Study on the Changes of Antioxidant Activity of Fruit Enzymes with Different Formulas Resistance to Environmental Pollution

Tianli Sun¹, Shengnan Cui¹, Wanying Ma¹, Chuan Rong¹, Hongling Wang¹, Yuhong Yang², *
¹School of Life Engineering, Shenyang Institute of Technology, Fushun, China
²College of Bioscience and Biotechnology, Shenyang Agricultural University, Shenyang, China
*Corresponding author: 2001500032@syau.edu.cn

Abstract. Enzymes were made with different fruit combinations, and their total phenol content and free radical scavenging ability (DPPH·, ·OH, O2-) were determined. The results show that the enzyme has good antioxidant properties. And with the extension of the fermentation time, total phenol content and free radical scavenging ability all show a trend of increasing first and then decreasing. From the comprehensive point of view of total phenol content and free radical scavenging effect, Group 3 has the best effect, and it is best to drink at 40-60 days.

Keywords: enzymes, antioxidant, free radicals.

1. Introduction
Many diseases have been proved to be associated with active oxygen and free radicals in the body. If the human body has too many free radicals, cells and proteins will be attacked and damaged, causing various diseases [1-3]. Therefore, the development of foods rich in natural antioxidants has become a research focus in this field at home and abroad.

Enzyme is a kind of functional food formed by the mixed fermentation of fruits and vegetables and a variety of beneficial bacteria [4-13]. In recent years, with the improvement of people's health awareness, it has received more and more attention, but the focus on enzymes has mostly stayed on regulation [7-13]. Gastrointestinal function, prevention and treatment of constipation, etc. This research is based on the kiwi fruit enzyme product of soft jujube, and in-depth research on its antioxidant, measuring the total phenol content, reducing power and free radical (DPPH·, ·OH, O2-) scavenging ability, so as to evaluate the enzyme The antioxidant capacity.

2. Materials and equipment

2.1. Materials
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Soft date kiwi, apple, red dragon fruit, pear, hawthorn, lemon, rose, cordyceps, passion fruit, Kyoho grape, little bee grape, white sugar

2.2. Main instruments
Table 1 shows the main instruments used during the test.

Table 1. Main instruments’ name, models and manufacturers.

| Equipment name                  | Type         | Manufacturer                                      |
|---------------------------------|--------------|---------------------------------------------------|
| Shangmin electronic balance     | FA1104       | Shanghai Jingke Balance                           |
| Visible spectrophotometer       | RR1509034    | UniCo (Shanghai) Instrument Co., Ltd.             |
| Digital display constant temp.  | HH-2         | Changzhou Zhiborui Instrument Manufacturing Co., Ltd. |
| High-speed centrifuge           | HC-3518      | HKUST Innovation Co., Ltd. Zhongjia Branch        |
| Clean bench                     |              | Shanghai Boxun Industrial Co., Ltd. Medical Equipment Factory |
| Vertical pressure steam sterilizer| LDZX-50KBS   | Shanghai Shen'an Medical Instrument Factory       |

3. Test method

3.1. Preparation of different formula enzymes
Wash all kinds of fresh fruits with sterile water while keeping them aseptic, dry them in an ultra-clean workbench (peel and core), and cut them into even slices for later use. Group the fruits (as shown in Table 2), and spread the total amount of fruits and rock sugar in the sterilized fermentation tank at a ratio of 1:1, repeat the placement until 7 minutes or 8 minutes full, and put another on the top Layer lemon. Fermentation temperature is 28-32°C, fermentation is 1-3 months, until no gas is produced, the pH value is 2-4, that is, fermentation is stopped. Naturally fermented fruit enzymes are obtained. During the production of enzymes, relevant operations must be performed under strict aseptic conditions.

Table 2. Composition of different groups of enzymes

| Groups  | Composition                                      |
|---------|--------------------------------------------------|
| Group 1 | apple, red dragon fruit, pear, soft date kiwi, hawthorn, lemon |
| Group 2 | red dragon fruit, soft date kiwi, hawthorn, lemon |
| Group 3 | rose, Cordyceps, passion fruit, pear, Jujube Kiwi, lemon |
| Group 4 | apple, red dragon fruit, pear, hawthorn, Kyoho grape, lemon |
| Group 5 | apple, red dragon fruit, pear, hawthorn, bee, lemon |
| Group 6 | apple, pear, red dragon fruit, bee, lemon |
| Group 7 | apple, pear, red dragon fruit, hawthorn, lemon |

3.2. Determination of total phenol content [14]
Add distilled water to 450μL sample to make a constant volume. The total volume is 46mL. However, add 1mL of Folin's reagent to it, mix well, and let it stand for 3min. However, add 3mL of 20% sodium carbonate solution at a temperature of 25°C. The water bath was shaken for 2 hours, distilled water was adjusted to zero, the absorbance A of the sample was measured at 760nm, and the test was repeated 3 times. According to the regression equation of the standard curve, the total phenol content is calculated.

3.3. Determination of O2- scavenging ability
When the temperature is set to 25°C, place 4.5mL Tris-HCl buffer (concentration of 0.05mol/L, pH=8.2) in the water bath. Water bath for 20 minutes, then add 1mL sample solution and 0.4mL 25mmol/L pyrogallic acid solution, mix well, water bath for 5 minutes, and then add 1.0mL HCl with a concentration of 8mol/L to stop the reaction. Adjust to zero with Tris-HCl buffer, and measure the
absorbance $A$ of the sample at 299nm. At the same time, the clearance rate was calculated, and 1 mL of solvent was substituted for the sample as a blank control. The $O_2^-$ clearance rate is calculated by formula (1):

$$O_2^-$ scavenging rate (%) = \frac{(A_1-A_2)}{A_1} \times 100$$  \hspace{1cm} (1)

Among them: $A_1$——The absorbance value of the blank control group;  
$A_2$——The absorbance value of the enzyme samples of the experimental group.

3.4. Determination of ·OH scavenging ability
Add water to 0.45mL sample to make the volume up to 2mL, then add 1.4mL of 6mmol/L hydrogen peroxide, 0.6mL of 20mmol/L sodium salicylate, and 2mL of 1.5mmol/L FeSO$_4$·7H$_2$O, and finally place it at 37$^\circ$C. In the water bath for 60 minutes. Perform zero adjustment with distilled water, and measure the absorbance $A$ of the sample at 760 nm, and the number of repetitions is 3 times. The ·OH radical scavenging rate is calculated by formula (2):

$$\cdot OH \text{ scavenging rate } (%) = \frac{(A_1-A_2)}{A_1} \times 100$$  \hspace{1cm} (2)

Among them: $A_1$——The average absorbance of the blank;  
$A_2$——The average absorbance of the sample solution.

3.5. Determination of DPPH·scavenging ability [15]
Take 2mL of the sample solution and transfer it to a 10mL test tube, and then add 2mL of DPPH ethanol aqueous solution with a molar mass concentration of $2 \times 10^{-4}$mol/L with a mass concentration of 80%. After mixing well, let it stand for half an hour and use 80% ethanol solution. Perform zero adjustment and measure the absorbance at 517nm and record it as $A_1$; after mixing 2 mL each of DPPH solution and 80% ethanol solution, the absorbance is measured as $A_0$ under the same conditions; after mixing 2 mL each of the sample and 80% ethanol solution evenly, The absorbance measured under the same conditions is $A_2$. Repeat the test 3 times. The scavenging rate of DPPH·radical is calculated by formula (3):

$$DPPH\cdot \text{ scavenging rate } (%) = \left[1-(A_1-A_2)/A_0\right] \times 100$$  \hspace{1cm} (3)

Among them: $A_0$——the absorbance of 2mL of DPPH solution and 2mL of 80% ethanol mixture.  
$A_1$——The absorbance of 2mL DPPH80% ethanol aqueous solution and 2mL sample solution;  
$A_2$——The absorbance of the mixed solution of 2mL sample solution and 2mL of 80% ethanol;

4. Results and analysis

4.1. Total phenol content
From the beginning of fermentation, take a small amount of samples every 10 days, measure the total phenol content, and obtain the total phenol content change curve of 7 groups with different formulas and different fermentation time, as shown in Figure 1.
Figure 1. The variation curves of total phenol content.

According to Figure 1, with the extension of the fermentation time, the changes in the total phenol content of different formula enzymes tend to be roughly the same. The total phenol content has a significant upward trend before its fermentation for 60 days, and all reach the total phenol content on the 60th day. The highest value. Among all the formulas, Group 3 has the highest total phenol content, followed by Group 2 and then Group 7. The main antioxidant properties are polyphenols, and macromolecular substances can be transformed into small molecular phenols under the fermentation of microorganisms [16].

According to the test results, the total phenol content of the formulas with kiwi jujube is higher than other formulas. On the 60th day of fermentation, the total phenol content reached the highest value. After that, the total phenol content gradually decreases, which means that the longer the fermentation time, the higher the total phenol content.

4.2. Superoxide anion free radical scavenging ability
From the beginning of fermentation, take a small amount of samples every 10 days to determine the O$_2^-$ scavenging rate, and obtain the change curve of total phenol content of different enzymes in different fermentation periods, as shown in Figure 2.
According to Figure 2, compared with other groups, Group 3 of formula enzymes has a stronger scavenging ability of superoxide anion free radicals, and the scavenging ability is the largest on the 50th day of fermentation, with a scavenging rate of 22.79%. On the whole, Group 4, 5, and 6 did not have a significant effect of eliminating O$_2^-$ Experiments. It has shown that phenols containing free hydroxyl groups and flavonoids substituted with 3-hydroxy or polyhydroxyl groups present on the A ring or B ring have a strong O$_2^-$ scavenging ability [17]. Group 3 of formula enzymes has a strong O$_2^-$ scavenging ability, which may be due to its high content of phenolic substances.

Therefore, from the perspective of O$_2^-$ scavenging ability, the formula of group 3 is the best, followed by group 2 and then group 7.

4.3. Hydroxyl radical scavenging ability
From the beginning of fermentation, take a small amount of samples every 10 days to determine the hydroxyl radical scavenging rate, and obtain the change curve of total phenol content of enzymes in different schemes during different fermentation times, as shown in Figure 3.

![Figure 3. The variation curves of ·OH scavenging ability.](image)

From Figure 3 above, it can be seen that compared with other groups, the first, second, and fourth formulas have weaker ·OH radical scavenging ability. The underlying reason may be that the bacteria degrade part of the active substances during the fermentation. Group 7 of formula enzymes has the strongest scavenging ability, and the scavenging ability reached 69.19% of the test maximum after 40 days of fermentation. Existing studies have confirmed that ·OH free radicals can cause considerable damage in every living cell [18]. Therefore, it is necessary to improve its removal ability.

Therefore, from the perspective of ·OH scavenging ability, the formula of group 7 is the best, then Group 5, then Group 6, and then Group 3.

4.4. DPPH· scavenging ability
From the beginning of fermentation, take a small amount of samples every 10 days to determine the DPPH· scavenging rate, and obtain the total phenol content change curve of different enzymes in different fermentation periods, as shown in Figure 4.
Figure 4. The variation curves of DPPH· scavenging ability.

According to Figure 4, the DPPH and radical scavenging capacity of various formula enzymes all show a trend of first increasing and then decreasing. At the 60th day of fermentation, the enzymes of Group 1, 4, and 7 had the strongest DPPH-based scavenging effect, followed by 68.4%, 69.39%, and 66.57%. The remaining enzymes reach their maximum on the 50th day. Among them, Group 6 of formula enzymes DPPH· has poor scavenging ability, and Group 4 has the strongest DPPH·scavenging ability, reaching the test maximum value of 69.39% after 60 days of fermentation.

According to the test results, the DPPH· scavenging ability is Group 4> Group 5>Groups3> Group.1

5. Conclusion
The content of this chapter is based on the research in the previous chapter. It conducts in-depth and meticulous research on the antioxidant activity of the enzyme liquid. The total phenol content, O₂·, DPPH· and ·OH are determined and compared among the 7 groups.

With the extension of the fermentation time, its total phenol content and free radical scavenging ability all showed a trend of increasing first and then decreasing. At the fermentation time of 60 days, the total phenol content reached the highest; at the fermentation time of 40 days; at the fermentation time of 50 days, the O₂· scavenging ability reached a maximum of 22.79%; at the fermentation time of 40 days, the ·OH scavenging rate reached the maximum of 69.19%; After 60 days of fermentation, DPPH· reached the experimental maximum value of 69.39%. It shows that enzymes have different effects in different periods.

According to the experiment, the total phenol content of different formula enzymes is Group 3, 2, 7, 7, 5, 4, 6 from high to low. The O₂· scavenging effect of different formulas is Group 3, 2, 7, 7, 4, 5, 6 from high to low; ·OH scavenging effect is Group 7, 5, 6, 3, 4, 1, 2 from high to low; DPPH· scavenging rate from high to low is Group 4, 5, 3, 1, 2, 7, 6. In summary, in terms of total phenol content and free radical scavenging effect, the best option is the Group 3, which is best consumed at 40-60 days.

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