Fermentation of Sweet Corn Produced A Very High Concentration of Vitamin K_2

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INTRODUCTION

Corn is one of the world's major cereal crops, with a production volume comparable to that of wheat and rice.

AIM

In this study, we report on a fermentation test using Bacillus subtilis that showed high production of vitamin K_2, which is used as a preventive drug for osteoporosis, as well as thrombolytic activity.

METHOD

Whole kernel corn from Meidi-ya (Tokyo, Japan) was used as the corn, and two types of *Bacillus subtilis*, M and N, cultured in the laboratory, were used.

Fibrin plate

Thrombolytic activity was measured by using the fibrin plate method. Amounts of 10ml of 0.5% fibrinogen solution (0.17M of borate-saline buffer: pH7.8) and 500µl of 50U/ml thrombin were used to prepare fibrin plates, where 30µl of each specimen was placed. The solution was pre-incubated at 37ºC for 24hr and then the lysis area formed was measured (mm^2).

Vitamin K_2

For vitamin K_2, the following procedures were carried out according to the method previously reported. HPLC and an ODS column (4.6×250mm) were used to operate the system at an excitation wavelength of 320nm and fluorescent wavelength of 430nm through the reduction fluorescent coloring method with a platinum-alumina catalyst column.

Amidase activity

An amount of 0.8ml of BSB (0.17M of borate-saline buffer: pH7.8) was added to 0.1ml of nattokinase and underwent pre-incubation at 37ºC for 2min. An amount of 0.1ml of the synthetic substrate, conditioned for a final concentration of 5×10^{-4}M, was added to this mixture and incubation was performed at 37ºC for 250sec. The absorbance of the *p*NA generated was measured at 405nm with spectrophotometer.

RESULTS

Fermentation of sweet corn was performed with M bacteria at 37ºC for 48 hours. The results showed a very high concentration of vitamin K_2 (1,890µg/100g: dry weight).

The thrombolytic activity of the ferment was quite resistant to heat at 100-150ºC for 30 min, but the substrate I/substrate II was about 2.0 in resolution against the synthetic amide substrate, indicating that it was nattokinase.

N bacteria were also used for comparison under various conditions. The morphology was better puree, pH increased, and fermentation at 45ºC for 4-8 days showed a maximum vitamin K_2 value of 406.0µg/g (dry weight).

Fig.1 Thrombolytic activity

Sweet corn fermented product showed a strong thrombolytic activity on fibrin plate, upper: fermented corn, lower: control corn.

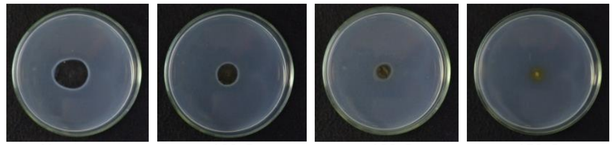

Fig.2 Thermal stability of nattokinase

Table Substrate specificity of nattokinase

Substrate	µmol/min/g
Bz-Ile-Glu-(OR)-Gly-Arg-*p*NA (I)	3440.46
Suc-Ala-Ala-Pro-Phe-*p*NA (II)	1775.72
MeO-Suc-Arg-Pro-Tyr-*p*NA	549.36
H-D-Ile-Pro-Arg-*p*NA	0.00
H-D-Val-Leu-Lys-*p*NA	0.00

Substrate concentration:5×10^{-4}M; 0.17M BSB (pH 7.8)

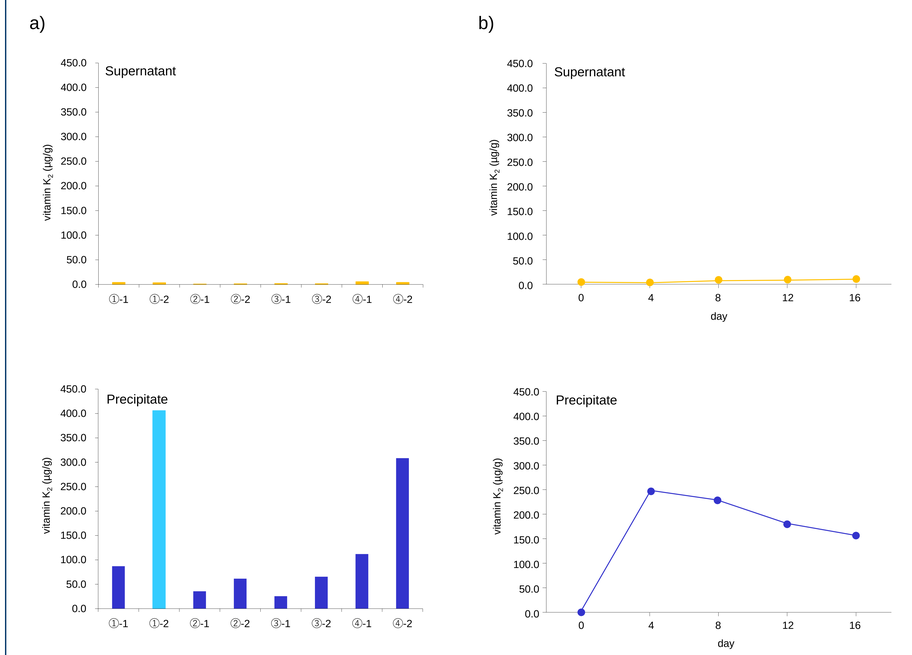

Fig.3 Vitamin K_2 content in fermented sweet corn

Bacillus subtilis (N bacteria) were used for comparison under various conditions.
a) After fermentation for 4 days at 45ºC, more than 99% of vitamin K_2 were observed in precipitate fraction.
-1, Whole mixture or -2, Puree was used for applied sample.
b) Maximum arise of vitamin K_2 were detected at 4-8 days, in contract 8-16 days gradually decreased.

CONCLUSIONS

The main feature of sweet corn fermented product is that it has less odor or stickiness compared to ordinary stringy natto. In addition, the thrombolytic activity of this ferment was extremely stable under heat.

The vitamin K_2 content of natto (wet weight) in 600µg/100g, the maximum value of vitamin K_2 was 40,600µg/100g (dry weight) of the corn fermented product is calculated to be several tens of times higher than that. This is equivalent to approximately 0.37g of our fermented product, based on the daily requirement of 150µg for adults, and is considered to be a very good functional food.

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