INCREASE DISSOCIATIVE CALCIUM AND AMINO ACID NITROGEN IN SMASHED YAK BONE PASTE BY FERMENTATION OR ENZYMOLYSIS

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Introduction

China has more than 90% of all the Yaks in the world. In recent years the amount of yaks increased dramatically but a large number of yak bone was little utilized and discarded. Yak bone is a non-contaminated source for foods and pharmaceuticals with higher amount of calcium and nutrients. Accordingly yak bone may be processed into a better supplementary resource of calcium than other animals' bone can be. *Lactobacillus bulgarian* can generate nisin and lactic acid to prevent the growth of the intestine putrefying bacteria. Therefore the fermentation process of yak bone paste by *L. bulgarian* would combine the virtues of *L. bulgarian* and the high amount of calcium in yak bone paste, and thus produce health foods and food additives of high quality.

In our study, the smashed yak bone paste of abundant protein and suitable ratio of calcium to phosphorus was subjected to the fermentation by *L. bulgarian* or enzymolysis. The time course for the quantitative development of dissociative calcium (Ca^{2+}), amino acid nitrogen and pH value during the treatment was followed. The influence of two concentrations of bone paste (10% and 20%), and the amount of dissociative calcium, amino acid nitrogen and pH value were compared between fermentation and enzymolysis. Hence a rapid and efficient method of increasing dissociative calcium in yak bone was explored.

Materials and Methods

The Preparation of Smashed Yak Bone Paste. The preparation of smashed yak bone paste was modified from Xia et al. (2005). Hair, blood, meat, fat, ligaments and periosteum were removed from yak bone. The bone was rewashed and frozen at -20°C for at least 12 hr before being smashed. Frozen bone was crushed and minced by bucker into the blocks of 5 - 10 mm. Some ice cubes were added into bone blocks to keep the low temperature during the grinding process. The coarse grinded bone paste was then smashed finely into bone paste preparation.

Enzymolysis of the Yak Bone Paste. Yak bone paste was diluted into 10% with distilled water, and then 0.03% trypsinase, dispase and caroid were added respectively. The treatment was incubated in the water bath at the optimal temperatures of 40°C, 55°C and 65°C for trypsinase, dispase and caroid respectively. The concentration of Ca^{2+}, amino acid nitrogen and pH value were measured at 2 hr and 4 hr of enzymolysis.

Fermentation of the Yak Bone Paste. L. bulgarian was inoculated into the sterilized MRS medium, and the culture was kept shaking at 140 r/min, 37°C for 12 hr. Yak bone paste was diluted into 10% and 20% respectively with distilled water, and 5% sucrose was added in. Then sterilized at 121°C for 30 min before being inoculated with *L. bulgarian* at the concentration of 1%. Shake all treatments at 140 r/min, 37°C. The concentration of Ca^{2+}, amino acid nitrogen and pH value were measured every 6 hr. At the end of the fermentation, microbiological plate count was performed to determine the quantity of the viable *L. bulgarian*.

Measurement of the Concentration of Ca^{2+}. The concentration of Ca^{2+} in the solution was measured by atomic absorption spectrophotometry according to National Standard of the People's Republic of China "Methods for determination of calcium in foods" (GB12398－90).

Measurement of the Concentration of Amino Acid Nitrogen. Measurement of the concentration of amino acid nitrogen by potentiometric titration was modified from "Food Analysis" (Hu, 1992). Five milliliter of the solution was sampled and put into the 150 ml beaker, and 45 ml distilled water was added under stirring. Standard natrium hydroxydatum solution of 0.01 mol/L was used to titrate the solution until the pH value reached 8.2 by pH meter. Five ml of formaldehyde was added and the titration was continued with 0.01 mol/L natrium hydroxydatum until the pH value reached 9.2 and recorded the volume of natrium hydroxydatum as V_1.For blank, 50 ml of distilled water was used to substitute the sample and the final volume was recorded as V_2.

Concentration of amino acid nitrogen（mg/100g）=（V_1-V_2）× C_{NaOH} × Dilution Factor

Statistic Analysis. The data were analyzed with SPSS statistical analysis software program, version 13.0 (SPSS Inc., USA). Paired-Samples T Test was performed to compare differences between means. Significance was defined at P <0.05.

Results and Discussion

The Change of the Concentration of Ca^{2+} and Amino Acid Nitrogen during the Fermentation. The concentration of Ca^{2+} and amino acid nitrogen of the bone paste increased dramatically as the fermentation time

increased (Figure 1). The concentration of Ca^{2+} in 10% bone paste fermented for 36 hr was about 90 times higher than the control, while for 20% bone paste, the increase was about 70 times higher than the control. The concentration of amino acid nitrogen in 10% bone paste was higher than that in 20% bone paste at any time point. Considering the dramatically increased amount of Ca^{2+} and amino acid nitrogen, due to the higher efficiency in 10% paste than that in 20% paste, it is cost-saving to use 10% but not 20% bone paste in the fermentation application. We compare our results with those in other animal's bone paste by previous studies. In our study the concentration of Ca^{2+} and amino acid nitrogen in the fermented yak bone paste increased to 2145.3 mg/100g and 233.4 mg/100g from 12.3 mg/100g and 117.7 mg/100g, but in the previous study on the fermented pig-bone paste, the concentration of Ca^{2+} and amino acid nitrogen increased to 120.3 mg/100g and 55.0 mg/100g from 5.0 mg/100g and 123.4 mg/100g (Xia et al., 2005). In a similar study by Gao et al.(2002) and Tang et al.(2002), the concentration of Ca^{2+} and amino acid nitrogen in the fermented yak bone paste was higher than that in other livestock's bone paste.

The Change of the Concentration of Ca^{2+} and Amino Acid Nitrogen during the Enzymolysis. After 4 hr of enzymolysis, the amount of Ca^{2+} increased from 23.3 mg/100g to 89.3 mg/100g by trypsinase, to 62.3 mg/100g by dispase and to 52.3 mg/100g by caroid respectively, while the amount of amino acid nitrogen increased from 111.2 mg/100g to 133.6 mg/100g, 147.6 mg/100g and 153.2 mg/100g respectively (Figure 2). Compared with the control, all the increases were significantly greater (P <0.05).

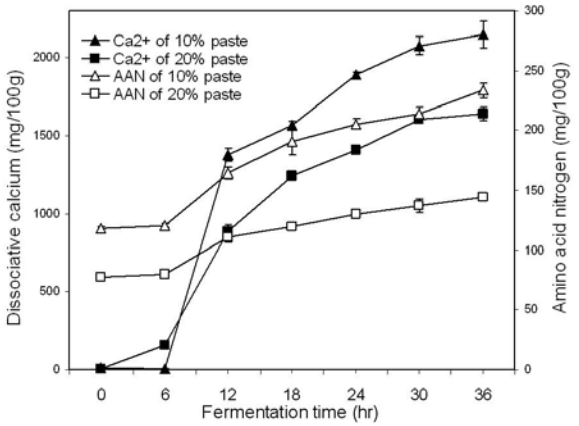

Figure 1. The change of the concentration of Ca^{2+} and amino acid nitrogen during the fermentation of yak bone paste

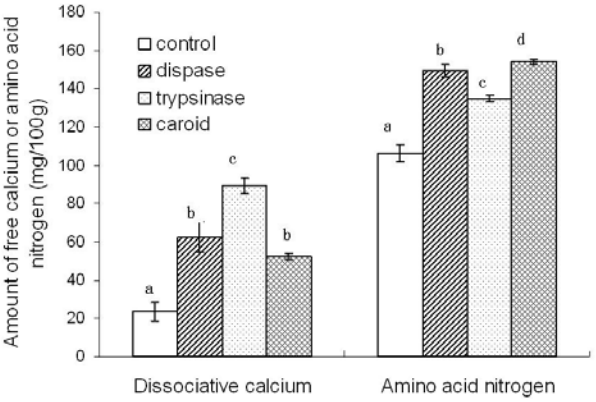

Figure 2. The change of the concentration of Ca^{2+} and amino acid nitrogen during the enzymolysis of the yak bone paste for 4 hours.

The Change of pH Value during the Fermentation. The pH value of bone paste dropped sharply from 8.2 to 4.5 during the first 6 hr of fermentation, and then kept decreasing slowly and finally maintained at about 3.9. The results from microbiological plate count of the fermented solution showed that the number of *L. bulgarian* in either 10% or 20% bone paste was about 5.5×10^6 CFU/ml. So there was a large amount of viable *L. bulgarian* in the bone paste after the fermentation, which could improve the gastro intestinal system, was reproduced during the fermentation of yak bone paste.

Conclusions

Our results prove that the fermentation process is much more effective to increase the amount of free calcium and amino acid nitrogen than enzymolysis process. Different concentrations of the bone paste caused the amount of Ca^{2+} and amino acid nitrogen to increase at different scales. In our experimental condition, 10% bone paste was utilized more effectively by *L. bulgarian* than 20% of bone paste. Our study is the most effective one among all the similar studies published so far and thus hold a good potential for application in the food industry.

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