Study on the Changes of Antioxidants and their Activities of Tomatoes during the Fermentation Process

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Abstract. In this study, the total phenolic and antioxidant index (DPPH free radical scavenging ability, ABTS free radical scavenging ability and reducing power) were investigated. The results showed that the total phenol content increased gradually with the extension of fermentation time, and reached the maximum at 60 days (p<0.05); the lycopene content decreased first, then increased and then decreased during the fermentation of tomato, and reached the maximum at 20 days (p<0.05); the vitamin C continued to decrease (p<0.05); In terms of antioxidant activity, the scavenging capacity of DPPH radical, ABTS radical and reducing power were significantly increased after tomato fermentation (p<0.05), while the scavenging capacity of hydroxyl radical was decreased (p<0.05). Moreover, polyphenols were positively correlated with scavenging capacity of DPPH and ABTS radicals, respectively. Tomato enzymes have good antioxidant activity, especially scavenging ABTS and DPPH free radicals. Total phenol content can be used as an index to evaluate the antioxidant activity of tomato enzymes. This study provides useful information for the fermentation of tomatoes into an ingredient that is rich in phenolics for producing functional foods with increased antioxidant activities.

1. Introduction
Plant enzymes are microbial fermented products containing raw material leachate, microorganism itself and various bioactive metabolic components, which are made from one or more fresh vegetables, fruits and cereals, algae, herbs and other edible materials and added (or not added) sugars after a long period of fermentation by various fermenting bacteria [1,2]. Enzymes have some functions such as bacteriostasis, antioxidant, hypoglycemia, hypolipidemia, improving intestinal environment [2,3]. In recent ten years, with people's attention to health, enzymatic products with wide raw materials, rich taste, diverse forms and remarkable effects have rapidly become popular in the United States, Japan, South Korea, Taiwan, China and other countries. Fermentation is an ancient technology used to enhance the nutritional and organoleptic qualities of food [4].

Tomato is one of the most widely planted vegetables in the world [5], and one of the most consumed vegetables in China. It is rich in nutritional active substances with strong antioxidant capacity, such as polyphenols, vitamin C, flavonoids and lycopene. It can supplement a variety of vitamins and minerals [6-10], which can not only prevent cancer, but also protect liver, at the same time, it also has the function of enhancing memory and self-immunity. Therefore, fermented products made from tomatoes are considered as functional foods containing tomato derivatives [11]. However, there is no report on the preparation of tomato enzymes in China. In this paper, tomato was used as raw material to prepare enzymes, the changes of VC, total phenols and lycopene during fermentation were detected regularly. The changes of antioxidant activity during fermentation were detected by DPPH free radical scavenging capacity, ABTS free radical scavenging capacity, reductive capacity...
and hydroxyl radical scavenging, so as to scientifically and reasonably analyze the antioxidant activity of tomato enzymes and provide theoretical basis for the development of its products.

2. Materials and Methods

2.1 Preparation of Tomato Enzyme
In this experiment, fresh fruits with uniform color, uniform size and full fruit were washed with sterile water and dried. Then it is added to the sterilized fermentation tank with white sugar (ultraviolet sterilization) at a mass ratio of 3:1, sealed and fermented in a dry and cool place. Sample regularly and store it in a cryogenic refrigerator for later use.

2.2 Analysis of active substances in tomato enzyme

2.2.1 Total phenol content
Folin phenol method was used to determine the change of total phenol content during fermentation [12]. The enzymes were diluted to 1 mL and added with 0.25 mol/L folin phenol solution for 3 mL. They were fully shaken and blended. They were kept silent for 3 minutes at room temperature and away from light. At the end of the reaction, 12% Na₂CO₃ solution was added to 6 mL, and the shaking was even. Absorbance was determined at 760 nm after photoavoidance reaction at 2h.

2.2.2 Vc content
Ammonium molybdate method [13] was used to determine the change of Vc content during fermentation. The enzymes were diluted to 2 mL, added 8 mL oxalic acid-EDTA solution, 1 mL metaphosphate acetic acid solution, 2 mL 5% sulfuric acid solution and 4 mL 5% molybdic acid solution respectively. The absorbance was determined at 705 nm.

2.2.3 Lycopene content
GB/T 14215-2008 method was used to determine the change of lycopene content during fermentation. The methanol is added to the tomato enzyme, stirring, filtering, and then adding a little methanol to the remaining residue. Repeat the above steps until the filtrate is colorless. Remove the filtrate and reserve the residue. Add toluene until the filtrate is colorless. Collect the filtrate in a 25 mL brown volumetric bottle and volume it with toluene. Mix it well for use. The absorbance was measured at 485 nm by zero-setting toluene as blank.

2.3 Analysis of antioxidative properties in tomato enzyme

2.3.1 Reduction power
Making accurate shift polysaccharide samples from roxburgh rose pomace, followed by adding 2.5 mL 0.2 mol/L phosphate buffer solution and 2.5 mL 1% six potassium dicyanate solution, and rapid cooling after reaction 20 min at 50°C, then adding 2.5 mL 10% trichloroacetic acid and centrifugation at 3000 rpm for 10 min, the supernatant was added in 2.5 mL distilled water and 0.5 mL 0.1% trichloride solution, and then the absorption was measured at the wavelength of 700 nm after statically placing 10min.

2.3.2 Scavenging hydroxyl radical (·OH) activity
2 mL 9 mmol/L FeSO₄, 2 mL 9 mmol/L salicylic acid and 2 mL sample solution were successively added to the tube with stopper. The absorptivity was measured at 510nm after water bath reaction for 30 min at 37 °C by adding 2 mL 8.8 mmol/L H₂O₂ [12].

2.3.3 Scavenging activity of DPPH radical
0.1984g 2,2-diphenyl-1-picrylhydrazyl (DPPH) was accurately weighed and dissolved with anhydrous ethanol to 50 mL as reserve liquid, then 2 mL DPPH reserve solution was dissolved in absolute
ethanol. 2 mL DPPH solution and 2 mL ethanol as the test tube, the absorptivity was determined at 517nm after light avoidance reaction [14].

2.3.4 scavenging activity of ABTS free radical
ABTS liquor: add 88 µL 140 mmol/L potassium persulfate in 25 mL 7mmol/L ABTS solution, and place it in the dark for 12 hours at 25 °C. The absorbance of mother liquor diluted by PBS at 734 nm was 0.7 ± 0.02. The 200 µL enzymatic samples at different stages were added to the 5 mL ABTS liquor. The absorbance of A734 was determined at 734 nm after 10 min of reaction in constant temperature water bath at 30℃. Clearance/%= [1-(A734-Ai)/Ac]*100, Ac is the absorbance value of blank control; Ai is the absorbance value of sample background tube.

2.4 Statistical analysis
Values are expressed as mean +/- standard deviation. Statistical analyses were performed by one-way analysis of variance with the Duncan’s multiple range test and correlation of the data (SPSS 24.0, Illinois, USA). P values of less than 0.05 were considered statistically significant.

3. Results and Discussion

3.1 Changes of Active Substances in Natural Fermentation of Tomato

3.1.1 Total phenol content
As can be seen from Fig.1, the total phenol content of tomato enzymes increased gradually with the prolongation of fermentation time, and there was no significant difference in 0-20 days (p>0.05), and the polyphenol content reached the maximum at 60 days (p<0.05), which was about 3.5 times higher than that at the initial stage of fermentation. At this stage, microbial growth and metabolism activities are very vigorous, consuming a large number of other components of raw materials to metabolize to produce phenolic substances. This is consistent with the change of polyphenols in barley natural enzymes [15], red meat pitaya wine [16], while the polyphenols content in apple enzymes shows a bell-shaped change trend [17]. This may be due to the difference of fermentation raw materials and the different microbial flora, which makes the ability of phenols to be degraded and utilized by microorganisms in the fermentation process different. In summary, these results demonstrate that the fermentation and complex enzyme hydrolysis together significantly increased total phenolic contents in food [18]. Therefore, fermented tomatoes may have certain effects on delaying senescence and resisting inflammation.

Fig.1 Changes of total phenolic content in the fermentation of tomato

Values with different superscripts within a column are significantly different (P<0.05). All measures were performed in six independent samples.

3.1.2 Vc content
As can be seen from Figure 2, the continuous loss of vitamin c during fermentation (p<0.05). Xiong et al. [19] found that different proportion of sugar and Rosa roxburghii fermentation would lead to significant changes in Vc content. Vc is a water-soluble vitamin, the increasing water content of fermentation enzymes makes the content of vitamin c decrease continuously, which is similar to that of ascorbic acid in tomato after fermentation [20].
3.1.3 Lycopene content
Tomatoes are rich in carotenoids [10], of which lycopene is a unique carotenoid [21]. Lycopene is also an active substance only existing in tomatoes, and its antioxidant capacity is even higher than that of beta-carotene [7]. As shown in Fig.3, the lycopene content decreased sharply at the beginning of fermentation (p<0.05), increased rapidly after 10 days (p<0.05), reached the maximum at 20 days, about 1.9 times as much as that at the beginning of fermentation, and began to decrease again after 30 days (p<0.05). This shows that the lycopene content of tomato decreases obviously after fermentation in the early stage of fermentation, which is similar to that of Mou Qin [22]. With the growth of microorganisms, the pH value of the system decreases and the polyphenol content increases. The stability of lycopene was improved, which was similar to that of total carrot after fermentation [20]. Liu Qiaoning et al [23] reported that controlling pH and temperature during fermentation could increase the content of lycopene. In the later stage, the accumulation of fermentation products may inhibit the enhancement of lycopene.

3.2 Changes of Antioxidant Activity in Natural Fermentation of Tomato
At present, there is no uniform standard for the evaluation of antioxidant activity in vitro due to the different antioxidant mechanisms in different organisms, mainly because only one method is difficult to evaluate its antioxidant comprehensively. The four methods were used to determine the antioxidant activity of tomato enzymes in this research.
3.2.1 Reduction power
As a comprehensive antioxidant evaluation index, reducing power is related to many antioxidant mechanisms, including free radical scavenging, polymetallic ion catalysts, reduction energy, peroxide degradation [24]. The variation of the reducibility of tomato enzymes during fermentation is shown in Fig. 4. Similar to DPPH free radical scavenging capacity, the reducing power of tomato enzymes was stable 30 days of fermentation, increased by 149% compared with the initial fermentation stage on the 30th day, and reached 1.25 times of the initial fermentation stage after 90 days of fermentation, and was stronger than 0.02 mg/mL Vc solution (p<0.05). The results showed that natural fermentation significantly increased the antioxidant activity of tomatoes, which was consistent with the change of polyphenols content and lycopene content during fermentation.

Fig. 4 Changes of reducing power during fermentation

3.2.2 Scavenging hydroxyl radical (·OH) activity
From Fig. 5, it can be seen that the scavenging ability of hydroxyl radicals decreases with the increase of fermentation time during tomatoes natural fermentation. At the initial stage of fermentation (0-20 days), the scavenging capacity of hydroxyl radicals decreased rapidly (p<0.05); then it tended to be stable and the change curve was gentle. The hydroxyl radical scavenging rate of winter jujube [25], grape [26], blueberry [27] increased significantly after fermentation. It can be inferred that the change of hydroxyl radical scavenging capacity is different due to the different fermentation substrates and the different microbial flora composition.

Fig. 5 Changes of Scavenging hydroxyl radical (·OH) activity during the fermentation

3.2.3 Scavenging activity of DPPH radical
In general, antioxidants prevent the auto-oxidation of food components and neutralise the plethora of free radicals that are generated within the human body [28]. As can be seen from Fig. 6, the scavenging capacity of DPPH radicals increased gradually and then decreased with the increase of fermentation time from tomato enzymes. At the beginning of fermentation (0-20 days), the scavenging rate was stable (p>0.05). After 30 days, the scavenging rate of DPPH radicals increased rapidly, reaching the maximum at 60 days (p<0.05), about 1.46 times as much as that at the beginning of fermentation, and then began to decrease slightly (p<0.05). The scavenging capacity of blueberry enzymes increased continuously during 30 days fermentation [27]; the scavenging capacity of apple enzymes began to decrease after increasing continuously in the initial stage of fermentation [29]; although the scavenging capacity of tomato enzymes was different from other fruits during fermentation, The scavenging capacity was related to the change of polyphenol content during fermentation, which indicated that polyphenols had certain effect on scavenging DPPH free radicals.
3.2.4 scavenging activity of ABTS free radical

As can be seen from Figure 7, the fermentation time of tomato was positively correlated with its ability to scavenge ABTS free radicals, and the scavenging ability gradually increased with the extension of time (p<0.05). In the first stage of fermentation (0-10 days), the scavenging capacity of tomato enzymes was not significantly different (p>0.05); in the second stage (10-20 days), the scavenging capacity increased rapidly (p<0.05), and remained elevated until the later stage of fermentation (p<0.05). The scavenging capacity of tomato enzymes to ABTS free radicals was lower than the 0.02 mg/mL Vc, which might be the continuous loss the Vc during fermentation. In barley natural enzymes [15], ABTS free radical scavenging rate showed stable growth in the early stage, rapid growth in the mid-term, and gentle growth in the later stage, showing a continuous upward trend, which is basically consistent with the overall upward trend measured in this experiment. It can be seen that the rising trend of ABTS free radical scavenging capacity of tomato enzymes can shows good antioxidant capacity, improves the possibility of preventing cancer, and has certain development and application value.

3.3 Correlation analysis of parameters in fermentation process

Pearson method was used to analyze the correlation of each index in tomato fermentation process. The results showed as shown in Table 1: Vc was negatively correlated with ABTS scavenging rate and hydroxyl radical scavenging rate; polyphenols were positively correlated with ABTS scavenging rate and DPPH scavenging rate; This indicates that phenolic compounds are the main source of antioxidant capacity in tomato enzymes. This is consistent with the results of natural enzymes of grapes and blueberries [30,31]; it shows that phenolics can easily separate from a hydrogen ion and stabilize through resonance hybridization [32], which is one of the main factors of phenolic antioxidant. The results showed that tomato enzymes had certain development value and were rich in natural antioxidant ingredients.

| correlation coefficient | reducing power | ABTS• | -OH | DPPH | Vc | total phenol | lycopene |
|-------------------------|----------------|-------|-----|------|----|--------------|----------|
| reducing power          | 1              |       |     |      |    |              |          |
| ABTS•                   | 0.395          | 1     |     |      |    |              |          |
| -OH                     | -0.211         | -0.517* | 1   |      |    |              |          |
| DPPH                    | 0.078          | 0.741** | -0.246 | 1   |    |              |          |
| Vc                      | -0.096         | -0.883** | 0.378 | -0.789** | 1 |              |          |
4. Conclusion

The Vc of tomato by natural fermentation was losing continuously; the content of polyphenols increased gradually, reaching the maximum at 60 days; and lycopene showed the trend of decreasing first and then increasing, reaching the maximum at 20 days. With the increase of fermentation time, the scavenging ability of ABTS radicals increased gradually from tomato enzymes; the scavenging ability of DPPH radicals and reduction power increased gradually and then decreased; the scavenging ability to hydroxyl radicals decreased gradually; therefore, the antioxidant ability of tomato enzymes was relatively strong during 30-60 days of fermentation. The correlation analysis showed that polyphenols were positively correlated with ABTS and DPPH scavenging rates, indicating that polyphenols played a more important role in antioxidant activity of tomato enzymes than Vc and lycopene. The results of this experiment can lay a theoretical foundation for the development and utilization of tomato efficacy.

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| total phenol | 0.351 | 0.890** | -0.439 | 0.888** | -0.848** | 1 |
|-------------|-------|---------|--------|---------|----------|---|
| lycopene    | 0.529 | 0.084   | -0.142 | -0.280  | 0.283    | -0.046 | 1 |
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