

# Study on Optimization of fermentation technology of carambola enzyme

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**Abstract:** in this paper, a new type of Carambola enzyme was developed from tropical fruit carambola. Through single factor experiment and orthogonal experiment, the mixed fermentation was carried out by using yeast and plant lactobacillus. The effects of the concentration of mixed bacteria, the proportion of mixed bacteria, the fermentation temperature and the fermentation time on the fermentation effect of Carambola enzyme were studied. The results showed that the best technological parameters of Carambola enzyme: feed liquid The ratio is 1:3, the sucrose content is 7%, the concentration of strain is 5%, the proportion of inoculated strain is 1:1, the optimal fermentation temperature is 30 °C, and the fermentation time is 7 days. After verification, it has certain guiding significance for the development and industrial production of Carambola enzyme products.

## 1 Introduction

Carambola<sup>[1]</sup>, also known as wulianzi, yangtao, yangtao, belonging to the Oxalis family, is a plant rich in tropical or subtropical. Carambola fruit is rich in nutrition and sugar, including sucrose, fructose, glucose, malic acid, citric acid, oxalic acid and other organic acids, and rich in vitamins, potassium, magnesium, phosphorus and other minerals, which can promote the digestion of food and reduce the absorption of fat by human body. Therefore, carambola fruit plays the role of reducing blood lipid and cholesterol, and hypertension, Cardiovascular disease has the effect of prevention. Enzyme<sup>[2]</sup> is a kind of functional product with one or more fruits and vegetables as raw materials and a variety of probiotics fermented for a long time. The plant enzyme contains the effective ingredients of the plant itself. After a long-time fermentation by microorganisms, some substances that are difficult to use or cannot be used are degraded into small molecular substances.<sup>[3]</sup> The secondary metabolites of microorganisms and some small molecular ingredients can be better absorbed and utilized by the body Use<sup>[4]</sup>. During the fermentation of Carambola to produce carambola enzyme, macromolecular substances such as polysaccharides are gradually degraded into small molecular components such as oligosaccharides and monosaccharides which are easy to be absorbed.

At present, there are few studies on carambola enzyme at home and abroad, and there is no study on the production of Carambola enzyme by using complex strains.<sup>[5]</sup> In this paper, carambola, a tropical fruit, was used as the raw material to ferment with complex strains. Through orthogonal design, under the conditions of

different strains proportion fermentation temperature, strain concentration and fermentation time, acidity was as the detection indicators to optimize the fermentation process of Carambola enzyme.<sup>[6]</sup>

## 2 Materials and methods

### 2.1 materials and reagents

Carambola, yeast, *Lactobacillus plantarum*, sugar, distilled water, sodium hydroxide, 95% ethanol, hydrochloric acid, hydrogen peroxide, sodium salicylate, ferrous sulfate, deionized water, pipette.<sup>[7-9]</sup>

### 2.2 instruments and equipment

Conical flask, beaker, acid-base burette, test tube, beaker, water bath pot, volumetric flask, constant temperature incubator, analytical balance, pipette, electric constant temperature blast drying oven.

### 2.3 experiment design

#### 2.3.1 carambola enzyme preparation process<sup>[9]</sup>

Fresh carambola - peeled - seeded - blanched - added with a fixed proportion of sucrose and water - sterilized - inoculated with yeast and plant lactobacillus - placed in the incubator for culture - carambola fermentation liquid - dried sterilization - carambola enzyme powder.

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## 2.4 experimental method

### 2.4.1 single factor test<sup>[10-12]</sup>

Under the conditions of 1:3 ratio of material to liquid, 7% sucrose, 1:1 ratio of yeast to plant lactobacillus, 25 °C fermentation temperature and 1 day fermentation time, the effect of 3%, 4%, 5%, 6% and 7% concentration of bacteria on the fermentation of Carambola was determined, and the best concentration of bacteria was obtained.

Under the condition of 3% concentration, 25 °C fermentation temperature and 1 day fermentation time, the effects of 1:3, 1:2, 1:1, 2:1, 3:1 ratio of yeast to plant lactobacillus on the fermentation of Carambola were determined, and the best proportion of Carambola was obtained.

Under the condition of 3% concentration, 1:3 ratio and 1 day fermentation time, the effect of fermentation temperature on the fermentation of Carambola was determined, and the optimal fermentation temperature was determined. The effect of fermentation time on carambola enzyme was determined under the condition of 3% concentration, 1:3 ratio and 25 °C fermentation temperature. The effect of fermentation time on carambola enzyme was determined at 1d, 3d, 5d, 7d and 9d, and the optimal fermentation time was determined.

### 2.4.2 orthogonal experiment

According to the results of single factor experiment, three influencing factors and five levels were determined, orthogonal experiment was carried out, and the optimal fermentation combination was determined through the analysis of experimental data<sup>[13]</sup>

## 2.5 determination method<sup>[14-16]</sup>

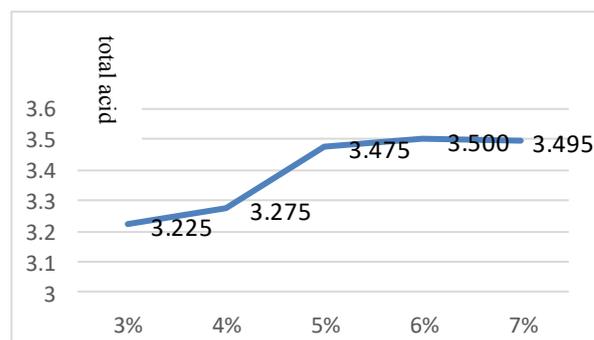
Determination of total acid value: add 2-3 drops of phenolphthalein solution to 10ml of sample solution each time, then titrate with 0.1mol/l NaOH solution until the sample solution is reddish and does not fade within 30s, and record the data. The concentration of each strain was titrated three times. Formula:

$$X = \frac{C \times K \times F}{M} \times V \times 1000 \quad (K = 0.075)$$

## 3 Experimental data analysis

### 3.1 data analysis of single factor experiment

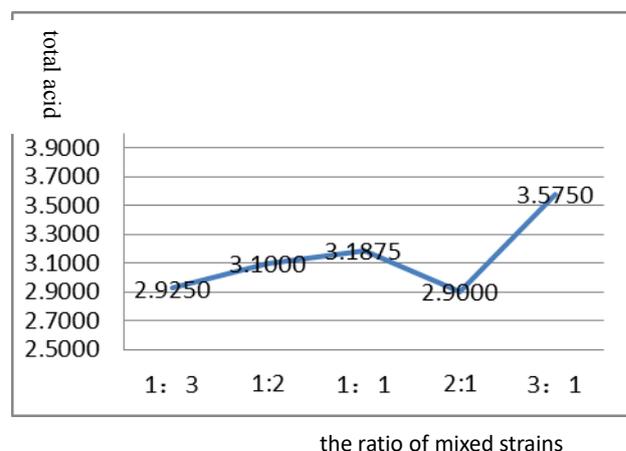
#### 3.1.1 effect of inoculation concentration on carambola enzyme fermentation



**Figure 1.** effect of inoculum concentration on carambola enzyme fermentation

It can be seen from fig 1 that with the increase of bacterial concentration, the nutrients mainly composed of carambola and sucrose in the fermentation broth are consumed by yeast and plant lactobacillus, and the total acid content increases accordingly. When the bacterial concentration reaches 5%, the total acid content of Carambola enzyme tends to be gentle, so the mixed bacterial concentration is 5%, and the total acid is 3.475g/kg.

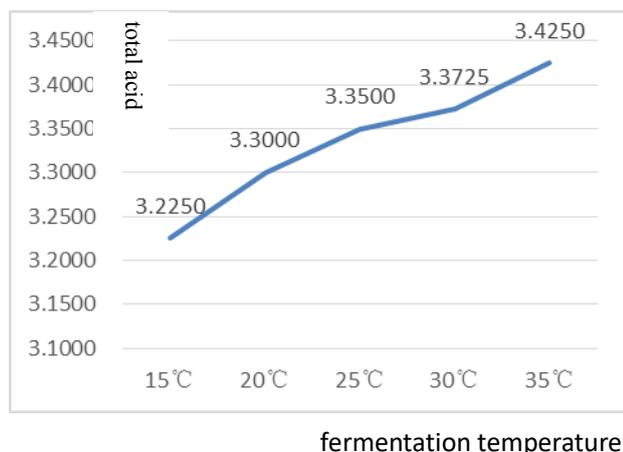
#### 3.1.2 effect of the proportion of mixed strains on the fermentation effect of Carambola



**Figure2.** Effect of the proportion of mixed strains on the fermentation

It can be seen from Fig. 2 that when the proportion of yeast is large, the total acid content is high, which may be due to the production of ethanol by yeast fermentation. Under certain conditions, a series of chemical reactions occur and a small amount of acetic acid is produced, which makes the total acid content in the fermentation liquid increase. When the proportion of plant lactobacilli increases, the total acid content in the fermentation liquid decreases, when the ratio of mixed strains was 3:1, the total acid content of Carambola fermentation broth was the highest.

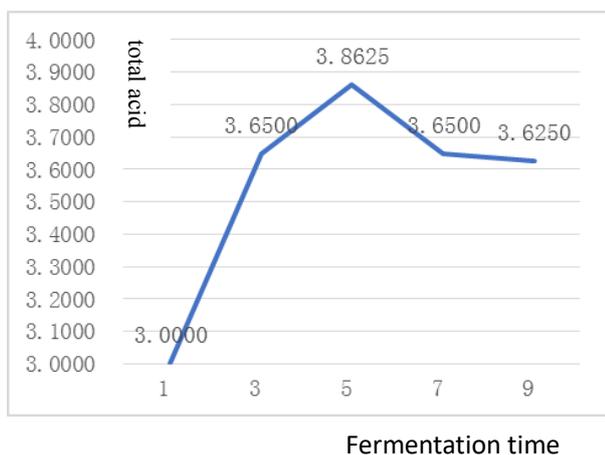
### 3.1.3 Effect of fermentation temperature on carambola fermentation



**Figure 3.** effect of fermentation temperature on carambola fermentation

According to Fig.3, with the increase of temperature, the total acid content shows a straight-line growth trend. At 15 °C, the minimum total acid content is 3.225g/kg; at 35 °C, the peak value is 3.425g/kg. With the increase of temperature, the growth temperature and fermentation temperature of Lactobacillus and Saccharomyces are more and more close to the optimum temperature. The fermentation degree of Lactobacillus and Saccharomyces is higher and higher, and the acids produced are also increased.

### 3.1.4 Effect of fermentation time on carambola fermentation



**Figure4.** effect of fermentation time on carambola fermentation

It can be seen from Figure 4 that with the extension of fermentation time, the total acid content of fermentation broth gradually increases. When the fermentation time is 5d, the total acid content is up to 3.863g/kg; then the total acid content starts to decrease, and the growth rate is the fastest when the fermentation time is 1-3d, and the growth rate starts to decrease when the fermentation time is 3-5d, because the longer the fermentation time is, the more the fermentation substrate is consumed, and by 7d, a large number of the main nutrients are consumed. A

small number of lactobacilli began to grow and propagate with organic acids as energy materials, and the total acid content began to decrease.

### 3.2 Data analysis of orthogonal experiment<sup>[12]</sup>

**Table 1** The table of factors and levels of orthogonal test

Level	test factor		
	A	B	C
1	1:3	15	1
2	1:2	20	3
3	1:1	25	5
4	2:1	30	7
5	3:1	35	9

Note: A-strain proportion B-fermentation temperature / °C  
 C-fermentation time / d

The values of strain proportion, fermentation temperature and fermentation time in carambola enzyme process are shown in Table 1:

The orthogonal test results of Carambola fermentation are shown in Table 2:

**Table 2** The table of orthogonal test results

Test No.	strain proportion	fermentation temperature / °C	fermentation time / d	total acid
1	1	1	1	2.63
2	1	2	2	3.43
3	1	3	3	4.1
4	1	4	4	5.4
5	1	5	5	5.85
6	2	1	2	3.13
7	2	2	3	3.5
8	2	3	4	3.63
9	2	4	5	3.86
10	2	5	1	3.18
11	3	1	3	3.55
12	3	2	4	3.55
13	3	3	5	3.65
14	3	4	1	4.467
15	3	5	2	4.367
16	4	1	4	4.067
17	4	2	5	3.733
18	4	3	1	2.833
19	4	4	2	4.500
20	4	5	3	4.300
21	5	1	5	1.367
22	5	2	1	5.667
23	5	3	2	3.267
24	5	4	3	4.300
25	5	5	4	3.900
K1	21.41	16.19	15.14	
K2	17.3	17.11	17.61	
K3	17.05	18.58	20.33	
K4	19.41	21.36	20.79	
K5	19.31	21.24	20.61	
Extreme difference R	0.872	1.034	1.13	
Better level	3	4	4	
Factors primary and secondary		C>B>A		
Optimal combination		A <sub>3</sub> B <sub>4</sub> C <sub>4</sub>		

**Table3** ANOVA table of orthogonal test analysis

Factor	Sum of squares of deviations	degree of freedom	mean square	F ratio	F critical value	Significance
A	2.542	4	0.636	1.07	2.866	
B	4.436	4	1.109	1.872	2.866	
C	4.869	4	1.217	2.054	2.866	*
Error	11.846	4	0.592			

$\alpha = 0.05$

According to the analysis of table 3 orthogonal test results, under the conditions of 1:3 feed liquid ratio and 7% sucrose addition, the primary and secondary order of the main factors affecting the fermentation effect of Carambola enzyme is C > B > A, that is, the fermentation time > fermentation temperature > the proportion of mixed strains; and from the visual analysis, it can be concluded that the optimal combination is a3b4c4, that is, the proportion of strains is 1:1, the fermentation temperature is 30 °C, and the fermentation time is 7 days.

### 3.3 validation test of Fermentation Conditions Optimization

According to the above optimization conditions for experimental verification, repeated three times, the carambola enzyme product obtained moderate acid and sweet, carambola unique aroma, good taste, achieve the desired fermentation effect, has the value of guiding the actual production.

## 4 conclusion

To sum up, the mixed fermentation with yeast and plant lactobacillus can obtain better fermentation effect than that with a single strain. When the inoculated strain is mainly yeast, the fermentation of fermentation broth is sufficient and the total acid content is increased, but the growth and reproduction of plant lactobacillus are inhibited. When the inoculated strain is mainly plant lactobacillus, the wind of Carambola enzyme can be improved Taste, but the fermentation is not enough, so the two have complementary effect. The best fermentation conditions of Carambola are: the ratio of material to liquid is 1:3, the added amount of sucrose is 7%, the concentration of strain is 5%, the ratio of inoculated mixed strain is 1:1, the best fermentation temperature is 30 °C, the fermentation time is 7 days. Through the experimental verification, the expected fermentation effect is achieved, and the technology is feasible, which has certain guiding significance for the development and industrial production of Carambola enzyme products.

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