobacillus rhamnosus and Lactobacillus plantarum. The experiment showed that the content of soluble dietary fiber (SDF) increased significantly after the action of the mixed lactic acid bacteria, and the increments rate was 21.1%. The inhibition rate of α-amylase and α-glucosidase increased by 25.9% and 16.6% respectively after fermentation. Therefore, after fermentation seaweed can enhance the soluble dietary fiber and improve inhibition rate of enzyme activity related to digestion. Dietary fiber of seaweed has potential of hypoglycemic.

1. Introduction

China has a long coastline and seaweed resources abundantly. It is an important part of marine living resources. The fibers of seaweed are considered as “the third fiber” [1]. With the improvement of living standards, “rich disease” and “sub-health” have become a major health problem for people [2]. The dietary fiber was seen as the seventh nutrient [3]. It was divided into soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) according to its solubility [2]. Soluble dietary fiber of seaweed can bind to carbohydrates in the intestine, and reduce the rate of amylase hydrolysis. In addition, it can improve the biological activity and enhance sensitivity of insulin [5,6]. Therefore, it has a good regulatory effect on blood sugar and blood lipids and has important significance.

With the use of seaweed resources, a large amount of insoluble dietary fiber is produced during the seaweed exploited. At present, we mainly use chemical methods [7], physical methods, chemical-enzyme binding method [8,11] to extract and enrich soluble dietary fiber. The chemical method destroys the activity of dietary fiber and the environment Contaminated [12,13], Physical methods can significantly reduce the loss of dietary fiber and environmental damage, and can also significantly improve the rate of product extraction and product quality, but the expense of extraction process is higher and the equipment requirements are high. In this experiment, the kelp was enzymatically hydrolyzed, and then fermented by lactic acid bacteria to prepare soluble seaweed dietary fiber, and the effect of lowering blood sugar was evaluated by detecting the inhibition rate of α-amylase and α-
2. Materials and methods

2.1. strains and materials

The seaweed used in this study were purchased from Fujian. Lactic acid bacteria culture media (MRS) were obtained from AOBOXING BIOTECHNOLOGY. Probiotic strains of *Lactobacillus plantarum* and *Lactobacillus rhamnosus* is from Microbiological Culture Collection Center of Shandong provincial.

2.2. The process of Seaweed fiber extraction

Seaweed were washed with water and cut into small pieces for mechanical separation. These seaweeds were mixed with dipotassium phosphate, acid cellulase, acid pectinase to react at 50 °C for 2 h. The reaction system was adjust with hydrochloric acid to pH4.8. 10% sodium carbonate and 0.3% alkaline pectinase were add, stirred at 60℃ for 1.5h. Finally, the water is added, so that the total system is 30 times of seaweed material, stirring is continued for 50 minutes. The liquid is centrifuged at 3700 r/min. We will obtain precipitation. The seaweed fiber was precipitated and dried in an oven at 50℃.

2.3. Probiotic fermentation process

Preparation of strain solution: *Lactobacillus plantarum* and *Lactobacillus rhamnosus* stored in laboratory glycerol tubes were respectively activated on MRS medium plate, cultured at 37 ℃ for 24 h. *Lactobacillus plantarum* and *Lactobacillus rhamnosus* was inoculated in MRS liquid medium separately. They are cultured in biochemical incubator at 37 ℃. The OD600 was controlled at about 0.9.

Fermentation culture process: Weighing 5g of the above-mentioned dried algae fiber precipitate was add to 100ml of liquid medium. Then the liquid medium was sterilized at 121℃ for 20 min. It was seen as fermentation medium. *Lactobacillus plantarum* and *Lactobacillus rhamnosus* strain solution was mixed (1:1) and inoculated into the fermentation medium, the inoculum amount was 2% and made three parallels. The seaweed fiber was continuously fermented for 2 days under the condition of 37℃, 180 r/min. The fermentation liquid was centrifuged at 3700 r/min for 10 min and after centrifugation, the supernatant and centrifuge were used to determine the fiber content.

2.4. The content of Dietary fiber determination

After the end of fermentation, the precipitate obtained by centrifugation is dried at 60℃. All the fibers fermented and 5g fibers obtained by enzymolysis are treated as follows. According to the national food safety standard GB-5009.88-2014, the fiber was treating with amylase, protease and amylglucosidase. After enzymatic hydrolysis, we would carry out precipitation, suction filtration, washing, dry weighing, and determination of the protein and ash quality. Finally, we calculate the content of the total fiber (TDF), insoluble fiber (IDF) and soluble fiber (SDF) in the sample.

After fermentation the supernatant add amylase, glucoamylase, and protease in an amount of 1% to carry out enzymatic hydrolysis. These enzymes can remove other polysaccharides such as protein and starch. The dietary fiber freely was released, and the solution was treated with 98% ethanol for 1 hour to precipitate soluble fiber. The soluble fiber was washed with 78% ethanol and acetone, and dried at 105℃ overnight to determine the content of soluble fiber therein.

2.5. Determination of inhibition of α-amylase and α-glucosidase activity

Determination of α-amylase inhibition rate [14,15]: the seaweed fiber obtained by enzymatic hydrolysis and fermentation was determine the inhibition of α-amylase separately. Simultaneously, we set up blank medium to culture only lactic acid bacteria comparing with seaweed fiber of fermentation and enzymatic hydrolysis. Three samples above were centrifuged. The supernatant was diluted 100 times and taken 1 mL to place in a test tube. In addition, 50 μL 1% soluble starch solution and 100 μL of α-amylase solution were added as an experimental group, and 1 mL of the diluted fermentation and...
100 μl of α-amylase solution were taken as background control tubes. Adding 50μl 1% soluble starch solution and 100μl α-amylase solution to test tube as blank tube. Adding 50μl 1% soluble starch solution as blank control tube. Each tube was added to 3mL with phosphate buffer, and placed at 37°C in water bath for 10min. 1 ml of DNS reagent was added and placed at boiling water for 7 min. After cooling to room temperature, the absorbance was measured at a wavelength of 540 nm. We calculate the inhibition rate of enzyme activity according to the following formula:

$$X = 1 - \left( \frac{A - B}{C - D} \right)$$

Note: A-experimental group; B-background control group; C-blank group; D-blank control tube group.

Determination of α-glucosidase inhibition rate: 6 mL 67mmol/L potassium phosphate buffer(pH6.8), 0.2mL 3mmol/L glutathione solution, 0.3mL 0.04 mg/mL α-glucosidase solution, 80μL of extract were mixed and placed at 37°C for 10min. 10mL 0.01mol/L PNPG solution was added to react for 20 min. We used 10mL 0.1 mol/L Na2CO3 solution to terminate the reaction. Then measuring the absorbance at 400 nm. According to the following formula. Calculating enzyme activity inhibition rate:

$$X = 1 - \left( \frac{A - B}{C} \right)$$

Note: A - inhibition group; B - background control group; C - blank control group.

3. Results

3.1. Determination of dietary fiber

According to the fermentation process above, 60 mL of the supernatant was obtained after fermentation of Lactobacillus plantarum and Lactobacillus rhamnosus and the precipitate obtained 4.08 g after dried. The algae fiber of Fermentation (-) and fermentation (+) was measured, respectively. The results are shown below:

### Table 1. The content of total fiber, soluble fiber, insoluble fiber before and after fermentation.

|                | TDF (g) | IDF (g) | SDF (g) |
|----------------|---------|---------|---------|
| Fermentation (-)| 3.31g<sup>a</sup> | 2.28g<sup>a</sup> | 0.84g<sup>a</sup> |
| Fermentation (+)| 3.82g<sup>b</sup> | 1.89g<sup>b</sup> | 1.94g<sup>b</sup> |

Note: Different letter means ± SD (n = 3) within the same column with different superscripts were significantly different at P b 0.05 (Duncan).

By comparing the content of total fiber, insoluble fiber and soluble fiber, the results showed that the total fiber content of seaweed fiber was 3.31g, the soluble fiber content was 0.84g, and the insoluble fiber content was 2.28g after enzymatic hydrolysis. After fermentation of the probiotics, the total fiber content was 3.82 g and the soluble fiber content was 1.94 g. It can be found that after probiotic fermentation, the total fiber content increased by 10.34%, and the soluble dietary fiber content increased by 21.97% significantly.
3.2. Determination of inhibition of α-amylase and α-glucosidase activity
The inhibition rate of α-amylase was determined according to the method above. The fiber obtained by enzymatic hydrolysis had an inhibition rate of α-amylase of -4.5%; The seaweed fiber of enzymatic hydrolysis was fermented by probiotics. The inhibition rate of α-amylase was 21.4%. The results showed that the fiber improve the inhibition rate of α-amylase by 25.9% after probiotic fermentation

According to the above method, the α-glucosidase inhibition rate was determined. The fiber of enzymatic hydrolysis had an inhibition rate of 14.7%. The α-glucosidase was inhibited by probiotic fermentation and the rate of inhibition was 31.3%. The results showed that the fiber inhibition rate of α-glucosidase increased 16.6% after probiotic fermentation.

Table 2. Inhibitory activities of fiber against α-amylase and α-glucosidase

| Fermentation | α-amylase inhibition rate | α-glucosidase inhibition rate |
|--------------|--------------------------|------------------------------|
| (-)          | -4.5%                    | 21.4%                        |
| (+)          | 14.7%                    | 31.3%                        |

4. Discussion
China's seaweed resources are abundant, and a large amount of seaweed fiber is produced during the development and utilization of seaweed. In this experiment, the seaweed fiber was biotransformed by Lactobacillus plantarum and Lactobacillus rhamnosus, and the soluble dietary content increased by 21.97%. The amylase and α-glucosidase inhibition rates were increased by 25.9% and 16.6%, respectively. In addition, Lactobacillus plantarum and Lactobacillus rhamnosus are widely recognized as probiotics. Fermentation of probiotics can not only increase the content of soluble dietary fiber, but also produce a large amount of beneficial nutrients for the human body during the fermentation process. It will provide a certain theoretical basis for the development and utilization of seaweed fiber.

During the fermentation process, the increase of total fiber and soluble fiber content may be some polysaccharides produced by lactic acid bacteria and these polysaccharides is kind of soluble fibers. In addition, lactic acid bacteria produce a large amount of organic acids, which break the insoluble cellulose glycosidic bonds and produce new reducing ends. Therefore, the content of soluble fiber would increase.

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