

# Comparative Study on Antioxidant Capacity of Self-fermenting Enzyme and Commercial Enzyme

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**Abstract.** In order to provide consumers with a more reasonable method of using Enzyme, UV-visible spectrophotometry was used to study the antioxidant ability of self-fermenting Enzyme and commercial Enzyme. By measuring the ability of fourteen kinds of self-fermenting Enzyme and three commercial Enzyme to scavenge DPPH free radicals, superoxide anion and hydroxyl radicals and the number of living bacteria, the following conclusions were drawn: (1) Commercial Enzyme has more properties than self-fermenting Enzyme of antioxidant capacity; (2) Compound fruit Enzyme has stronger antioxidant ability than single fruit Enzyme; (3) Self-fermenting Enzyme is not sterilized including more living bacteria; (4) Commercial Enzyme has a higher drinking value than self-fermenting Enzyme.

## 1 Introduction

Enzyme is a product containing specific bioactive components prepared by microbial fermentation with animals, plants, bacteria and other materials<sup>[1]</sup>. According to the product application field, the Enzyme is divided into six categories: edible Enzyme, daily Enzyme, feed Enzyme, agricultural Enzyme, environmental Enzyme, and other Enzyme. In recent years, more and more studies have been done on edible Enzyme. Xin Liu et al. obtained the best fermentation conditions by optimizing the fermentation process of blueberry Enzyme, under which the content of bioactive substances was the highest<sup>[2]</sup>. The formula research of other raw material Enzyme is also a hot spot, such as mulberry Enzyme<sup>[3]</sup>, wheat seedling Enzyme<sup>[4]</sup>, wolfberry Enzyme<sup>[5]</sup>, bamboo leaf Enzyme<sup>[6]</sup>, blueberry Enzyme<sup>[7]</sup>, grape Enzyme<sup>[8]</sup> and so on.

Enzyme has become a popular topic because of its rich nutrition and the experience of the beauty of brewing process. On the one hand, the report of the 19th National Congress pointed out that 'when socialism with Chinese characteristics enters a new era, the principal contradiction in our society has been transformed into the contradiction between the growing needs of the people for a better life and unbalanced and inadequate development'. People's growing need for a better life is to eat healthy food. Therefore, edible Enzyme have rapidly developed<sup>[9]</sup>, and the prevalence of micro-business has stimulated food enthusiasts (such as leavens) to adhere to the self-fermenting of Enzyme. On the other hand, the market price of edible Enzyme varies greatly, ranging from CNY hundreds to CNY hundreds of thousands<sup>[10]</sup>. This phenomenon further promotes the firm confidence of

Enzyme lovers in the self-fermenting Enzyme. They believe that the selection of good raw materials and the training of micro-businessmen will certainly make Enzyme with high nutritional value. In fact, there is no inspection agencies or specialized agencies to determine whether their products are qualified. In this paper, 14 kinds of self-fermenting Enzyme were collected from Enzyme lovers in Jinan City. A series of data were obtained by comparing and analyzing the antioxidant capacity and living bacteria index of three kinds of commercially available Enzyme. These data provide a method for Enzyme lovers to select Enzyme, and have a certain understanding of their self-fermenting Enzyme products, so as to provide a correct consumption orientation for the public.

## 2 Materials and methods

### 2.1 Test materials

14 kinds of self-fermenting Enzyme (collected from self-fermenting Enzyme lovers) were pitaya Enzyme (abbreviation A), apple Enzyme (B), strawberry Enzyme (C), pear Enzyme (D), barley seedling Enzyme (E), garlic Enzyme (F), seabuckthorn Enzyme (G), mulberry Enzyme (H), blueberry Enzyme (I), pitaya and apple Enzyme (J), strawberry and lemon Enzyme (K), barley seedling and lemon Enzyme (L), mulberry and apple Enzyme (M), wolfberry and jujube Enzyme (N). Three kinds of commercial Enzyme (purchased in supermarket) are recorded as O, P, Q.

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## 2.2 Test method

Self-fermenting Enzyme was collected and filtered with 0.45µm filter membrane, then stored in refrigerator at 4°C. The commercially available Enzyme were kept and reserved according to the instructions.

## 2.3 Project determination

### 2.3.1 DPPH scavenging rate

The determination of DPPH free radical reference Yang Yang and OMP S' method [11-12]. DPPH dissolved in anhydrous ethanol solution was purple and had a strong absorption at 517nm. When there was a free radical scavenger, the absorption gradually disappeared due to its single electron pairing. UV spectrophotometer was used for rapid quantification, and the scavenging ability of the sample solution to DPPH free radicals was calculated according to the clearance rate formula. Each sample was measured three times in parallel, and the average value was calculated.

$$\text{DPPH free radical scavenging rate (\%)} = \frac{(1+A_2-A_1)/A_3 * 100\%}{(1)} \quad (1)$$

In the formula:

A<sub>1</sub> -- Absorbance of 4 mL DPPH solution and 4 mL sample solution;

A<sub>2</sub> -- Absorbance of 4 mL sample solution and 4 mL 95% ethanol;

A<sub>3</sub> -- Absorbance of 4 mL DPPH solution and 4 mL 95% ethanol.

### 2.3.2 hydroxyl radical scavenging effect

The hydroxyl radical scavenging effect was determined according to the method of Yang Yang and Hesheng Li [11,13]. Hydroxyl radical has a strong oxidation ability. Hydroxyl radical is generated by electric Fenton method, and salicylic acid is added in the reaction process. The absorbance of the reaction solution containing the measured substance is measured at 510 nm using the fixed reaction time method. Each sample was measured three times in parallel and averaged.

$$\text{Hydroxyl radical scavenging rate (\%)} = \frac{(A_1+A_3-A_2)/A_1 * 100\%}{(2)} \quad (2)$$

In the formula:

A<sub>1</sub> -- blank control;

A<sub>2</sub> -- Determination sample;

A<sub>3</sub> -- without adding chromogenic H<sub>2</sub>O<sub>2</sub>.

### 2.3.3 Superoxide anion radical scavenging effect

Determination of superoxide anion radical scavenging activity reference Yang Yang [11] determination of papain method. Under weak alkaline conditions, superoxide anion can react with pyrogallol to form O<sup>2-</sup> and colored intermediate, which has a characteristic absorption peak at 325nm. In the initial test stage, the amount of intermediate products was linear with time. When superoxide anion scavenger is added, it can react with superoxide anion rapidly, thereby preventing the accumulation of

intermediate products and weakening the optical absorption of the solution at 325nm. Therefore, the scavenging effect of scavenger on superoxide anion was evaluated by measuring A<sub>325</sub> value. Each sample was measured three times in parallel and the average value was calculated.

The superoxide anion radical scavenging rate E(%) was calculated as follows

$$E(\%) = (A_1 - A_2) / A_1 * 100\% \quad (3)$$

In the formula:

A<sub>1</sub> -- average absorbance of blank;

A<sub>2</sub> -- the average absorbance of the sample.

### 2.3.4 Number of viable bacteria

The number of viable bacteria was determined by referring to the determination of total number of colonies in the national standard of food safety microbiology inspection [14]. By gradient dilution method, 25 mL sample was absorbed by sterile pipette and put into a sterile conical flask containing 225 mL phosphate buffer solution or normal saline (with an appropriate amount of sterile glass beads in the flask). The sample homogenate of 1:10 was prepared by fully mixing. During the test, 2~3 sample homogenates with appropriate dilution (the liquid sample may include the original solution) were selected. During the 10-fold increasing dilution, 1 mL sample homogenate was absorbed into the sterile plate, and two plates were made for each dilution. At the same time, 1 mL of blank diluent was added into two sterile plates for blank control. Pour the plate-counting AGAR culture medium (cooled to 46°C for 15 mL~20 mL) into the plates in time, and rotate the plates to mix evenly. After 48±2 h of culture, count. Each sample was measured three times in parallel and averaged.

$$N = \sum C / [(n_1 + 0.1n_2)d] \quad (4)$$

In the formula:

N -- the number of colonies in the sample;

∑C -- plate (plate containing the number of colonies in a suitable range);

n<sub>1</sub> -- the first dilution ( low dilution ) plate number;

n<sub>2</sub> -- number of plates with second dilution ( high dilution multiple );

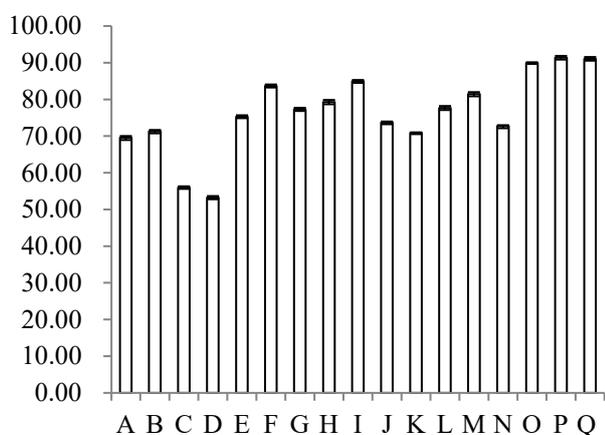
d -- Dilution factor ( first dilution ).

## 2.4 Data analysis

There were more than three replicates in the experiment, and the data was the average of multiple replicates. Excel software was used to analyze the difference significance and correlation, and Origin Lab8.5 software was used to draw.

### 3 Results and analysis

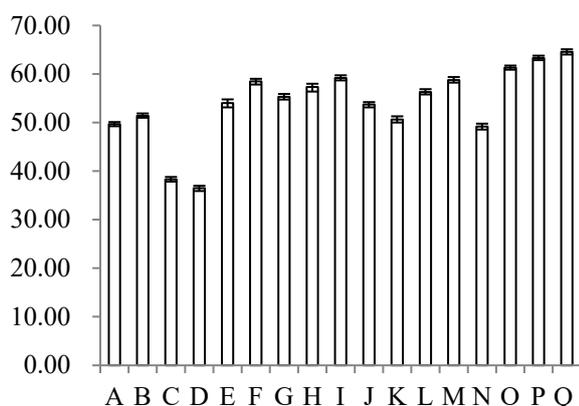
#### 3.1 Scavenging capacity of Enzyme on DPPH free radical



**Fig.1** Scavenging capacity of different Enzyme on DPPH free radical (%)

The DPPH radical scavenging capacities of 14 self-fermenting Enzyme and 3 commercially available Enzyme are shown in Fig.1. According to the analysis in Fig.1, the average scavenging capacity of 14 self-fermenting Enzyme on DPPH radical was 73.35%, and the average scavenging capacity of 3 commercially available Enzyme on DPPH radical was 90.78%. The scavenging capacity of 14 self-fermenting Enzyme on DPPH radical was significantly lower than that of commercially available Enzyme. Among the 14 commercially available Enzyme, pear Enzyme had the lowest DPPH radical scavenging capacity, and blueberry Enzyme had the highest DPPH radical scavenging capacity. The DPPH radical scavenging ability of compound fruit Enzyme was significantly higher than that of single fruit Enzyme.

#### 3.2 hydroxyl radical scavenging ability of the enzyme

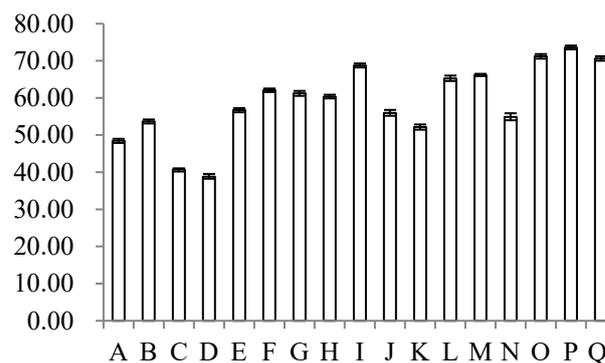


**Fig.2** Hydroxyl free radical ( $\cdot\text{OH}$ ) scavenging ability of different Enzyme(%)

The hydroxyl radical scavenging capacities of 14 self-fermenting Enzyme and 3 commercially available Enzyme are shown in Fig.2. According to the analysis in Fig.2, the average scavenging capacity of 14 self-fermenting Enzyme on hydroxyl radical was 52.04%, and that of 3

commercially available Enzyme was 63.04%. The scavenging capacity of 14 self-fermenting Enzyme on hydroxyl radical was significantly lower than that of commercially available Enzyme. Among the 14 commercially available Enzyme, pear Enzyme had the lowest scavenging capacity on hydroxyl radical, and blueberry Enzyme had the highest scavenging capacity. The hydroxyl radical scavenging ability of compound fruit Enzyme was significantly higher than that of single fruit Enzyme.

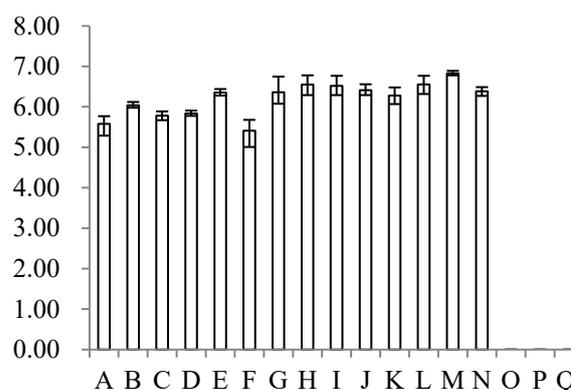
#### 3.3 Superoxide anion radical scavenging ability



**Fig.3** Superoxide radical scavenging ability of different Enzyme(%)

The scavenging capacities of 14 self-fermenting Enzyme and 3 commercially available Enzyme on superoxide anion free radicals are shown in Fig.3. According to the analysis in Fig.3, the average scavenging capacity of 14 self-fermenting Enzyme on superoxide anion radical was 56.08%, and that of 3 commercially available Enzyme was 71.81%. The scavenging capacity of 14 self-fermenting Enzyme on superoxide anion radical was significantly lower than that of commercially available Enzyme. Among the 14 commercially available Enzyme, pear Enzyme had the lowest scavenging capacity on superoxide anion radical, and blueberry Enzyme had the highest scavenging capacity. The scavenging capacity of compound fruit Enzyme on superoxide anion free radical was significantly higher than that of single fruit Enzyme.

#### 3.4 Number of viable bacteria



**Fig.4** The number of living bacterium of different Enzyme  
The number of viable cells of 14 self-fermenting Enzyme and 3 commercial Enzyme is shown in Fig.4. According

to the analysis in Fig.4, the average number of viable cells of 14 self-fermenting Enzyme was  $6.21 \times 10^8$ , and no viable cells were detected in 3 commercially available Enzyme. Among the 14 commercially available Enzyme, garlic Enzyme had the lowest number of live bacteria, and mulberry apple Enzyme had the highest number. The number of viable cells of compound fruit Enzyme was significantly higher than that of single fruit Enzyme.

#### 4 Discussion and conclusion

The antioxidant capacity of the self-fermenting Enzyme was significantly lower than that of the commercial Enzyme. The DPPH radical scavenging ability, hydroxyl radical scavenging ability and superoxide anion radical scavenging ability of the self-fermenting Enzyme were lower than those of the commercial Enzyme. The DPPH radical scavenging ability, hydroxyl radical scavenging ability and superoxide anion radical scavenging ability of the self-fermenting Enzyme were 73.35%, 52.04% and 56.08%, respectively. The scavenging capacities of commercially available Enzyme on DPPH free radical, hydroxyl free radical and superoxide anion free radical were 90.78%, 63.04% and 71.81%, respectively, which was consistent with the results of the antioxidant properties of *Tremella fusiformis* blueberry Enzyme studied by Luo et al.<sup>[15]</sup>. According to the investigation, the fermentation time of self-fermenting Enzyme in the brewing process is generally 7-10d, and the fermentation time is short, which is not conducive to the leaching of nutrients in fruits and the metabolism of microorganisms. In addition, the self-fermenting Enzyme focus on the natural fermentation process, and the main components are only fruit, sugar or honey, without any addition, which is adverse to the protection of the nutritional value of fruit itself<sup>[16-17]</sup>.

The number of viable cells of self-fermenting Enzyme is generally  $6.21 \times 10^8$ , and the number of viable cells is relatively high. Most of the commercially available Enzyme has gone through pasteurization or sterilization process, and no live bacteria were detected<sup>[18-19]</sup>. Beneficial live bacteria are beneficial to regulate intestinal function and have more benefits to the body<sup>[20-21]</sup>. However, self-fermenting Enzyme is naturally fermented, and the types of live bacteria are complex. Fermenters generally do not drink after testing, so there is a certain risk of drinking self-fermenting Enzyme directly.

Based on the above results, it is suggested that Enzyme lovers should drink their self-fermenting Enzyme after they are tested by regular testing institutions. And citizens should choose more regular enterprises to produce commercial Enzyme, rather than casual drink of self-fermenting Enzyme.

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