A Comparative Analysis of the Bioactive Ingredients and Antioxidant Capacity of 3 Enzymes

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Abstract

As a fermented mixture with multiple active ingredients, enzymes play a probiotic function and are closely related to their composition. In this experiment, the contents of organic acids and oligosaccharides in the three enzyme samples were determined using HPLC technology. Among the organic acids, the content of lactic acid was the highest, ranging from 29 mg/mL to 66 mg/mL. The oligosaccharide was glucose Mainly, the highest can reach 56 mg/ml; methanol and ethanol are only present in low content in individual samples; the content of total flavonoids and total polyphenols is higher, the highest is 8.2 mg/ml and 17.6 mg/ml, respectively. The scavenging ability of ABTS free radicals, hydroxyl free radicals and DPPH free radicals of each sample was measured, and their antioxidant capacity was comprehensively evaluated. The results showed that the difference in antioxidant capacity between the samples was small, but the overall performance was poor.

Keywords

Enzymes; Active Ingredients; Flavonoids; Polyphenols; Antioxidant Capacity.

1. Introduction

Enzyme is a product containing a variety of biologically active ingredients, which is produced by fermentation of animals, plants, fungi, etc., with or without auxiliary materials, using lactic acid bacteria, yeasts and other microorganisms[1]. It contains a variety of secondary products that affect physiological functions. Metabolites and active ingredients, such as oligosaccharides, organic acids, polyphenols, etc. A large number of studies have shown that enzymes have physiological activities such as promoting body metabolism, regulating the balance of intestinal flora, and enhancing anti-oxidation[2].

The enzyme uses a variety of probiotics for mixed fermentation and undergoes complex intermediate cell metabolism to achieve biotransformation between metabolites, which can significantly improve the abnormal flavor of the fermentation raw materials, and can also produce new active ingredients, which can improve the performance of enzyme products[3]. The flavor and mouthfeel can effectively increase its potential physiological regulation activity at the same time[4].

This research takes 3 kinds of enzyme products as the research object, and analyzes and evaluates the content of organic acids, oligosaccharides, flavonoids, polyphenols and the ability to scavenging free radicals to provide data for the theoretical research and product development of enzyme foods. basis.

2. Experimental materials

2.1 Experimental materials

3 kinds of homemade enzyme products, namely wolfberry enzyme, blueberry enzyme and jujube enzyme.

2.2 Experimental reagents

Rutin standard, ascorbic acid standard, ABTS standard, DPPH standard: Shanghai Aladdin Biochemical Technology Co., Ltd.

Other commonly used chemical reagents are purchased from Sinopharm Chemical Reagent Co., Ltd.

2.3 Experimental equipment

Desktop high-speed centrifuge, high-performance liquid chromatograph, ultraviolet-visible spectrophotometer, ultrasonic cleaner, digital display constant temperature water bath, common laboratory equipment.

3. Experimental method

3.1 Determination of the content of biologically active ingredients.

For the determination of organic acids, oligosaccharides, methanol and ethanol, a liquid chromatograph is used for accurate determination.

3.1.1 Sample pretreatment.

Measure a certain volume of enzyme sample and centrifuge at 10000 r/min for 3 minutes, then take the supernatant, and filter it with 0.22 μm membrane after proper dilution.

3.1.2 Configuration of standard products.

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

3.1.3 Chromatographic analysis conditions.

Carbomix H-NP type chromatographic column (7.8×300 mm) was used, 2.5 mM H2SO4 solution was used as the mobile phase, the flow rate was 0.6 mL/min, the column temperature was 55°C, the injection volume was 10 μL, and the refractive index detector was used.

3.2 Determination of total flavonoids and total polyphenols

3.2.1 Determination of total flavonoids

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

Standard curve drawing: accurately draw 1 g/L rutin standard working solution 1 mL, 2 mL, 3 mL, 4 mL, 5 mL, add absolute ethanol to 15 mL respectively, 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 a 30% ethanol solution as a blank, and measure the absorbance.

Sample determination: accurately draw 1 mL of the sample solution to be tested, operate according to the standard curve experiment method, check the standard curve or calculate the total flavonoid content in the solution through the regression equation. The total flavonoid content in the sample is calculated as follows:

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

In the formula, X is the total flavonoid content in the sample; M is the total flavonoid content in the solution calculated from the standard curve or the regression equation; W is the mass of the sample; D is the 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 to make it blue. The phenol content shows a linear relationship within a certain range[5].

The drawing of the standard curve of gallic acid: After putting the standard gallic acid in the oven for 4 hours for dehydration, accurately weigh 0.011 g and distilled water to make the volume to 100 mL, which is the standard working solution. Accurately pipette 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6 mL of working solution into a 25 mL volumetric flask, add distilled water to make up to 6 mL, add 0.5 mL of HUF reagent, shake and mix, and then add 1.5 mL 20% Na2CO3 solution, after mixing well, make the volume constant, and place it in the dark for 0.5 h. Use an ultraviolet spectrophotometer to read the absorbance at 760 nm, use distilled water as a blank control, and draw a standard curve based on the relationship between the absorbance and the volume of the working fluid.

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

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°C. Take the supernatant and determine its antioxidant capacity.

3.3.2 ABTS free radical scavenging rate

Prepare 7 mmol/L ABTS solution and 2.45 mmol/L potassium persulfate solution in advance, mix them in a ratio of 1:1, place them in the dark for 12 h-16 h, and dilute them by 30 times during use. Mix 0.5 mL of the enzyme sample diluent with 4.5 mL of ABTS solution thoroughly, put it in the dark and let it stand for 6 min, and measure the absorbance at 734 nm.

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

In the formula: X is the ABTS free radical scavenging rate; A1 is the absorbance of the mixture of 0.5 mL of distilled water and 4.5 mL of ABTS solution; A2 is the absorbance of the mixture of 0.5 mL of enzyme sample and 4.5 mL of ABTS solution; A3 is the absorbance of 0.5 mL of enzyme sample The absorbance of the mixture with 4.5 mL of absolute ethanol.

3.3.3 Hydroxyl radical scavenging rate

The ability to clear hydroxyl radicals is determined according to the Fenton reaction method, H2O2+Fe2+=·OH+H2O+Fe3+. Hydroxyl radicals react with salicylic acid to generate pyrotheric acid, which has a maximum absorption wavelength at 510 nm. Can detect and calculate the hydroxyl radical scavenging rate.

Prepare 6 mmol/L ethanol-salicylic acid solution, 6 mmol/L FeSO4 solution and 6 mmol/L H2O2 solution in advance. Take the enzyme samples and mix them with FeSO4 solution and H2O2 solution respectively. After standing for 10 minutes, add salicylic acid solution. After standing for 30 minutes to react, measure the absorbance of the reaction solution at 510 nm and use deionized water instead of salicylic acid solution. As a control group, deionized water was used as a blank group instead of enzyme samples, and the hydroxyl radical scavenging rate was calculated.

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

In the formula: X: hydroxyl radical scavenging rate

A1: Absorbance of a 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 DPPH free radical scavenging rate is basically determined according to the method of Yang Yang et al. for the determination of papaya enzyme. First prepare 8% DPPH solution, measure 3 mL enzyme diluent and 3 mL DPPH solution, mix well and react for 30 min in the dark. After the reaction, the absorbance was measured at 517 nm, 95% ethanol was added as a control, and the DPPH radical scavenging rate was calculated according to the formula.

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

In the formula: X is the DPPH radical scavenging rate; A1 is the absorbance of the mixture of enzyme sample and DPPH solution; A2 is the absorbance of the mixture of enzyme sample and absolute ethanol; A3 is the absorbance of the mixture of DPPH solution and absolute ethanol Absorbance.

4. Results and analysis

4.1 Measurement results of the content of biologically active ingredients

4.1.1 Types and content of organic acids

Organic acids not only enrich the flavor of enzyme products, but also play an important role in maintaining the acid-base balance of body fluids, improving the vitality of intestinal digestive

enzymes, promoting the growth of beneficial intestinal flora, and regulating the balance of intestinal microbial colonies[6].

This study detected a variety of organic acids in enzyme products, including formic acid, acetic acid, propionic acid, butyric acid, metabutyric acid, valeric acid, pivalic acid, oxalic acid, malic acid, lactic acid, etc. The test results showed that there were 7 kinds of organic acids in the enzyme samples, of which lycium barbarum enzyme and jujube enzyme contained many kinds of organic acids. There are organic acids acetic acid, propionic acid, and lactic acid in the three kinds of enzymes. Among them, the content of lactic acid is the highest, ranging from 29 mg/mL to 66 mg/mL. These organic acids work together to make the enzyme product rich in flavor and the ability to regulate the flora. Studies have used HPLC to detect organic acids in 5 kinds of enzyme samples including green plum enzymes. There are 4 to 11 kinds of organic acids, and tartaric acid, acetic acid and lactic acid are all detected. There are big differences in the types and contents of organic acids among different types of enzyme products, which are importantly related to the source of raw materials.

4.1.2 Types and content of oligosaccharides

Oligosaccharides are a class of compounds composed of 2-10 glycosidic bonds. Some oligosaccharides are easily digested and absorbed by the human body, and have the effects of anti-fatigue and regulating insulin balance; the other part is difficult to digest, but can effectively promote The growth and reproduction of bifidobacteria, while inhibiting the growth of spoilage bacteria, have the effects of slowing down aging, preventing cancer, anti-cancer, reducing the burden on the liver, and increasing the rate of nutrient absorption. The higher the content of oligosaccharides, the greater the effect on human nutrition and health care.

In this study, four common oligosaccharides, fructose, maltose, glucose, and galactose were tested. The results showed that the enzyme samples all contained maltose and glucose, and the glucose content was generally higher, and only the blueberry enzyme had a lower glucose content. Fructose and galactose are only present in some samples, and the content is low.

4.1.3 The 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 anesthesia effects, and can cause brain edema.

After being absorbed by the human body, ethanol will stimulate the central nervous system to be highly excited, and then inhibit the nervous system's response. Regular intake of ethanol can also cause addiction and cause chronic diseases such as fatty liver and alcoholic liver.

After testing, only the wolfberry enzyme in the enzyme samples contained a lower concentration of methanol, about 2 g/L, and no methanol was detected in the others. The concentration of ethanol in the samples was low or undetectable.

4.2 The determination results and analysis of the content of total polyphenols and total flavonoids

Flavonoids have the functions of lowering blood sugar, lowering lipids, preventing and treating cardiovascular diseases, calming and anti-inflammatory, antibacterial and insecticide, anti-oxidation and delaying aging, preventing cancer and anti-tumor, etc., and are added to food, medicine and other products as health functional factors[7]. Therefore, this class Substances have broad development prospects as medicines and functional foods.

Polyphenol compounds can act on oxidizing substances to terminate the oxidation reaction, thereby exerting its antioxidant activity. Research on the polyphenol extracts of banana peels found that the ability of polyphenols to scavenge DPPH free radicals is lower than that of ascorbic acid, but the ability to scavenge hydroxyl free radicals is higher than that of the same concentration of ascorbic acid. Most polyphenols have antibacterial ability. Studies have shown that the phenolic hydroxyl group in polyphenols can promote the passage of polyphenols through the cell membrane, destroy the integrity of the cell membrane structure, and interfere with the normal function of the cell

membrane to exert antibacterial effects. At the same time, researchers found that the protective effect of polyphenols on blood lipids is related to its antioxidant activity, and at the same time has a protective effect on acetaminophen-induced liver damage[8].

The detection of flavonoids and polyphenols in the samples showed that the total flavonoids content in the samples was between 3.6 mg/ml and 8.2 mg/ml. The highest total polyphenol content was blueberry enzyme, which was 17.6 mg/ml.

4.3 Test results and analysis of antioxidant capacity

Free radicals in the human body can damage human cells due to oxidative stress, causing a series of harmful biochemical reactions, causing protein damage, lipid peroxidation, DNA mutations and enzyme inactivation, and ultimately leading to a series of problems such as human aging. Therefore, the ability to scavenge free radicals is an important indicator for enzyme samples to exert their beneficial physiological functions[9]. Regarding antioxidant activity, there are multiple detection methods, and the corresponding reaction principles are different. Therefore, in order to conduct a comprehensive comprehensive evaluation of the antioxidant capacity, this experiment measured and evaluated the antioxidant capacity of the enzyme samples in terms of ABTS scavenging ability, hydroxyl radical scavenging ability, and DPPH free radical scavenging ability.

All three enzyme samples have a certain level of free radical scavenging ability. Among them, wolfberry enzyme has the lowest ABTS free radical scavenging ability, which is 34.28%; the highest is blueberry enzyme, which is 52.37%. The hydroxyl radical scavenging ability is between 20% and 40%. The DPPH free radical scavenging rate is the highest in the sample blueberry enzyme, which can reach 58.24%, and the DPPH free radical scavenging ability of jujube enzyme is only 24.35%. Blueberry enzyme has the best performance in terms of ABTS scavenging ability, hydroxyl free radical scavenging ability, and DPPH free radical scavenging ability, and it can exert its antioxidant ability better.

5. Conclusion and discussion

Enzymes contain different kinds of biologically active substances, which have different biological effects. This study analyzed the types and contents of organic acids and oligosaccharides, the contents of methanol and ethanol, the contents of total flavonoids and total polyphenols, and the antioxidant capacity of the three enzyme products. The results show that all three enzyme products contain 3 to 5 organic acids, among which lactic acid content is generally higher, which is an important factor that affects the taste and flavor of enzyme products and promotes its regulation of intestinal flora. Oligosaccharides mainly exist in the form of glucose and contain a small amount of maltose. Methanol was not detected in all samples, and the ethanol content was kept at a low level, which met the safety and quality requirements of enzymes. The total flavonoids and total polyphenols content of different samples are quite different, but they are generally better than the results in other studies. There is little difference in antioxidant capacity, but generally low. Some indicators of enzyme products are quite different, 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 enzyme products, while fermentation processes and storage conditions have a greater impact on the antioxidant capacity . According to the above conclusions, the enzyme products can be optimized to improve their quality and active function.

References

[1] Qiang P, Hang L, Lei H, et al. Relationship between structure and immunological activity of an arabinogalactan from Lycium ruthenicum[J]. Food Chemistry, 2016, 194:595-600.

[2] Pagliai Giuditta, Venturi Manuel, Dinu Monica, Galli Viola, Colombini Barbara, Giangrandi Ilaria, Maggini Niccolò, Sofi Francesco, Granchi Lisa. Effect of consumption of ancient grain bread leavened with sourdough or with baker's yeast on cardio-metabolic risk parameters: a dietary intervention trial [J]. International Journal of Food Sciences and Nutrition, 2021,72(3).

[3] Van der Sman R.G.M. Thermodynamic description of the chemical leavening in biscuits[J]. Current Research in Food Science, 2021(prepublish).

[4] Katsi Pavlina, Kosma Ioanna S., Michailidou Sofia, Argiriou Anagnostis, Badeka Anastasia V., Kontominas Michael G. Characterization of Artisanal Spontaneous Sourdough Wheat Bread from Central Greece: Evaluation of Physico-Chemical, Microbiological, and Sensory Properties in Relation to Conventional Yeast Leavened Wheat Bread[J]. Foods, 2021,10(3).

[5] Owade Joshua Ombaka, Abong' George Ooko, Okoth Michael Wandayi, Mwang'ombe Agnes Wakesho, Jobor Jared Omondi. Comparative profiling of lactic acid bacteria isolates in optimized and spontaneous fermentation of cowpea leaves.[J]. Food science & nutrition, 2021,9(3).

[6] Alzuwaid Nabeel T, Pleming Denise, Fellows Christopher M, Laddomada Barbara, Sissons Mike. Influence of Durum Wheat Bran Particle Size on Phytochemical Content and on Leavened Bread Baking Quality.[J]. Foods (Basel, Switzerland), 2021,10(3).

[7] Huangtong Kang Jinjoo, Lee Soyoung, Kim Namkyung, Dhakal Hima, Choi YoungAe, Kwon Taeg Kyu, Khang Dongwoo, Kim SangHyun. Hispidulin alleviates 2,4-dinitrochlorobenzene and house dust mite extract-induced atopic dermatitis-like skin inflammation.[J]. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie,2021,137.

[8] Mihanfar Aynaz, Nouri Mohammad, Roshangar Leila, Khadem-Ansari Mohammad Hassan. Polyphenols: Natural compounds with promising potential in treating polycystic ovary syndrome [J]. Reproductive Biology, 2021,21(2).

[9] Opuwari Chinyerum, Setumo Mmaphulane, Adefolaju Gbenga, Henkel Ralf. Aqueous extracts of black tea (Camellia sinensis) enhanced human sperm functions in vitro[J]. The FASEB Journal, 2021,35.