ISSN: 1813-4890

Comparison of Bioactive Components and Antioxidant Capacity of Different Enzymes Analyze

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Abstract

Enzyme is a kind of fermentation product with specific active ingredients. The biological active ingredients and organic small molecules are more concentrated and easier to be absorbed by the human body. Moreover, enzyme has physiological activities such as promoting metabolism, regulating intestinal flora balance and enhancing antioxidant activity. In this study, HPLC was used to determine the content of organic acids and oligosaccharides in 8 enzyme samples. The contents of methanol and ethanol; ABTS free radical, hydroxyl free radical and DPPH free radical scavenging ability were determined. The results showed that lactic acid was the main organic acid, with the content ranging from 12mg/ mL to 51mg/ mL. The main oligosaccharides were glucose, up to 52mg/ml. Methanol and ethanol are adverse ingredients for enzyme products and are present at lower levels only in individual samples. Total flavonoids and total polyphenols were highest at 7.4 mg/ml and 15.1 mg/ml, respectively. Comprehensive evaluation of its antioxidant capacity, the results showed that there was little difference in antioxidant capacity between samples, but the overall performance was not good. The focus of this study is to provide data basis for the optimization and theoretical research of enzyme food follow-up products.

Keywords

Enzyme; Active Ingredients; Polyphenols; Antioxidant Capacity.

1. Introduction

Enzyme, also known as enzymes, is made up of fruits, plants, animals, fungi, etc as the raw material, adding or not adding auxiliary materials, By lactic acid bacteria, yeast and acetic acid bacteria and other beneficial bacteria fermentation of containing biological active ingredients of a particular product. Which is rich in polysaccharide, widowed sugar, polyphenols, polypeptide, amino acids and vitamins, and other physiological functions of secondary metabolites and biological activity compounds. Compared with the raw material for fermentation, enzyme of bioactive components and organic small molecule is more concentrated, and are more likely to be absorbed by human body, which is research focus in the enzyme.

Enzyme due to the diversification of production of raw materials and product variety, use a variety of probiotic mixed fermentation through complex bacteria somatic intermediary metabolism, implementation of metabolites between biological transformation, can significantly improve the metabolic substrate in the original abnormal flavor, also can produce new probiotic active ingredients, increase enzyme production [1].

In this study two kinds of commercially available products and 6 kinds of homemade laboratory enzyme as the research object. Based on its ability to remove DPPH free radicals, and organic

ISSN: 1813-4890

acid content of flavonoids, polyphenols, oligosaccharides and other active substances such as indexes.

2. Materials and Methods

2.1. Experimental Materials

In this study two kinds of commercially available enzyme products and six kinds of homemade laboratory enzyme as experimental material.

2 kinds of commercially available enzyme number respectively, product fine red Chinese wolfberry enzyme for YH-1, the product fine black Chinese wolfberry enzyme for YE-2. 6 kinds of homemade laboratory enzyme were numbered mulberries enzyme for S-3, Jujube enzyme for Z-4, Kumquat enzymes for J-5, red Chinese wolfberry enzyme for H1-6, Red Chinese wolfberry II enzymes for H2-7, black Chinese wolfberry enzymes for HQ-8.

2.2. Experimental Reagents

The standard and ascorbic acid standard rutin standard: the standard and DPPH and ABTS Shanghai Aladdin biological technology co., LTD.

Fructose and galactose and glucose, maltose, formic acid, acetic acid, propionic acid, oxalic acid, aluminum nitrate, potassium acetate, Gallic acid, potassium persulfate, FeSO4: analysis of pure, 99% or higher, its group chemical reagent co., LTD.

Malic acid, lactic acid, H2O2: analysis of pure, 99.5% or higher, tianjin branch close the chemical reagent co., LTD.

1,1 diphenyl-2-trinitrobenzene, butyric acid, butyric acid, valeric acid, valeric acid, Na2CO3: analysis of pure, winds in Shanghai science and technology development co., LTD.

Methanol, ethanol, the forint reagents, salicylic acid: chromatographic pure, national medicine group chemical reagent co., LTD.

2.3. Experimental Equipment

Centrifuge table high speed: TG16-WS, Changsha high-tech industrial development zone xiang instrument centrifuge instrument co., LTD. High performance liquid chromatograph: LC-20A., island ferry company.

Uv-vis spectrophotometer, UV-5500PC, Shanghai yuan analysis instrument co., LTD.

Ultrasonic cleaning device: KQ2200B type, kunshan ultrasonic instrument co., LTD.

Digital constant temperature water-bath water: HH - 4, guohua electric appliance co., LTD.

3. Experimental Method

3.1. Determination of Biological Active Ingredient Content

The determination of organic acids, oligosaccharides and methanol and ethanol by liquid chromatography analyzer for precise measurement.

3.1.1. Chromatographic Conditions

Using Carbomix H-NP type of chromatographic column (7.8 x 300 mm), to 2.5 mM-H2SO4 solution, flow rate 0.6 mL/min, column temperature 55 ℃, sample quantity 10 μL, adopting refractive index detector.

3.1.2. Sample Pretreatment

Amount of samples from a certain volume of enzyme by 10000 r/min, the centrifugal supernatant, 3 min after diluted properly used after 0.22 μm membrane filtration.

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3.1.3. Standard Configuration

Accurately weigh the standard, dilute it to an appropriate concentration, and filter it using a 0.22 μm membrane.

3.2. Determination of Total Flavonoids and Total Polyphenols

3.2.1. Determination of Total Flavonoid Content

For the determination methods of flavonoids content in SN/T4592-2016 about on the basis of the relevant method for the determination of flavonoids.

Sample pretreatment: draw 5-10 mL of enzyme sample, weigh (accurate to 1 mg), add about 30 mL of absolute ethanol, extract by ultrasonic for 1 h, and filter the extract to a volume of 50 mL.

Drawing of the standard curve: Accurately draw 1 mL, 2 mL, 3 mL, 4 mL, 5 mL of 1 g/L rutin standard working solution, add absolute ethanol to 15 mL, and then add aluminum nitrate solution (100 g /L) 1 mL, potassium acetate solution (98 g/L) 1 mL, dilute to 50 mL, and let stand for 1 h. Use a 1 cm cuvette at 420 nm with 30% ethanol solution as a blank to measure the absorbance.

Determination of the sample: Accurately draw 1 mL of the sample solution to be tested, operate according to 2.2, check the standard curve or calculate the total flavonoid content in the solution through the regression equation. The total flavonoid content in the sample was calculated as follows:

$$X = \frac{m}{W \times \mathrm{d} \times 1000} \times 100\%$$

X in the formula: total flavonoid content in the sample.

m: the amount of total flavonoids in the solution calculated from the standard curve or by the regression equation.

W: the quality of the sample.

D: dilution ratio.

3.2.2. Determination of Total Polyphenol Content

The determination of total polyphenol content refers to the Folin phenol method, that is, under alkaline conditions, polyphenols can reduce the phosphotungstic acid and molybdic acid in the Folin reagent, making it blue, its color depth is related to the total polyphenol. There is a linear relationship within a certain range of phenol content.

Drawing of the gallic acid standard curve: After the gallic acid standard is dehydrated in the oven for 4 hours, accurately weigh 0.011 g and add distilled water to make up to 100 mL, which is the standard working solution. Accurately pipette 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, and 1.6 mL of the working solution into a 25 mL volumetric flask, add distilled water to make up to 6 mL, add 0.5 mL of Fulin reagent, shake and mix, and then add 1.5 mL 20% Na2CO3solution, fully mixed and then fixed to volume, and placed in the dark for 0.5 h.Use an ultraviolet spectrophotometer to read the absorbance at 760 nm, and use distilled water as a blank control to draw a standard curve according to the relationship between the absorbance value and the volume of the working solution.

Sample determination: Take 1 mL of properly diluted enzyme sample and operate according to the above method, and calculate the total polyphenol content with reference to the standard curve.

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3.3. Determination of Antioxidant Capacity

3.3.1. Sample Processing

Dilute each enzyme sample as appropriate to make it have a certain fluidity, centrifuge at 6000 r/min for 5 min at 4 ℃, and take the supernatant to determine its antioxidant activity.

3.3.2. ABTS Free Radical Scavenging Rate

Use pre-prepared 7 mmol/L ABTS solution and 2.45 mmol/L potassium persulfate solution, mix them in a ratio of 1:1, place in the dark for 12 h-16 h, and dilute 30 times when using. Mix 0.5 mL of enzyme sample diluent with 4.5 mL of ABTS solution thoroughly, place it in the dark for 6 min, and measure the absorbance at 734 nm.

$$X = \frac{1 + A3 - A2}{\mathrm{A1}} \times 100\%$$

X: ABTS free radical scavenging rate.

A1: The absorbance of the mixture of 0.5 mL distilled water and 4.5 mL ABTS solution.

A2: The absorbance of the mixture of 0.5 mL enzyme sample and 4.5 mL ABTS solution.

A3: The absorbance of the mixture of 0.5 mL enzyme sample and 4.5 mL absolute ethanol.

3.3.3. Hydroxyl Radical Scavenging Rate

The scavenging ability of hydroxyl radicals was determined according to the Fenton reaction method, H2O2 +Fe2+ =·OH+H2O+Fe3+ , the reaction between hydroxyl radicals and salicylic acid can generate pyroteacateate, pyroteacateate at 510 There is a maximum absorption wavelength at nm, from which the hydroxyl radical scavenging rate can be detected and calculated.

Prepare 6 mmol/L ethanol-salicylic acid solution, 6 mmol/L FeSO4 solution and 6 mmol/L H2O2 solution in advance. The enzyme samples were mixed with FeSO4 solution and H2O2 solution respectively, and after standing for 10 min, salicylic acid solution was added, and after standing for 30 min, the absorbance of the reaction solution was measured at 510 nm, and deionized water was used instead of water. The salicylic acid solution was used as the control group, and the deionized water was used instead of the enzyme sample as the blank group, and the hydroxyl radical scavenging rate was calculated [3].

$$X = \frac{1 + A3 - A2}{\mathrm{A1}} \times 100\%$$

A1: The absorbance of the mixture of deionized water and salicylic acid solution.

A2: The absorbance of the mixture of enzyme sample and salicylic acid solution.

A3: The absorbance of the mixture of enzyme sample and deionized water.

3.3.4. DPPH Free Radical Scavenging Rate

The determination of DPPH free radical scavenging rate is basically in accordance with the method of Yang Yang et al. to determine papain. First prepare 8% DPPH solution, measure 3 mL of enzyme diluent and 3 mL of DPPH solution, mix thoroughly and react in the dark for 30 min. After the reaction, the absorbance was measured at 517 nm, 95% ethanol was added as a control, and the DPPH free radical scavenging rate was calculated according to the formula.

$$\mathrm{X} = \frac{1 + A2 - A1}{\mathrm{A3}} \times 100$$

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X: DPPH free radical scavenging rate.
A1: The absorbance of the mixture of enzyme sample and DPPH solution.
A2: The absorbance of the mixture of enzyme sample and absolute ethanol.
A3: The absorbance of the mixture of DPPH solution and absolute ethanol.

4. Results and Analysis

4.1. Determination Results of Biologically Active Ingredients

4.1.1. Types and Content of Organic Acids

Play an important role in maintaining the acid-base balance of body fluids, improving the activity of intestinal digestive enzymes, promoting the growth of beneficial intestinal flora , and regulating the balance of intestinal microbial colonies [2,3].

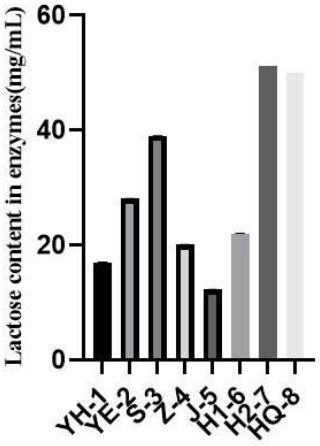

Figure 1. Content of lactic acid in different enzymes

Table 1. Quantification of organic acids in enzyme samples

sample	Formic Formic acid	Acetic Acetic acid	propioPrOpionic acid	Butyric Butyric acid	metabutyPartial butyric acid	Valeric Pentanoic acid	Pivalic Partial pentanoic acid	oxalic Oxalic acid	malic Malic acid	Lactic Lactic acid
YH-1		√	√		√			√	√	√
YE-2		√	√							√
S-3		√	√						√	√
Z-4		√	√		√				√	√
J-5		√	√					√		√
H1-6		√	√							√
H2-7		√	√						√	√
HQ-8		√	√							√

Note: "√" indicates the presence of this substance.

The types of organic acids in the detection enzyme products mainly include formic acid, acetic acid, propionic acid, butyric acid, metabutyric acid, valeric acid, metavaleric acid, oxalic acid,

ISSN: 1813-4890

malic acid, and lactic acid. The test results showed that the enzyme samples contained a total of 6 kinds of organic acids, of which YH-1 Yipinjiahong wolfberry enzyme and Z-4 jujube enzyme contained the most types of organic acids, followed by S-3 mulberry enzyme and YH-1. Red wolfberry enzyme. Acetic acid, propionic acid and lactic acid are the common organic acids of 8 enzymes, of which lactic acid has the highest content, ranging from 12.23 g/L to 51.18 g/L . These organic acids work together to give the enzyme product a rich flavor and the ability to regulate the flora. HPLC to detect organic acids in 5 kinds of enzyme samples including green plum enzymes. There were 4 to 11 kinds of organic acids, and tartaric acid, acetic acid and lactic acid were all detected. There are large differences in the types and contents of organic acids between different types of enzyme products, which are closely related to the source of raw materials.

4.1.2. Type and Content of Oligosaccharides

Oligosaccharides are compounds composed of 2-10 glycosidic bonds. Some oligosaccharides are easily digested and absorbed by the human body and have the effect of anti-fatigue and insulin balance regulation; The growth and reproduction of fidobacteria, while inhibiting the growth of spoilage bacteria, has the functions of slowing down aging, preventing cancer, anti-cancer, reducing the burden on the liver, and improving the rate of nutrient absorption. The higher the content of oligosaccharides, the greater the effect on human nutrition and health care [4].

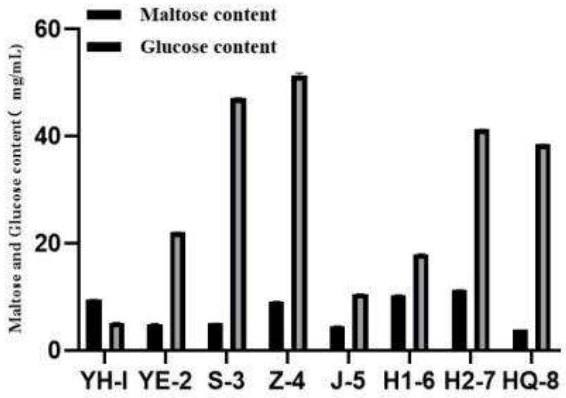

Figure 2. Maltose and glucose content in enzymes

In this study, four common oligosaccharides including fructose, maltose, glucose and galactose were detected. The results show that maltose and glucose are generally contained in each enzyme sample, and the content of glucose is generally high. Only YH-1 Yipinjia Red Lycium barbarum enzyme has a low level of glucose, which is 5.2 mg /mL.Fructose and galactose were only present in some samples, and the content was low.

4.1.3. Content of Methanol and Ethanol

Methanol can be converted into toxic formaldehyde and formic acid through metabolism in the human body, so it has low toxicity. Methanol mainly acts on the nervous system, has obvious anesthetic effect, and can cause cerebral edema.

After ethanol is absorbed by the human body, it will cause the central nervous system to be highly excited, and then inhibit the response of the nervous system. Regular intake of ethanol can also produce addictive reactions and lead to chronic diseases such as fatty liver and alcoholic liver.

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After testing, among the eight enzyme samples, only S-3 contained a lower concentration of methanol, about 1.922 g/L, and no methanol was detected in the other seven. Ethanol reached a concentration of 15.62 g/L in YH-1 and J-5, while the concentration was lower or not detected in other samples.

4.2. Determination Results and Analysis of Total Polyphenols and Total Flavonoids

Flavonoids have the functions of hypoglycemic, lipid-lowering, prevention and treatment of cardiovascular diseases, sedative and anti-inflammatory, antibacterial and insecticide, anti-oxidation, anti-aging, anti-cancer and anti-tumor, and are added to food, medicine and other products as health-care functional factors.Therefore, such substances have broad development prospects as medicines and functional foods.

Polyphenols can act on oxidative substances to terminate the oxidation reaction, thereby exerting their antioxidant activity. Under the action of enzymes, microorganisms can release phenolic compounds related to other components in the matrix, such as sugars, so that more biologically active ligand forms may be released from the corresponding glycosides [5,6]. Chen Chen [7] studied the polyphenol extract of banana peel and found that the ability of polyphenols to scavenge DPPH free radicals is lower than that of ascorbic acid, but the ability of scavenging hydroxyl radicals is higher than that of ascorbic acid at the same concentration. Most polyphenols have antibacterial properties. Studies have shown that the phenolic hydroxyl groups in polyphenols can promote the passage of polyphenols through the cell membrane, destroy the structural integrity of the cell membrane, and interfere with the normal function of the cell membrane, thereby exerting an antibacterial effect. At the same time, researchers found that the protective effect of polyphenols on blood lipids is related to its antioxidant activity, and paracetamol-induced liver injury has a protective effect [8].

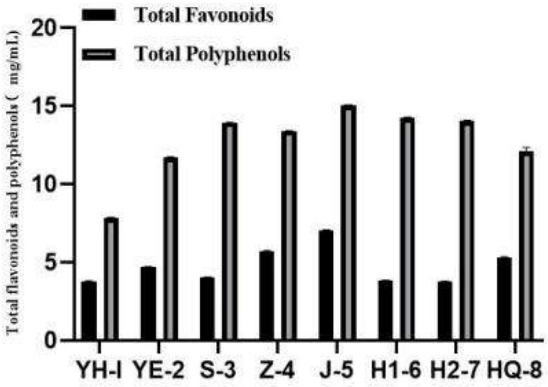

Figure 3. Total flavonoids and total polyphenols in the samples

The detection of flavonoids and polyphenols in the samples showed that the total flavonoid content in the eight samples was between 3.6 mg/ml and 7.4 mg/ml, and the highest total polyphenol content was J-5, which was 15.1 mg/ml. The lowest YH-1 was 7.4 mg/ml, and the content difference between samples was obvious.

4.3. Determination Results and Analysis of Antioxidant Capacity

Free radicals in the human body will damage human cells due to oxidative stress, causing a series of harmful biochemical reactions, resulting in protein damage, lipid peroxidation, DNA mutation and enzyme inactivation, and eventually lead to a series of human aging and other series. question. Therefore, the ability to scavenge free radicals is an important indicator for the

ISSN: 1813-4890

enzyme samples to exert their beneficial physiological functions. Regarding the antioxidant activity, there are various detection methods, and the corresponding reaction principles are different. Therefore, in order to comprehensively evaluate the antioxidant capacity, this experiment measured and evaluated the antioxidant capacity of the enzyme samples from the aspects of ABTS scavenging capacity, hydroxyl radical scavenging capacity, and DPPH radical scavenging capacity.

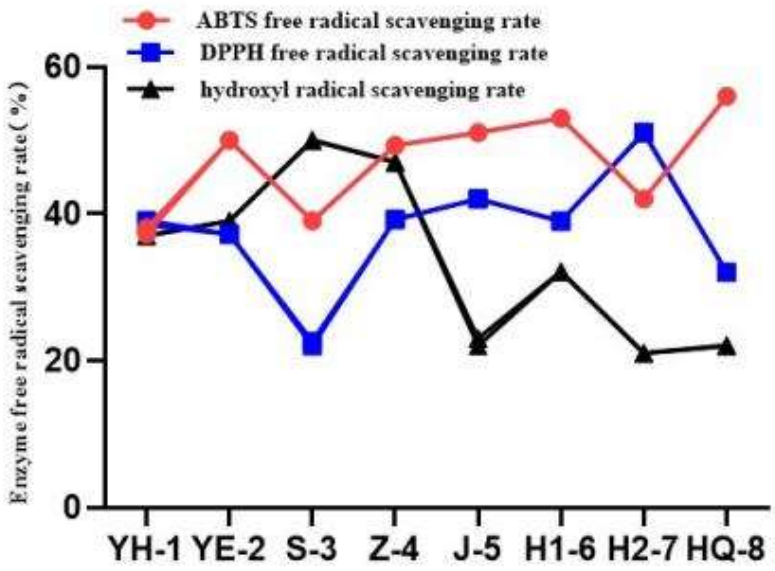

Figure 4. Free radical scavenging ability of samples

Can be seen from figure 4, 8 kinds of enzyme samples have a certain level of radical scavenging ability. Among them, the free radical scavenging ability of ABTS is the lowest, which is YH-1 Yipinjiahong wolfberry enzyme, which is 37.28%; the highest is HQ-8, which is 56.37%. The hydroxyl radical scavenging ability is generally between 20% and 40%, and the mulberry enzyme S-3 and the jujube enzyme Z-4 can reach a level close to 50%. The highest DPPH free radical scavenging rate is the red wolfberry II H2-7, which can reach 58.24%, and the DPPH free radical scavenging ability of mulberry enzyme S-3 is only 24.35%. Z-4 Jujube Enzyme ABTS scavenging ability, hydroxyl radical scavenging ability, DPPH free radical scavenging ability is the best in balance, and it can better exert its antioxidant capacity.

5. Conclusion and Discussion

Enzymes contain different kinds of biologically active substances and thus have different biological effects. In this study, the types and contents of organic acids and oligosaccharides, as well as the contents of methanol and ethanol, total flavonoids and total polyphenols, and antioxidant capacity of 8 enzyme products were analyzed. The results of the study showed that a total of 10 kinds of organic acids were detected in 8 kinds of enzyme products, and all of the 10 kinds of organic acids contained 3 to 5 kinds of organic acids, including acetic acid , propionic acid and lactic acid. The lactic acid content is generally high, which is an important factor affecting the taste and flavor of enzyme products and promoting its regulation of intestinal flora. The research on fructose, maltose, glucose and galactose in oligosaccharides in enzymes found that oligosaccharides mainly exist in the form of glucose and contain a small amount of maltose. A lower concentration of methanol was detected in S-3, and no methanol was detected in the other 7 enzymes.The ethanol content is also kept at a low level, which meets the safety and

ISSN: 1813-4890

quality requirements of enzymes. The total flavonoids and total polyphenols contents varied widely among the different samples, but were generally better than the results in other studies. Antioxidant capacity varied little, but was generally low. There are big differences in some indicators of the 8 enzyme products, which may be related to raw materials and processes. For example, different raw materials have a greater impact on the organic acids and oligosaccharides of the enzyme products, while the fermentation process and storage conditions have a higher antioxidant capacity. Great connection. According to the above conclusions, the enzyme products can be optimized to improve their quality and active function.

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