Research on the Change of Protease Activity in the Process of Chili Sauce Fermentation

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Abstract. In order to better meet the needs of people's taste of chili sauce, to meet the needs of more consumers. In this paper, pepper sauce was used as the research object, and the change trend of the protease activity value of the pepper sauce during fermentation was observed and analyzed at a specific time and temperature to find out the suitable fermentation temperature range and time of the pepper sauce. The experimental data analysis shows that in the process of koji making, the suitable fermentation temperature of the bean koji is 35 °C, and the suitable fermentation days are 3 days. In the process of making sauce, the suitable fermentation temperature of the chili sauce is 40 °C, and the suitable fermentation days are 20 days.

1. Introduction
Chili sauce is a very good local specialty food that has been passed down to the present day. It is made by microbial fermentation. Chili sauce is not only nutritious, but also has good health benefits. In this experiment, Xinji's chili sauce was used as the research object. [1-4] During the specific time, the change trend of the protease activity value of the chili sauce in the process of koji making and sauce preparation was observed and analyzed to find out the suitable fermentation temperature range of bean curd and chili sauce, and time.

2. Experimental principle
Spectrophotometry is a commonly used method for enzymatic studies. Substrates in the enzymatic reaction often contain unsaturated radicals which are optically absorptive in the spectral violet region. The concentration of the reaction substrate is measured in the amount of absorption of the spectrum, and the progress of the enzyme reaction is measured in accordance with the change in the light absorption. This test mainly uses this principle to determine the protease activity in the fermentation of chili sauce.

Pick a certain amount of soybeans and ask for fullness, no decay, and damage. Rinse it and then soak it with water. The soaking time is about 6 hours. The soaked soybeans are filtered out of water.
and placed in a pan until they are almost soft. Sprinkle the soybeans into the pan, let cool, then sprinkle the flour evenly, and dry it on the gauze to let it ferment naturally. Until the hyphae turn yellow-green. Finally, dry and dry each soy bean and wait for it.

Pick the right size peppers, require maturity, no decay. Clean it, cut it in half from the middle, use the spoon to dig out the flesh, cut into small pieces of about 2 cm and put it in the tray for later use. Finally, we used a balance to weigh 225g of small pieces of pepper and 45g of bean curd, 10g of ginger, and put a proper amount of pepper and star anise to mix. The well-mixed raw materials were placed in a tank and fermented under the conditions required for the test.

3. Drawing of standard curve

Accurately weigh 1 kg of chili sauce solid enzyme, dissolve it with a small amount of phosphate buffer and grind it with a glass rod, then pour the supernatant into a 100 ml volumetric flask, and add a small amount of buffer to the sediment again with a glass rod. In the end, all of them were transferred to a volumetric flask, diluted to the specified scale, and filtered with four layers of gauze. The enzyme has been diluted 100-fold and the filtrate can be used directly as a test enzyme.

Prepare the L-tyrosine standard solution according to the following table:

| Pipe number | 1 | 2 | 3 | 4 | 5 | 6 |
|-------------|---|---|---|---|---|---|
| Distilled water, mL | 10 | 8 | 6 | 4 | 2 | 0 |
| 100μg/ml Tyrosine, mL | 0 | 2 | 4 | 6 | 8 | 10 |
| Final concentration of tyrosine, μg/ml | 0 | 20 | 40 | 60 | 80 | 100 |
| Absorbance (A) | 0.18 | 0.37 | 0.56 | 0.78 | 0.95 |

![Figure 1. Tyrosine standard curve change diagram](image)

4. Sample determination

Take 1.00 ml of each of the above solutions, add 5.00 ml of 0.4 mol/L sodium carbonate solution to each solution, 1.00 ml of forint reagent solution, and place in a 40±0.2 °C constant temperature water bath for 20 min, then remove Using a spectrophotometer at a wavelength of 680 nm, colorimetric, with a tyrosine-free 0 tube as a blank tube to adjust the zero point, respectively, the absorbance value, the absorbance value as the ordinate, the concentration of tyrosine as the abscissa, draw the standard
Curve or calculate regression equations. Calculate the amount (μg) of tyrosine when OD is 1, that is, the absorbance constant K value, and its K value should be in the range of 95 to 100.

a). The 2% casein solution was placed in a constant temperature water bath of 40 ± 0.2 °C and preheated for 5 min.

b). Take 4 tubes and add 1 ml of enzyme solution.

c). Take one as a blank tube, add 2ml of trichloroacetic acid solution, add 3ml of casein solution to each of the other 3 test tubes, shake well, and keep at 40 °C for 10min.

d). The test tube was taken out, 2 ml of trichloroacetic acid solution was added to each of the three test tubes, and 1 ml of casein solution was added to the blank tube.

e). After standing for 10 min, the precipitate was filtered.

f). 1 ml of the filtrate was taken from each tube, and 5 ml of a Na2CO3 solution and 1 ml of a forint reagent solution were separately added. The color was developed in a constant temperature water bath at 40 °C for 20 min. The OD value was measured at a wavelength of 680 nm. Adjust the zero point with a blank tube.

5. Data analysis

Table 2. Watermelon sauce protease activity value measured during the koji making process

| Temperature | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 |
|-------------|------|------|------|------|------|
| 30±1 °C     | 5.61 | 15.21| 19.56| 20.41| 13.2 |
| 35±1 °C     | 5.83 | 19.57| 25.38| 20.78| 15.37|
| 40±1 °C     | 6.15 | 18.3 | 23.45| 21.98| 16.65|

Table 3. Watermelon paste protease activity value measured during the process of making sauce

| Temperature | Day 1 | Day 5 | Day 10 | Day 15 | Day 20 | Day 25 | Day 30 |
|-------------|------|------|--------|--------|-------|-------|-------|
| 30±1 °C     | 4.21 | 15.46| 30.12  | 45.97  | 63.41 | 60.43 | 56.84 |
| 35±1 °C     | 5.11 | 16.35| 31.64  | 53.87  | 68.47 | 67.35 | 60.45 |
| 40±1 °C     | 5.61 | 19.57| 34.76  | 57.39  | 74.98 | 71.46 | 60.31 |

Figure 2. Growth graph of protease activity during koji making
Figure 3. Growth index of protease activity during the process of making sauce

It can be seen from Table 2 and Figure 2 that in the process of koji making, under the same fermentation days, the protease activity value of the Bean Curd measured at three different temperatures on the first day was not large, and the protease activity on the second day was the rate of change of the content increased, and the activity of the protease measured at the three temperatures on the third day continued to rise, reaching the peak and the highest value of the activity. Among them, the protease activity value was the highest at 35 °C, followed by 40 °C, and the protease activity value was the lowest at 30 °C at room temperature. On the fourth day, the activity values decreased, but the changes were not obvious. On the fifth day, it began to drop sharply and the activity value was low. Under the same temperature conditions (30 °C, 35 °C or 40 °C), the first day to the third day, the change showed a straight upward trend, the third day reached the peak, which is the highest value of the protease. The fourth day showed a downward trend, and the change value on the fifth day tended to be flat.

It can be seen from Table 3 and Figure 3 that under the same fermentation days, the protease activity value of the watermelon paste measured at three different temperatures on the first day is not large, and the change rate of the protease activity value on the fifth day is accelerated, on the 10th day. On the 20th day, the protease activity value measured at three temperatures continued to rise linearly, and reached the peak on the 20th day, which was the highest value of activity. Among them, the protease activity value was the highest at 40 °C, followed by 35 °C, and the protease activity value was the lowest at 30 °C at room temperature. On the 25th day, the activity values decreased, but the changes were not obvious. On the 30th day, the activity values began to be flat.

Under the same temperature conditions (30 °C, 35 °C or 40 °C), from day 1 to day 20, basically a steady upward trend, reaching the highest value on the 20th day, the highest value of watermelon paste protease activity. The 25-day change value decreased slightly, and the 30-day change value tended to be flat.

6. Conclusion
In this paper, the activity values of watermelon paste protease were determined in the process of koji making and in the process of making sauce, under different temperature conditions and different fermentation days. In summary, in the process of koji making, the activity value of the koji protease was the highest on the third day under the same temperature conditions, and the protease activity value at 35 °C was the highest under the same fermentation days. It can be seen that in the process of making the watermelon sauce, the suitable fermentation temperature is 35 °C, and the suitable fermentation days are 3 days. In the process of making sauce, the activity of protease activity of watermelon paste was the highest on the 20th day under the same temperature condition. Under the same fermentation days, the activity of protease activity of watermelon paste at 40 °C was the highest.
It can be seen that in the process of making the watermelon sauce, the suitable fermentation temperature is 40 °C, and the suitable fermentation days are 20 days.

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