Characteristics analysis of sugar beet powder fermented by staphylococcus xylobose

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Abstract: As a color and antiseptic additive, nitrite has been widely added to meat products. The large amount of nitrite remaining in meat products will generate carcinogenic nitrosamines in the body after eating, so natural sources of nitrate-rich fermented vegetables Powder has certain potential as a substitute for nitrite. In this paper, fermented beet powder rich in nitrite as the research object, spectral characteristics were analyzed. The stability of its pigments under the conditions of temperature, time, pH, metal ions, oxidants and preservatives were studied. The results showed that the maximum absorption wavelength of fermented beet powder measured with an ultraviolet spectrophotometer was 538 nm. The conditions that most affect the content of betacyanins were temperature, pH, and metal ions.

1. Introduction
As one of the important sensory quality, color determines the value of food. It affects consumers' desire to purchase, predetermines consumers' expectations of flavor and taste, and regulates the appetite. It is believed that natural pigment derivatives are safe and therefore more popular. Consumers in the United States, Japan and the European Union are increasingly using natural pigments. As a natural pigment, betaine in sugar beet not only has a good color, but also has the ability to maintain a good color in the processing and preservation of meat products.

Sugar beets are rich in nitrates. Some microorganisms can convert nitrates in sugar beet powder into nitrates under certain conditions, replacing inorganic nitrates and controlling meat corruption. At present, the most researches are mainly about fermentating celery powder. However, since celery is a common cause of food allergies, patients with celery allergy must completely avoid it[1]. The color of celery itself is green, so if it is used in industry, celery needs to be decolorized, and an additional production process is added, which brings inconvenience to the producer and relatively increases the cost. Beet has excellent color and its own properties provide convenience for production. The literature shows that the beet powder prepared by spray drying is basically the same as the celery powder, and the beet does not contain allergens[2].

The stability of betaine pigments is influenced by a number of factors similar to other natural pigments, including pH, temperature, oxygen, water activity and light. In this experiment, the pigment stability of betacyanins was explored.
2. Materials and Methods

2.1. Materials and Reagents
Red beet root, which was from Xuzhou city of Jiangsu province. Staphylococcus (101445) was purchased by CICC company. All reagents were of the chemical reagents.

2.2. Preparation of Fermented Beet Powder Rich in Nitrite
The beet was washed and crushed with a pulsator, and 2.2 g of beet homogenate was weighed and dissolved in 4% glucose solution. The beet was inoculated with 7.8×10^6 CFU/g of Staphylococcus xyloglycosis into the fermentation broth, which was incubated in a constant temperature incubator at 30 °C for 27 h. The fermentation broth was concentrated in a vacuum rotary evaporator at 55 °C and centrifuged. The supernatant was pre-frozen in a refrigerator at -40 °C for 12 h and then lyophilized in a vacuum freeze-dryer to obtain fermented beet powder, which was stored in a fresh-keeping bag in a dryer for later use.

2.3. Spectral Characteristics of Fermented Beet Powder Rich in Nitrite
The 0.1 g sugar beet product was dilute to a 10 mL volumetric flask with pure water, and the maximum absorption wavelength was determined by scanning with a visible spectrophotometer in the range of 330-760 nm.

2.4. Determination of betacyanins (BC) in nitrite-rich fermented beet powder [3]
The 0.1 g sugar beet product was made up to a 10 mL volumetric flask with pure water, and the pigment content was measured with an ultraviolet spectrophotometer.

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BC=\frac{A \times DF \times MW \times 1000}{e \times l}
\]

A represents the absorbance, DF represents the dilution factor, l represents the thickness of the cuvette, MW represents the molecular weight, with a value of 550 g/mol, and e represents the molecular extinction coefficient with a value of 60000 L/mol cm in H2O.

2.5. Determination of the influencing factors of BC in nitrite-rich fermented beet powder

2.5.1. Effect of temperature on betacyanins stability
Weighed 5 equal parts of fermented beet powder to prepare a 0.1% aqueous solution and placed them under indoor light at 20 °C, 40 °C, 60 °C, 80 °C, and 100 °C for 1 h, and measured the absorbance at 538 nm with an ultraviolet spectrophotometer to calculate the betacyanins content.

2.5.2. Effect of acidity and alkalinity on betacyanins stability
Weighed 5 equal parts of fermented beet powder, diluted them with different pH solutions to 0.1% aqueous solution, and after fully dissolving, measured the absorbance at 538 nm with an ultraviolet spectrophotometer, calculated the betacyanins content.

2.5.3. Effect of metal ions on betacyanins stability
Weighed 5 equal parts of fermented beet powder to prepare a 0.1% aqueous solution in 100 mg/L Fe^{2+}, Fe^{3+}, Cu^{2+}, Al^{3+}, measured the absorbance at 538 nm with an ultraviolet spectrophotometer and calculated the betacyanins content.

2.5.4. Effect of oxidants on betacyanins stability
Took 5 equal parts of fermented beet powder to prepare a 0.1% pigment solution and dissolved it in hydrogen peroxide solutions of different concentrations (0%, 0.3%, 0.6%, 0.9%, 1.2%), and different concentrations (0%, 0.12%, 0.15%, 0.18%, 0.21%) of sodium hypochlorite solution, placed it in the
room under normal light source conditions. Measured the absorbance at 538 nm with an ultraviolet spectrophotometer and calculated the betacyanins content.

2.5.5. Effect of Preservatives on betacyanins stability
Took 5 equal parts of fermented beet powder to prepare 0.1% pigment solution, dissolved in different concentration gradient (0%, 0.3%, 0.6%, 0.9%, 1.2%) of potassium sorbate solution, fully dissolved and placed in the room under the condition of normal light source. The absorbance of the solution at the maximum absorption wavelength was measured.

3. Results and Discussion

3.1. Spectral characteristics of nitrite-rich fermented sugar beet powder
The visible spectrophotometer scans betacyanins with a maximum absorption wavelength of 538 nm.

3.2. Effect of Different Factors on the Stability of betacyanins in Fermented Beet Powder

3.2.1. Effect of temperature on betacyanins stability
It could be seen from Fig. 1, that the temperature was significantly different at 70 °C and relatively stable at 60 °C. In the process of food processing and storage, temperature was the most important factor affecting the stability of sugar beet. Betacyanins were generally considered to be heat-susceptible pigments and lose their stability at high temperatures [4], and their degradation speed increases with increasing temperature. In addition, the stability of betatin decreased slightly at 50 °C-60 °C and 70 °C-80 °C, which was consistent with the results of other people's studied [5]. The thermal degradation reaction of betatin in sugar beet follows first-order kinetics [4]. During the heat treatment process, betaine may be isomerized, decarboxylated or cracked (degraded by heating, resulting in a gradual reduction in red color) [6]. The C17-decarboxylation caused a blue shift of the absorption peak from 538 to 505 nm, resulting in orange-red [7]. The yellowing of betaine may be due to the dehydrogenation of betaine leading to the formation of dehydrobetaine [8]. Therefore, in the industrial processing process, tried to avoid high temperature heating, otherwise it would easily lead to thermal degradation. But thermal degradation would not cause loss of its activity.

![Fig. 1. Effect of temperature on betacyanins](image)

3.2.2. Effect of acidity and alkalinity on betacyanins stability
It could be seen from Fig. 2, that the difference between pH 4-7 was not significant (P>0.05), indicating that it was stable in this range. When the pH was less than 3, the difference was significant when the pH was greater than 7, indicating that it was not resistant to strong acids and alkalis. The acid-base degradation mechanism is the same as the heating degradation mechanism mentioned above. When the pH was less than 3, the betarubin content was less. It meant that betacyanins was unstable under acidic conditions [9], and acidification would cause the reaggregation and isomerization of betacyanins, led to the production of dehydrobetaine [4,10]. The pH value between 3 and 7 did not
significantly affect the color. Below pH 3, the color of betacyanins shifted to violet, while above pH 7, the color shifted to blue. Outside this range (pH 3-7), it could lead to the degradation of betacyanins, which was consistent with the results of other researchers [11]. The pH value of low-temperature meat products is pH 3-7, and the stability of betarubin is also the optimum pH range for low-temperature meat products.

**Fig. 2. Effect of pH on betacyanins**

3.2.3. Effect of metal ions on betacyanins stability

It could be seen from Fig.3, that Al³⁺ had no effect on the pigment, while the effect of Cu²⁺ was greater than that of Fe²⁺ and Fe³⁺, and the difference was significant (P<0.1). Previous studies on the influence factors of metal ions on betacyanins showed that the addition of metal ions at 1 mmol concentration in beet juice would not significantly reduce the retention of betacyanins. The reduction of pigment occurs only when the metal concentration was higher than 2.5 mmol [12], so when it was applied to products containing metal ions, the effect of appropriate betacyanins concentration on the degradation of pigment should be considered during application. It could be used in industrial applications. Consider adding as far as possible to avoid mixing of ions or adding acid or metal ion chelating agents.

**Fig. 3. Effects of ions on betacyanins**

3.2.4. Effect of oxidants on betacyanins stability

As the concentration of hydrogen peroxide and sodium hypochlorite increase, the value of betacyanins showed a slow downward trend, the results were shown in Fig. 4 and Fig. 5. When the concentration of hydrogen peroxide was 1.8%, the difference was not significant (P>0.05), it indicated that the effect was small before the concentration of hydrogen peroxide was 1.8%, and it would not cause pigment degradation 1.8%. However, when the sodium hypochlorite concentration was 0.18%, the difference had little effect and would not cause degradation of the pigment. The two oxidant concentrations had a negative effect on betacyanins, but the effect of the two oxidants varies with the concentration. The effect of oxidation on betarubin should be considered in the application research.
3.2.5. Effect of Preservatives on betacyanins stability
The results were shown in Fig. 6, that with the increase of potassium sorbate concentration, the content of betacyanins showed a downward trend, and the difference was significant when the potassium sorbate concentration was 0.2%, $P_{0.2\%}<0.01$, and the concentration of potassium sorbate before 0.2% had no effect on the experiment. According to the national standard GB2760-2016, the limit standard of potassium sorbate in ham meat products is 0.0075% [13], which is significantly lower than the concentration of 0.2%. Therefore, it could be concluded that the concentration of potassium sorbate had no effect on the content of betacyanins.
4. Conclusions
The maximum absorption wavelength of betarubin was 538 nm, which was consistent with the literature description [14]. The effects of temperature, time, acidity and alkalinity, metal ions, oxidants, and preservatives on stability had been studied. The experimental results showed that temperature was a major factor affecting the degradation of betacyanins, and the pH was stable between 4-7 (partially acidic), which was more suitable for the pH range of low-temperature meat products. The metal ion Al$^{3+}$ had no effect on the pigment, while the Cu$^{2+}$ affected more than Fe$^{2+}$ and Fe$^{3+}$. Fe$^{3+}$ had a significant difference, and metal ion chelating agents such as citric acid, EDTA, etc. could be added during application to eliminate the impact. The oxidant Sodium hypochlorite concentration had little effect before 0.18%, the hydrogen peroxide concentration had an effect before 1.8%, and it had little effect when the concentration of potassium sorbate was lower than 0.2%.

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