Study on Preparation Technology of Taurine in Rice Peptide Feed

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Abstract With rice as experimental material, the multi-strain solid-state fermentation method was used to study the preparation technology of taurine, and the analysis of variance was employed to determine the optimal process of taurine preparation as follows: fermentation time (72 h); amount of cysteinyl acid (2.5 g), amount of methionine (2.5 g); ratio of *Bacillus subtilis* YS-45 to yeast S-78 to *Aspergillus niger* PL-39 (1∶2∶1). Under the optimal process, the taurine content reached 117.8 mg/g.

Key words Taurine, Solid-state fermentation, *Bacillus subtilis*, Yeast, *Aspergillus niger*

1 Introduction

With the rapid development of China's feed industry, the price of conventional protein raw materials such as soybean meal and fish meal is rising, the cost of feed is increasing and the income of breeding is decreasing. How to rationally and efficiently use unconventional protein feed resources as substitute for conventional protein feed to reduce feed costs becomes a problem of market attention[1]. Rice protein as plant source protein has low price and balanced amino acid composition, but compared with fish meal, it lacks an important nutritional factor—taurine. For livestock, poultry and fish, taurine is conditionally essential amino acid, because it only synthesizes 30% to 40% of what physiology requires by itself, and it must be supplemented from food. Taurine can improve animal growth, reproductive performance, immunity feeding and other biological functions[2]. Studies have shown that the lack of taurine source protein diets can cause slow growth and pathological phenomena in fish such as green liver syndrome and hemolytic anemia[3]. Cysteinyl acid and methionine, as the precursor materials of taurine synthesis pathway, can effectively improve the content of taurine in rice peptide feed under the combined effect of *Bacillus subtilis*, yeast, *Aspergillus niger* multi-strain. With rice as experimental material, the multi-strain solid-state fermentation method was used to study the preparation technology of taurine.

2 Materials and methods

2.1 Materials

2.1.1 Strains. *Bacillus subtilis* YS-45, yeast S-78 and *Aspergillus niger* PL-39 were all provided by Microbiology Laboratory of Changsha University.

2.1.2 Raw materials. Rice, soybean meal and bran were commercially available.

2.1.3 Culture medium. Slant culture medium: potato juice was prepared according to the mass ratio of potato: water = 1∶4-6; after potato was boiled in water for 25 min - 35 min, the potato juice was obtained by filtration, and 2% glucose and 2% agar were added. Liquid activation culture medium: potato juice was prepared according to the mass ratio of potato: water = 1∶4 - 6; after potato was boiled in water for 25 min - 35 min, the potato juice was obtained by filtration, and 2% glucose was added. Fermentation culture medium: fermentation substrate was prepared according to the 35% water content; 10 g of rice, 5 g of soybean meal, 5 g of bran and a certain amount of cysteinyl acid and methionine were placed into 250 mL flask, respectively, sterilized for 20 min at 121 ℃; 8% subculturing was completed after cooling and it was fermented at 30 ℃.

2.1.4 Reagents. Anhydrous sodium acetate, triethylamine, glacial acetic acid, acetonitrile, phenyl isothiocyanate. The above reagents were of analytical grade.

2.1.5 Instrument. Shimadzu LC-6AD plus high performance liquid chromatograph.

2.2 Methods

2.2.1 Solid-state fermentation. 10 g of rice, 5 g of soybean meal, 5 g of bran and a certain amount of cysteinyl acid and methionine were sterilized and cooled, and *Bacillus subtilis*, yeast and *Aspergillus niger* were used for subculturing, respectively. The fermentation medium after subculturing was cultured in 30 ℃ constant temperature incubator, and the taurine content was determined after the fermentation process.

2.2.2 Taurine detection method. It was determined by HPLC method[4]. Solution A: 11.45 g of anhydrous sodium acetate (analytical grade) was dissolved in 800 mL double distilled water, and 100 mL of triethylamine was added to a constant volume of 1000 mL; pH was adjusted to 5.8 with glacial acetic acid, and 940 mL of the solution was added to 60 mL of acetonitrile; large particles were removed using 0.45μm film, and it was degassed with helium for HPLC analysis. Solution B: 600 mL of acetonitrile was diluted with double distilled water to 1000 mL, and prepared as 60% acetonitrile; it was degassed with helium for HPLC analysis. Derivatizing agent: phenyl isothiocyanate (PITC): acetonitrile: triethylamine: water = 1∶7∶1∶1. 30 g of prepared taurine-enriched rice feed was dissolved in 50 mL water, and supernatant was taken after being centrifuged. An equal volume of pro-

Received: February 11, 2017 Accepted: April 15, 2017

Supported by Changsha Municipal Bureau of Science and Technology (k1509012-21).

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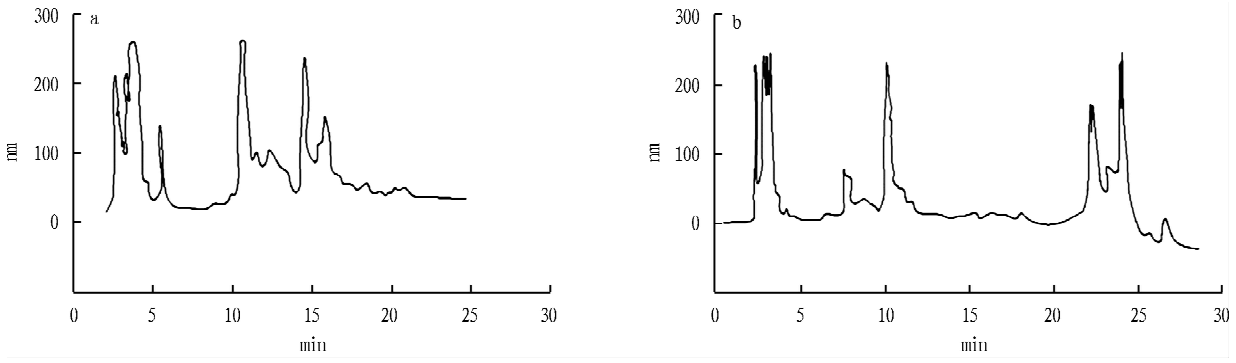
tein-removed trichloroacetic acid and defatted ether was lyophilized. 5 mg of lyophilized powder was dissolved in 100 μL water, and 300 μL of derivatized agent was added for 30 min of derivation, and then lyophilized. Using high performance liquid chromatography, the taurine was detected, and the liquid chromatogram of taurine standard substance was shown in Fig. 1 (a). Under optimized conditions, the liquid chromatogram of taurine in rice peptide feed was shown in Fig. 1 (b).



Note: (a) liquid chromatogram of taurine standard substance; (b) liquid chromatogram of taurine in rice peptide feed under optimized conditions.

Fig. 1 Taurine standard substance

3 Results and discussions

3.1 Fermentation time The culture medium was prepared according to **2.1.3**, and 2.5 g of cysteinyl acid and methionine (1∶1) were added, respectively. The inoculation ratio of *Bacillus subtilis* to yeast to *Aspergillus niger* was 1∶2∶1, and it was fermented under 30 ℃ culture conditions, to determine the content of taurine at different fermentation time. The results were shown in Fig. 2. From Fig. 2, it was found that in multi-strain fermentation process, taurine content gradually increased with time, peaked at 72 h and then began to decrease, so the mixed strains were chosen for 72 h of fermentation.

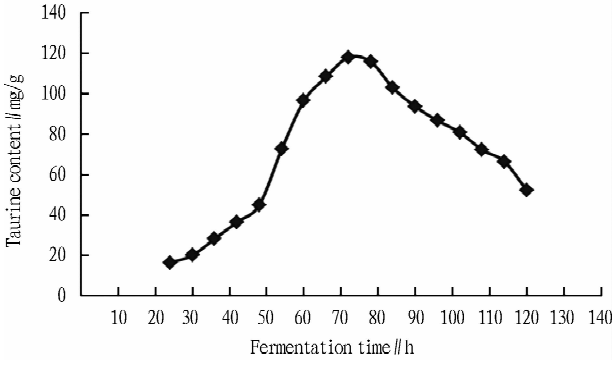


Fig. 2 Change in taurine content at different fermentation time in multi-strain fermentation

3.2 Addition of precursor Cysteinyl acid and methionine were regarded as precursors for taurine synthesis pathway, whose addition was important to increase of taurine content in rice peptide feed. Insufficient addition made it difficult to effectively improve taurine content; excessive addition would increase the cost of feed due to high price of cysteinyl acid and methionine, which was not conducive to widespread use. Fig. 3 showed that the optimum amount of cysteinyl acid and methionine was 2.5 g.

3.3 Effects of strain ratio This test used six different ratios of *Bacillus subtilis* to yeast to *Aspergillus niger* (1∶1∶1, 1∶2∶1, 1∶1∶2, 1∶2∶2, 2∶2∶1, 2∶1∶2) for fermentation, and Fig. 4 showed that 1∶2∶1 was the optimum mixing ratio, and the taurine content reached 117.8 mg/g under this ratio.

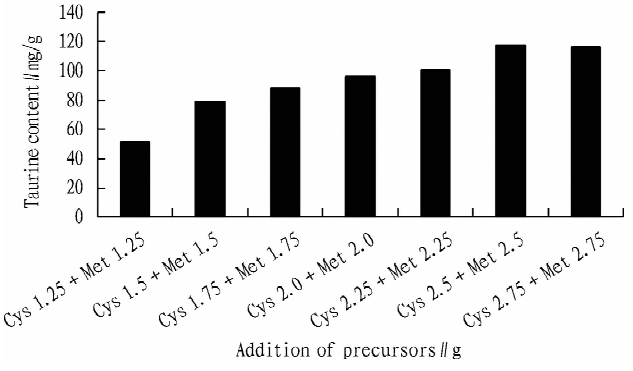


Fig. 3 Effect of addition of different precursors on taurine content

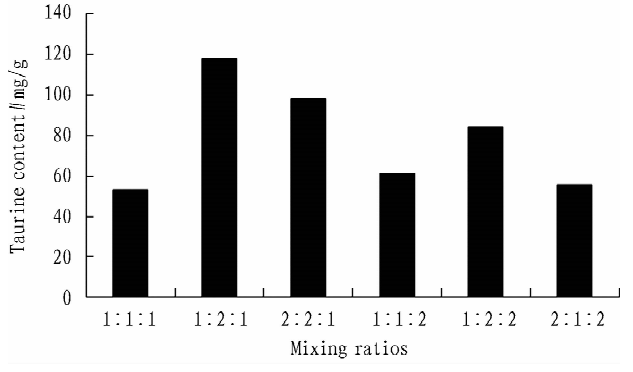


Fig. 4 Effect of different strains ratio on taurine content

4 Conclusions

By the single factor experiment on preparation of taurine in rice peptide feed, the optimal process of taurine preparation was determined as follows: fermentation time (72 h); amount of cysteinyl acid (2.5 g), amount of methionine (2.5 g); ratio of *Bacillus subtilis* YS-45 to yeast S-78 to *Aspergillus niger* PL-39 (1∶2∶1).

(To page 76)

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(From page 73)

Under the optimal process, the taurine content reached 117.8 mg/g. In the experiment, we first used cheap rice as material, and added the precursors cysteinyl acid and methionine for taurine synthesis. Under multi-strain combined action, the taurine content in peptide feed was greatly improved, which provided a good practical value for the wide application of plant-derived proteins in the feed industry.

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