Development and Bioactivity Evaluation of Antioxidant Jiaosu with Compound Fruit and Vegetable

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Abstract. The antioxidant Jiaosu selected the fruits and vegetables with strong antioxidant capacity as raw material, meanwhile, the strain of Lactobacillus rhamnosus 217-1, 217-3, 217-8, which had the ability to remove cholesterol was selected as the fermentation strain with high glutathione production. After fermentation time and other processes were optimized, the content of total sugar, polyphenol, proanthocyanidin, and riboflavin and antioxidant capacity of Jiaosu were tracked and tested. The results showed that the fermentation time of antioxidant Jiaosu developed was 14 days, the total sugar content of antioxidant Jiaosu was 13.31mg/ml, the content of polyphenol was 0.554mg/mL, the riboflavin was 0.0514mg/ml, the original anthocyanin was 1.975mg/mL, and the antioxidant capacity reached a high level. Therefore, the compound fruit and vegetable Jiaosu developed in this thesis retains the nutrients in the original fruits and vegetables, and simultaneously transforms the macromolecules into the small molecules that are beneficial to the body's absorption and metabolism through the biodegradation and conversion of probiotics. At the same time, a secondary active metabolite is also produced, further increasing the active ingredient and function of the developed antioxidant Jiaosu product.

Introduction

Fruits and vegetables contain many different anti-oxidants. They can remove oxygen free radicals. In addition to vitamin E (tocopherol), vitamin C (ascorbic acid), β-carotene, carotenoids. The recent study also found that Lycopene, Flavonoids, anthocyanin and Catechin also contain higher bioactivity [1]. We refer to the strength of antioxidant capacity in fruits and vegetables, select more than ten fruits and vegetables such as lemon, apple, mulberry, pineapple, strawberry, tomato, kiwi fruit, orange, yam and carrot as raw materials for the development of the original Jiaosu [2], and then through lactobacillus fermentation, ripening and later taste debugging and other steps to develop into a multi-function Jiaosu of composite fruits and vegetables.

Jiaosu refers to products containing specific bioactive components made by microbial fermentation, and using animals, plants and fungi as raw materials. It is contain natural nutrients, Jiaosu, beneficial microorganisms and active metabolites. Aiming at different people, the raw material compatibility and the process side of the Jiaosu product are different. The first Jiaosu products focus on targeted nutritional supplements, and second focus on targeted nutritionally balanced[3]. The growing popularity of Jiaosu products also reflects the general public's recognition of the health benefits of a reasonable diet, but there is a clear lack of functional clear Jiaosu studies. The project uses a variety of fruits and vegetables with strong antioxidation as the main raw material. By means of probiotic fermentation, a composite Jiaosu product that is rich in various nutrients and contains active probiotics and beneficial metabolites[4]. In this paper, we study the total sugar, proanthocyanidins, polyphenols and antioxidant capacity in the developed Jiaosu and provide more detailed data, which is instructive for the enrichment of Jiaosu products and the development of other Jiaosu products.
Materials and Equipment

Strain
Lactobacillus rhamnosus 217-1, 217-3, 217-8 used in the fermentation experiments was my laboratory-deposited strain.

Equipment
pH meter, model FE20; Spectrophotometer, model RS232-C; Beater, model DJ1- 0.12; Sterilizer, model LDZX-50KB; Incubator, Model SHP-150; High performance liquid chromatography, model LC-VP.

Reagents and Materials
1) Phenol, concentrated sulfuric acid, anhydrous ethanol, glucose, dipotassium hydrogen phosphate, disodium hydrogen phosphate, gallic acid, sodium carbonate, forint phenol reagent, DPPH(2,2-Diphenyl-1- picrylhy- drazyl): Sigma company.
2) Riboflavin (VB₂) insoluble in water, accurately weighed 50 mg of the above standard, a small amount of 0.25 mol/L NaOH solution was added dropwise until the solid was completely dissolved with 0.01mol/L HCl solution was fixed in 25mL brown volumetric flask, Prepared as a mass concentration of 2mg/mL standard stock solution. All stock solution should be stored in the refrigerator at 4°C.[⁶]
3) 2mol / L hydrochloric acid; 2% ammonium ferric sulfate solution; Proanthocyanidin standard solution (1.00mg/mL): Weigh 0.01g procyanidin standard (accurate to 0.0001g), with methanol 10mL brown volumetric flask, is now available.

Experiment Method
Selection and Fermentation of Compound Fruit and Vegetable Jiaosu Fruits
Taking the reference of the literature as reference, the proportion of fruits and vegetables was determined according to the seasonality of the fruits and the juice yield: 2500g of hawthorn, 3000g of orange, 2500g of grapefruit, 2500g of carrot, 2500g of tomato, 2500g of cucumber, 1500g of cucumber, 1000g of pineapple, 500g of lemon, 1500g of kiwifruit. The total fruit juice output rate is about 50%. After beating the above fruits, C source and N source were added, and then Lactobacillus rhamnosus was inoculated for fermentation.

Compound Fruit and Vegetable Jiaosu Live Bacteria and pH Value Changes
Count the viable count in Jiaosu using the plate count method. In the ultra-clean bench for compound fruit and vegetable Jiaosu gradient dilution, respectively take 10⁻⁴, 10⁻⁵, 10⁻⁶ concentration gradient of the Jiaosu dilution, with 10μL pipette point MRS plate medium. Each gradient try three times in parallel. After standing in a clean bench for 15 minutes, the plate was placed in a 37°C incubator and invertedly cultured for 36 hours. Take samples of Jiaosu and test pH every 5 hours on the clean bench.

Determination of Total Sugar Content of Compound Fruit and Vegetable Jiaosu
Determination of polysaccharides in Jiaosu by phenol sulfuric acid method.
Respectively draw 0mL, 0.2mL, 0.4mL, 0.6mL, 0.8mL, 1.0mL standard glucose working solution placed in a test tube, make up to 1.0ml with distilled water. Add 1.0 mL of 5% phenol solution to the test tube, quickly add 5.0 mL of sulfuric acid and let stand for 10 min. Using a vortex shaker so that the reaction mixture was thoroughly mixed, the tube was placed in a 30°C water bath reaction 20min, 490nm measured absorbance. Then the glucose concentration as abscissa and the absorbance value as the ordinate, to develop polysaccharide standard curve. Then take the Jiaosu sample, diluted to the appropriate concentration, take 1.0mL of Jiaosu diluted samples repeat the above operation, the absorbance of the Jiaosu dilution was measured to calculate the sample polysaccharide content[⁶].

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Detection of Polyphenols in Compound Fruit and Vegetable

The content of polyphenols in the Jiaosu was determined by gallic acid method. Pipette respectively Pipette1,0.2,0.3,0.4,0.5 mL standard stock solution of gallic acid in 100 mL volumetric flask, then the volume of water to the mark, and the concentrations were 10,20,30,40, 50 μg/mL. Measure gallic acid working solution and distilled water 1 mL each in a test tube, add 5.0 mL of 10% Forint phenol reagent, shake and react about 3-8 minutes. Adding 4.0 mL 7.5% Na2CO3 solution, shake and at room temperature place 60 min, absorbance at 765nm wavelength measurement. The micrograms of gallic acid for the vertical axis, the absorbance value of the abscissa, draw the standard curve. Measure 1 mL test solution, repeat the above operation, measured absorbance [7].

Compound Fruit and Vegetable Jiaosu Riboflavin Detection

High performance liquid chromatography was used to detect the content of riboflavin in the Jiaosu.

Take a small amount of riboflavin standard stock solution mixing, diluting, fixed volume formulated into a vitamin standard solution mass concentration of 100 μg/mL. This standard solution as the highest concentration, followed by 2 times, 4 times, 20 times, 100 times, 500 times, 5000 times, 5000 times diluted into a series of standard solution. Inject 20 μL of each concentration and record the peak area. The calibration curves were plotted with the injection concentration as abscissa (X) and peak area integral (Y) as ordinate, and the regression equation was calculated. Jiaosu samples were also taken for liquid chromatographic analysis, according to the standard curve to calculate the content of riboflavin [6].

Column: Agilent Zorbax Eclipse Plus-C18 (4.6mm×250mm, 5μm); Mobile phase A: 25 mmol/L Potassium dihydrogen phosphate buffer (adjusted to pH 2.5 with phosphoric acid), Mobile phase B: Acetonitrile; gradient elution conditions: 100% A within the first 5 min elution, followed by 20 min A phase volume ratio decreased linearly from 100% to 75%, maintained for 5 min, recovered to 100% A equilibration column 10 min; 1.0 mL/min; column temperature: 4°C; injector temperature: 4°C; injection volume: 20 μL; acquisition wavelength set at 267 m.

Study on the Antioxidative Activity of Complex Fruit and Vegetable Jiaosu

DPPH (2, 2-Diphenyl-1-picrylhydrazyl) test was used to test the antioxidant activity of the Jiaosu. DPPH is a stable organic free radical, when there is free radical scavenging material in the sample. It will be absorbed by the single electron pair of free radical to gradually fade away, and the sample can be removed freely by the change of absorption value by spectrophotometry Based activity, so as to evaluate the self-compound fruit and vegetable Jiaosu of the antioxidant activity.

Take 100 μL sample into the test tube, and then add 3.9 mL 0.1 mmol/L DPPH ethanol solution into each tube, mix darkness 37°C water bath for 1 h and then measure the absorbance value at 517 nm, with ethanol as a blank control, each sample three times were measured in parallel, the results expressed as IC50[8]. The required concentration of the sample for the DPPH radical scavenging rate of 50% is IC50 and the DPPH radical scavenging rate is calculated (Eq. 1):

\[
\text{Clearance Rate (\%) = \left[1 - \frac{(As/Ac)}{}\right] \times 100\%}
\]  

(1)

Ac-- absorbance value of blank control tube; As-- sample tube absorbance value

Study on the Complex Fruit and Vegetable Jiaosu Proanthocyanidins

Liquid phase conditions: Column: C18 column; column temperature 35°C; detector: UV detector; detection wavelength: 525 nm; injection volume: 10 μL; mobile phase: Water + methanol (chromatographic purity) + isopropanol + formic acid=73+13+6+8; Flow rate: 1.0 mL/min.

Sample Processing: According to the content accurately draw 1 ml~5 ml sample solution, placed in 50 ml volumetric flask, add methanol (chemically pure) to the mark.

Weigh the original anthocyanin 0.01 g dissolved in 10 mL of methanol, the solution was drawn 0.1, 0.25, 0.5, 1.0, 1.5 mL place in 10 mL brown volumetric flask (final concentrations were 0.01, 0.025, 0.05, 0.1, 0.15 mg/mL), add methanol to the mark and shake well. Take 2 mL each. After
n-butanol and concentrated hydrochloric acid were mixed in a volume ratio of 95:5, take 15mL of the solution was put into a stoppered flask, then 0.5mL of ammonium ferrie sulfate solution (2%) and 2 mL of sample solution were added, Set boiling water bath heated 40 minutes, immediately placed in ice water cooling, filtration by 0.45µm filter membrane, and be in progress high performance liquid chromatography analysis [9]

**Results and Discussion**

**Determination of Fermentation Time of Compound Fruit and Vegetable**

As can be seen from Fig.1, in the first week as the new access to lactic acid bacteria due to the conditions for rapid entry into the logarithmic phase, lactic acid also rapidly with the growth of lactic acid bacteria, pH value dropped from 6.00 to 3.50. With the continuous formation of lactic acid and nutrients are consumed, resulting in growth and metabolism was inhibited, the growth of lactic acid bacteria into a stable period, pH changes are no longer obvious. After 14 days, the pH value basically stable at about 3.00. From the curve of viable count, it can be seen that during the first week of fermentation, the number of viable cells increased rapidly during the key period of Jiaosu fermentation. After 14 days of fermentation, the data tended to be stable, indicating that the fermentation was basically completed and into the ripe stage.

![Figure 1. Compound fruit and vegetable enzymes PH value changes and viable count cure within 49 hours.](image1)

![Figure 2. Compound fruit and vegetable enzymes polysaccharid content of the standard curve.](image2)

**Compound Fruit and Vegetable Jiaosu Total Sugar Content Test Results**

The standard curve of glucose solution shown in Fig.2. Compound fruit and vegetable Jiaosu in the fermentation before the polysaccharide content is 120.5mg/mL .After 24 days of fermentation, the polysaccharide concentration of 24.37mg/mL. When the fermentation was about 48 days, the polysaccharide concentration dropped to 13.37mg/mL.

According to the experimental data show that: the early fermentation of sugar metabolism is relatively strong, is the key period for the production of lactic acid, so sugar is one of the main limiting factors in the initial fermentation restriction fermentation. According to research, excess sugar can cause long-term harm to health. Our compound fruit and vegetable Jiaosu also have less sugar, while reducing sugar intake, can effectively reduce the incidence of obesity and hypertension. This is very suitable for modern diet.

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Test results of Compound Fruit and Vegetable Jiaosu Polyphenol Content

Polyphenols, as natural antioxidative active components, supply hydrogen radicals such as hydroxyl radical, superoxide anion radical and lipid compound to themselves and convert themselves into phenolic radicals. Because the phenolic radicals have some stability, so it can reduce the automatic oxidation chain reaction of the transfer rate and inhibit the further oxidation reaction. According to the concentration of gallic acid produced polyphenols standard curve shown in Fig.3. The absorbance at 765 nm of the 20-fold diluted sample solution of composite fruit and vegetable Jiaosu was 0.318. According to the standard curve, the polyphenol content of the compound fruit and vegetable Jiaosu is 0.554 mg/mL.

Riboflavin in Compound Fruit and Vegetable Jiaosu Test Results

Studies have shown that riboflavin can catalyze a large number of oxidation-reduction reaction, riboflavin-based iso-alloxazine of N$_1$ and N$_{10}$ can be reduced to accept hydrogen, while the reduced state of the riboflavin and easy dehydrogenation is oxidized, so riboflavin has a reversible redox potential with a redox potential between the lower potential hydrogen donor (organic metabolite, nicotinamide coJiaosu) and higher potential hydrogen receptors (molecular oxygen, cytochromes) \cite{10}. After high performance liquid chromatography analysis, the Riboflavin peak time is 27-28min. Calculated by the standard curve (Fig.4), the composite fruit and vegetable Jiaosu riboflavin content of 0.0514mg/mL.

Compound Fruit and Vegetable Jiaosu to Scavenge Free Radicals Test Results

Using DPPH method to calculate the compound fruit and vegetable Jiaosu free radical scavenging capacity, clearance rate curve shown in Fig.5. According to the curve, it can be concluded that the compound fruit and vegetable Jiaosu has a high free radical scavenging activity and a high antioxidant activity, and the IC$_{50}$ value is 0.0715. Under the same conditions, the lower the IC$_{50}$ value, the stronger ability to scavenge free radicals. Yong-jie Cheng\cite{11} in the study on the Cudrania tricuspidata Bun Jiaosu, the IC$_{50}$ value was detected between 4.027-5.854. Compared with the composite fruit and vegetable Jiaosu antioxidant capacity was significantly higher than the market has reported a number of Jiaosu products. Under the conditions of 100 times dilution, it can achieve
radical scavenging rate of 92.9%. The study of Fessenden\cite{12} shows that the phenolic substances can give a hydrogen ion and by resonance and stability, affecting free radical scavenging rate. Combined with the change trend of polyphenol content, the trend of DPPH radical scavenging rate showed a certain correlation with the change of total phenolic content.

![Figure 5. Compound fruit and vegetable enzymes DPPH antioxidant test.](image)

![Figure 6. Compound fruit and vegetable enzymes proanthocyanidins detection.](image)

**Compound Fruit and Vegetable Jiaosu Procyanidins Test Results**

Experiments show that proanthocyanidins anti-free radical oxidation of vitamin E 50 times, 20 times the vitamin C, and quickly absorbed completely absorbed. Oraling 20 minutes to reach the highest blood concentration, metabolic half-life of up to 7 hours \cite{13}. For medicine, proanthocyanidins used to promote blood circulation, protect eyesight, and even improve the vision of night blindness patients; combined with vitamin C is conducive to lower cholesterol levels. For the skin disease patients, it have the role of anti-allergy, nourishing. As shown in Fig.6, the anthocyanin content of the compound fruit and vegetable Jiaosu was found to be 1.975 mg/mL. Rui Guan\cite{14} and other fermented in the blueberry Jiaosu tracking process, the initial procyanidins reached 0.70mg/mL. Man-li Zhu\cite{15} and other pitaya Jiaosu in the determination of proanthocyanidins detected pitaya procyanidins content between 3.42-1.32mg/mL. From this we find that the proanthocyanidin content of the composite fruit and vegetable Jiaosu is at a high level in all Jiaosu.

**Conclusion**

This dissertation aims to develop a compound fruit and vegetable Jiaosu product with high biological activity. By optimizing the activity of probiotic strains, tracking the fermentation process pH value and viable count to determine the fermentation time of composite fruit and vegetable Jiaosu, and through the detection of composite fruit and vegetable Jiaosu, the probiotic functions of the composite fruit and vegetable were detected. According to the experimental results, the fermentation time of compound fruit and vegetable Jiaosu is 14 days. After fermentation, its total sugar content was 13.31mg/mL, polyphenol content was 0.554mg/mL, riboflavin 0.0514mg/mL,

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procyanidin 1.975mg/mL, and antioxidant capacity reached a very high level. Experiments show that this product can potentially regulate intestinal balance in the human body, improve the body's antioxidant capacity and immune level[16], promote the digestion and absorption of nutrients, anti-aging, in line with the diet of modern people's health.

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