Optimization of nitrite content in beetroot fermented by staphylococci xylosus using response surface methodology (RS M)

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Abstract: In this experiment, staphylococci xylosus were used as the research object to determine the amount of nitrite in the fermentation process. It was studied in single factor experiment by means of changing the amount of bacteria addition, vegetable content, fermentation time and in response surface optimization method. The nitrite was analyzed according to GB5009.30-2016. The optimum conditions were as follows: vegetable content 2.2g, inoculation amount 7.8×10⁶CFU/g, fermentation time 27h, and the optimum nitrite content of fermented Beet Liquefied is 487mg/kg (Fermented beet Power is 12000mg/kg).

1. Introduction
Sodium nitrite is widely used in meat products, its main role is delaying and controlling meat spoilage[1,2], making meat products produce typical pink[3]. The nitrite as an antioxidant [4] can produce a unique flavor [5] and inhibit Clostridium botulinum[2,4,6,7]. Other studies have confirmed that the conversion of nitrate to nitrite under the action of microorganisms can also produce the same effect as described above [8]. Therefore, nitrite is used as an irreplaceable additive in the field of cured meat products [9]. As a natural substance, vegetables are rich in nitrates [10], so they are widely used as natural sources in organic cured meat products. The nitrate content of sugar beet is as high as 4500mg/kg. Microorganisms mainly use nitrate reducing bacteria such as Staphylococcus carnosus and xylosus to reduce nitrate. Micrococcus is the main nitrate reductase microorganism, and its nitrate reductase can catalyze nitrate ion reduction into nitrite ion at low oxygen concentration or anaerobic condition [11]. Considering the rapid transformation of nitrate in the vegetable in the post fermentation process, so it is impossible to measure and control the conversion, the fermentation time is longer and the production cost increase as well. Therefore, the vegetable known as the natural nitrite substance are fermented to produce high nitrite content, and directly used for natural pickling[12,13]. The proportion of vegetable adding, the temperature of fermentation, the time of fermentation and the amount of...
vegetable addition should be considered synthetically [14]. Since 1990, sales of natural organic meat products have increased at a rate of 20% per year due to consumer preference for natural organic meat products [15,16]. The red beetroot has good color because of its two major groups of water-soluble nitrogenous pigments: betalains and betacyanins which display red to purple color, and the yellow respectively [17]. Betalains is used in meat substitutes and sausages. Beetroot color is used also by the pharmaceutical industry as a coloring agent in drug formulation [18]. The bioactive substance of betaine is available easily to the human body[19,20,21]. Based on the content of sugar beet and nitrate, this experiment decided to ferment beetroot with Staphylococcus xylosus. Staphylococcus (101445) is purchased by CICC company.

2. Material and Reagent

2.1 Materials
Red beet root, which is from Xuzhou city of Jiangsu province.

2.2 Regent
As shown in reference[22].

3. Experimental Method

3.1 Drawing of standard curves
As shown in reference[22]. The standard curve is $y = 0.0156x + 0.0002$, $r = 0.9995$.

3.2 Nitrite extraction
The same as above 2.1.

3.3 Determination of nitrite content
The same as above 2.1.

3.4 Sample
Fresh beet is cleaned with tap water and deionized water 3 times later. Then it’s dried and the plate is scrub with 75% alcohol. The sample is grind it a homogenous and spared with the mortar.

3.5 Single-factor experiment

3.5.1 The effect of vegetable on fermentation
The 15 triangle(3 group) sterile bottle of 250ml (each containing 4%glucose solution) beet homogenate was added to each bottle in a sterile operation. The beets concentration in each group was 0.5%, 1%, 1.5%, 2%, 2.5%, and the inoculation amount was $1.5 \times 10^6$ cfu/g. The sample were placed in a 30°C incubator and allowed to stand for 24h.

2.5.2 Effect of fermentation time on fermentation
The same as above 2.5.1. 2% beet was added in each group, the inoculation amount is $1.5 \times 10^6$ cfu/g respectively, Samples were placed at 30°C for fermentation 6, 12, 24, 36, 48h in incubator.

3.5.3 Effect of salt on fermentation efficiency
The same as above 2.5. 1.2% beet was added and the inoculum is $1.5 \times 106$ cfu/g. It was added 0%, 2%, 4%, 6%, 8%, 10% NaCl solution respectively. Samples were placed in a 30°C incubator for 24 h.

3.5.4 Effect of inoculum on fermentation
The same as above 2.5. 1.2% beet was added and the inoculum is $6 \times 10^4$ cfu/g, $6 \times 10^5$ cfu/g, $6 \times$
10^6 cfu/g, 1.2 \times 10^7 cfu/g, 1.02 \times 10^8 cfu/g respectively. Samples were placed in a 30°C incubator for 24 h, and the nitrite content of the fermentation broth is measured.

3.6 Optimization of nitrite content
Design 3 factors 3 levels table to optimize. A total of 17 experiments were performed. Response surface result was analyzed in Design Expert 8.0.6.1 (Table 1).

3.7 Data processing method
The original data was entered into Excel and analyzed statistically with SPSS software for Windows Version 20.0 and was dealt with Excel later. As shown in Table 1.

| Factor            | -1  | 0   | 1   |
|-------------------|-----|-----|-----|
| Vegetable (%)     | 1.8 | 2   | 2.2 |
| Time              | 24  | 27  | 30  |
| Inoculum          | 6 \times 10^5 | 6 \times 10^6 | 6 \times 10^7 |

4. Results and Discussion

4.1 Single factor experiment results

4.1.1 Effect of vegetable content on fermentation
As shown in Figure 1. The difference in vegetable content between 2% and 2.5% is not significant, so the 2% concentration is the best point.

4.1.2 Effect of fermentation time on fermentation
As shown in Figure 2. The content is maximized between 24h and 30h, however over time, it may be due to the nitrite reductase is predominating and decomposing nitrite so that the nitrite content is degraded.

4.1.3 Effect of salt content on fermentation

4.1.4 Effect of salt content on fermentation
As shown in figure 3. The salt concentration played a negative role in the nitrite content. May be the target strain was inhibited by salt. So the salt in subsequent fermentation experiments removed.

4.1.4 Effect of inoculum on fermentation
As shown in figure 4. Before the inoculation was $6 \times 10^6$ cfu/g, it is increased with the increase of inoculation, So the nitrate reductase activity was the highest. As the concentration of inoculation increase, the nitrite content decreases, possibly it’s due to competitive inhibition between microorganisms.

4.2 Results and Analysis of RSM

4.2.1 Regression equation significance misfit test
The results are shown in Fig. 5. It can be seen that the response surface regression model reaches a highly significant level ($p < 0.001$), the influence of A, C, AB, A², B² on the test results is significant; The missing term $P=0.373>0.05$. The regression equation fits the actual experiment goodly.

$$Y=37141.25+5364.84A+3184.71B-10038.87C+7488.76AB-2744.47AC-3133.20BC-7758.52A^2-14076.63B^2-4817.35C^2$$

4.2.2 Principal factor effect analysis
Comparing the four groups of graphs as follows, the time was the most significant for nitrite, which showed that the surface was steep.

4.2.3 Interaction factor effect analysis
In the center of the elliptical region, the amount of nitrite formed gradually decreases from the center to the edge. The ellipse indicates that the interaction between the two factors is significant; while the circle is converse, the interaction between the two factors can be ignored. From the contour plot analysis in Figure 6, it can be seen interaction between vegetable concentration and fermentation time is significant.
4.3 Freeze drying of fermented beet juice

When the best nitrite content of the beet fermentation juice is obtained, centrifuged to get the supernatant and is evaporated by Vacuum rotary with cooling cycle later. It’s frozen at -40°C overnight and dried to powder in a vacuum freeze dryer, stored under -4°C refrigeration protecting from light.

5. Conclusions

Factors order was fermentation time > vegetable content > vegetable inoculum. The optimum conditions were as follows: vegetable content 2.2g, inoculation amount $7.8 \times 10^6$ CFU/g, fermentation time 27h, a the optimum nitrite content of fermented Beet liquied is 487mg/kg (Fermented beet Power is 1200mg/kg)

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