韓國營食誌 4(1) 7～12 1975 J. Korean Soc. Food Nutr. 4(1) 7～12 1975

Bacillus subtilis sporangiospore를 이용한 cheese 제조에 관한 연구

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Studies on manufacturing of cheese by using bacillus subtilis sporangiospore

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개 요

본 연구에서는 지금까지 대용 응유 효소로써 제시된 미생물 중에서 실용화되지 못하였던 bacillus subtilis를 분리 배양하여 sporangiospore의 상태로 cheese를 제조하여 봄으로써 응유작용의 일반적 성질을 natural rennen 및 bacillus subtilis enzyme과 비교 검토하였고 cheese의 제조법 숙성도 등에 관한 예비 실험을 하였다.[1)～4)]

Introdution

With the developing of dairy farming, the processing of milk is really needed and in many western cuntries, cheese is manufactured as not only a food of taste but also a chief food, therefore in our country home production is deeply needed. In manufacturing cheese it is only calf rennen that is used in the milk-clotting enzyme. So the study on developing new milk clotting enzymes is in progress in the world. During the past 20 years, studies on developing a substitute milk-clotting enzyme have been progressing by the search of zoologists, botanists and microbiologists in several countries. Until now, hog pepsin[5), 6)] developed by the zoologists has been known as a good substitute milk-clotting enzyme and such enzymes as chymotrysin have been reported to be good also.

In the aspect of botany milk-clotting enzymes from plants, such as ficin[8), 9)] in juices of ficas carica the fruit extracts of withania coagulans,[5)] as well as papain of carica papaya,[10)] strebulus kasper[5)] in cxnara carduncolus' flower, and enzymes in pumpkin[11)] have been reported.

And it has been reported that great quantities of milkclotting enzymes have been produced by microorganisms such as Aspergillus oryzae,[12] Mucor rouxii [13] Serrtia marcescens, [14] Bacillus-subtilis, [15]~[17] Endothia parasitica, [18] Mucor pusillus, [16] Rhizopus candidus, [16] Streptomycesalbus, [16] and Basidiomycetes [19] etc.

However, the necessary conditions of on effective milk-clotting enzyme are : (1) clotting activity as great as calf rennen and little degradation of protein, so that the yield of curd is not decreased in manufacturing cheese. (2) cheese manufactured must not have an unpleasant smell or taste.

In consideration of these points, only hog pepsin and mucor pusillus Var. Livdt among many substitute clotting enzymes from animals, plants, microorganisms are known to be promising substitute clotting enzymes, the majority of reported milk-clotting enzyme are not being utilized. So this study has, as its purpose, a preliminary examination of the method of manufacturing cheese, comparing two milk-clotting enzymes, Bacillus subtilis sporangiospore derived from the isolation and cultivation of bacillus subtilis, and calf rennen.

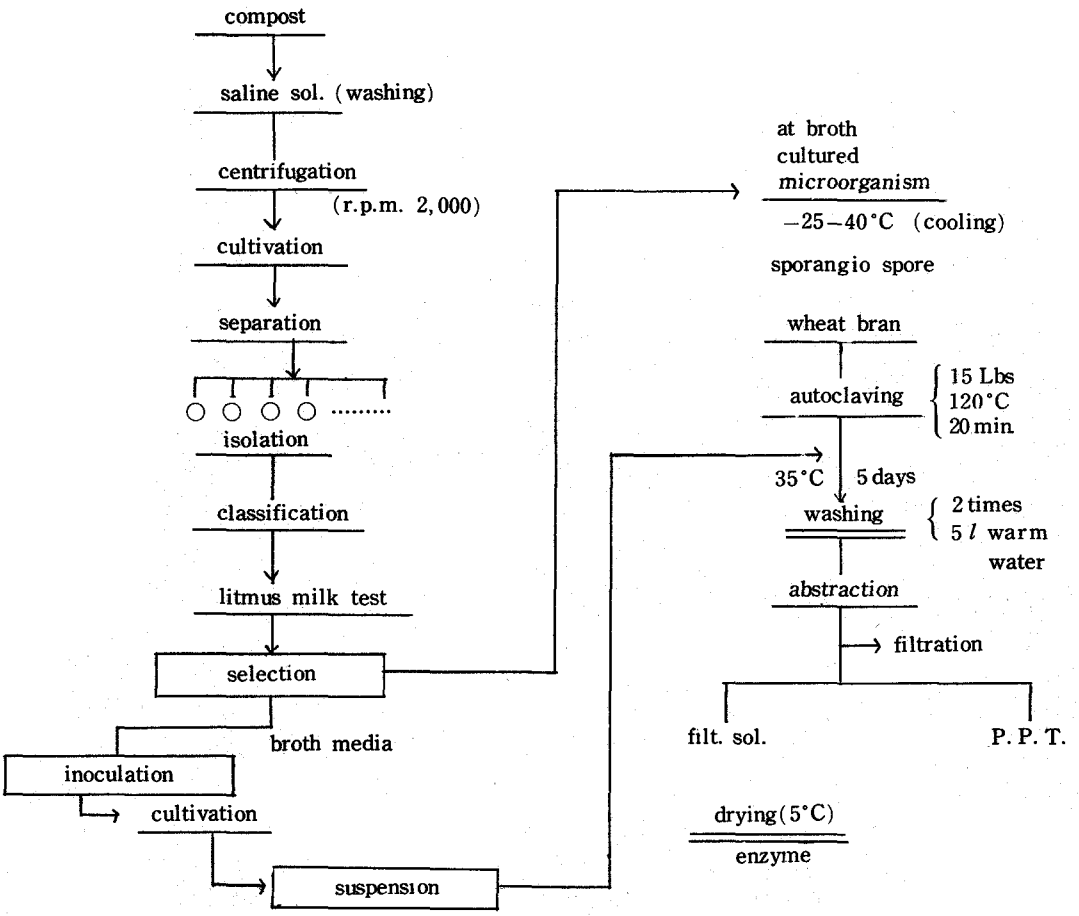

Fig. 1 Isolation of the microorganism producing milk-clotting enzyme

Experimental sample and method

1. sample

Milk used in all experiments was produced in Jeon Buk University meadow.

Milk-clotting enzyme were (1) calf rennen(CR) (2) Bacillus subtilis(B S) (3) Sporangiospore form of Bacillus subtilis(BSS) (4) Bacillus subtilis sporangiospore and calf rennen mixture (BSS+CR).

2. method

(1) Method of cultivation of starter

① 500 m*l* milk in flask was sterilized at 65°C for 30 min.

② It was cooled at 18°−30°C and then 1% (for milk) good lactobacillus (streptococus lactis : streptococus cremoris = 2 : 1) was innoculated, and then was standing at 18°C−30°C for 24 hours.

③ The coagulated milk was then cooled at 1−5°C and used for germ bacillus starter.

④ Then, milk containing 2% active starter was innoculated in sterilized milk. (A greater percentage of starter could have been used.)

The best acidity of germ starter was found to be 70°−80°D

(2) The process of isolation and cultivation of microorganisms by which milk-clotting enzyme were produced was as follows.

(3) Method of measurement of the activity of milk-clotting enzyme (22) (per unit gram)

① To 5 m*l* in a test tube added 0.01 M $CaCl_2$ and left standing at 30°C in a thermostat.

② When enzyme fluid (Min mg) at 30°C was added, a white line would appear between the glass rod and the test tube filled with red ink.

The time was them measured precisely. ($\frac{I}{M.T}$)

(4) Method of measurement of acidity.

Table 1. Processing of cheddar cheese

process	time	temp.	acidity	remarks
milk				
pasteurization	20 min.	65°C		$CaCl_2$ 0.01%
starter		30°C		2% to raw milk
fermentation	20 min.			(streptococcus lactis
1 kg/0.1 g 0.00		31°C	22°D	streptococcus cremolis)
cutting	40 min.		15°D	
Heating	20 min.			
filtration	2 hour	39°C	23°D	heating for 30 min.
pressing	30 min.	30°C	24°D	standing for 1.5 hour.
pressing			42°D	
salting	3 hour	20°C		22 hours. in saturated NaCl sol.
ageing	22 hour	10°C		cheddar 6 month.
		90 % (humidity)		soft 3 weeks

This was taken by the dornic method.

$$(D=\frac{0.1\,N-NaOH(ml)\times 0.009}{sample\,(ml)\times S.G.}\times 100)$$

(5) Measurement of degree of ageing of cheese.

① Total nitrogen sample (cheese) of 10 g was measured by micro kjeldahl method.

② Soluble nitrogen

After sample of 10 g was pulverised with 20 g sand in mortar, it was dissolved by 70 m*l* distilled water (70°C) and 3~4 drops 40% formaldehyde were added and filterated. Total volume of filterate solution was taken from the 100 m*l*, soluble nitrogen was measured by the micro kjeldahl method, and was calculated as follows.

$$\text{degree of ageing}\,(\%)=\frac{\text{solublen of cheese}\,(\%)}{\text{total N of cheese}\,(\%)}\times 100$$

(6) Process of manufacture of cheddar cheese. Process of manufacture of cheese was follows.

Results

(1) Activity of milk clotting enzyme.

CR : BS : BSS : BSS + CR = 1 : 0.4 : 0.8 : 1.1

Specially, strong activity of mixture is thought to represent the ascent phenomenon produced by the coenzyme reaction.

(2) Effect of amount of $CaCl_2$ added to milk-clotting activity was as follows.

Table 2. Effect of amount of $CaCl_2$ added to milk on milk-clotting activity of calf rennet (CR), B. subtilis enzyme (BS), B. subtilis sporangiospore (BSS) & CR + BSS.

amount of $CaCl_2$ add. to 5 m*l* of milk (mg) \ item	milk clotting activity (%)			
	CR	BS	BSS	CR + BSS
non	100	100	100	100
1.21	355	272	216	340
3.45	720	453	545	794
5.75	1,050	897	1,210	1,140
12.05	1,555	1,442	1,560	1,610
15.54	2,047	1,720	1,940	2,150

The more the quantity of $CaCl_2$ was increased, the activity of milk clotting (practically speaking, if the quantity of $CaCl_2$ is over 0.02%, its taste is bitter). There fore 0.01% $CaCl_2$ is best.

(3) Effect of amount of milk-clotting enzyme on clotting time was as follows.

Generally, milk-clotting time is on inverse proportion to the amount of the milk-clotting enzyme. However, CR + BSS mixture was sensitive compared with the other enzymes.

(4) Effect of temperature on milk-clotting activity was the as follows.

Clotting activity of CR was greatest at 45°C, the other enzymes increased as a rise of temp-

erature with 30°~50°C range but if temperature was raised over 50°C, they decomposed.

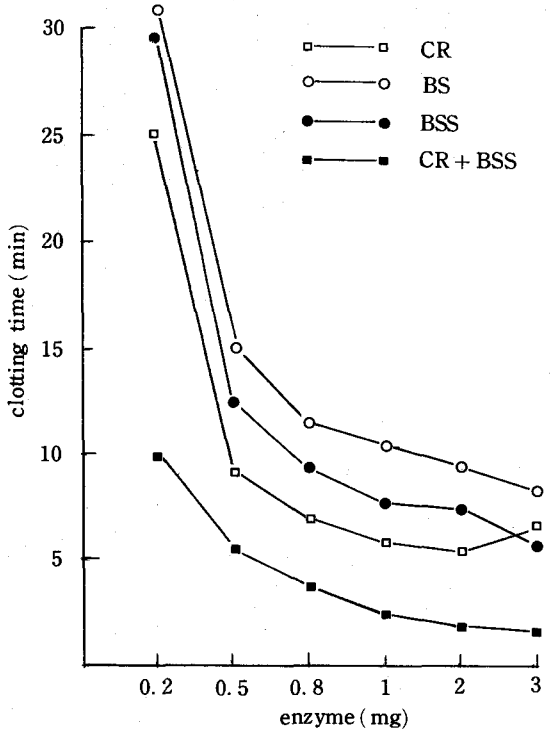

Fig. 2 Effect of amount of milk-clotting enzyme on clotting time (temp. 30°C)

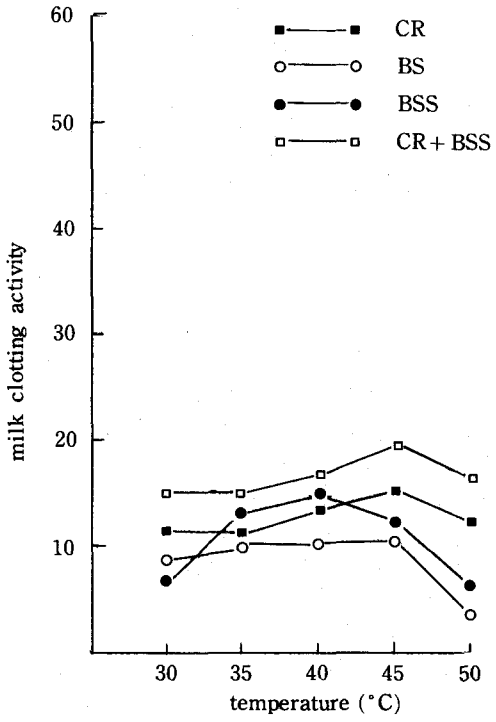

Fig. 3 Effect of temperature on milk-clotting activity of calf rennet (CR), **bacillus subtilis enzyme** (BS), **BS sporangio spore** (BSS) & **CR+BSS**

Surveys

The characteristics of this experiment is that cheese was manufactured by using a form of sporangiospore of enzyme expecting to produce strong enzyme activity during ageing. It has been known that a form of sporangiospore had stronger activity than other forms of enzymes.

So, if experiments using sporangiospore forms were applied to the various microorganisms producing clotting enzymes that have been tested to clote(Aspergillus oryzae, Mucor rouxii, Serratia marcescens, Rhizopus candidus, Streptomyces albus, Basidiomycetes, etc.) it is expected that stronger substitute to clotting enzymes can be developed.

The problem in this experiment is that the cheese was manufactured by using bacillus subtilis sporangiospore and could not be stored for a long time because sporangiospore in the stored cheese was increasing continuously. Also in its tissue, cheddar cheese manufactured by using BSS is not solid state in comparison with cheese manufactured by using calf rennen, but soft (camenbert) cheese has the advantages of being pleasant to Korean peoples' taste and of being able to be eaten as soon as it ages.

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