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The Microstructures of Soybean Milk Curds Prepared by Different Coagulation Methods

-Research note-

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Abstract

The microstructures of soybean milk curds, prepared by different coagulation methods, were observed by the scanning electron microscope. The curd coagulated by the addition of bacterial soybean milk clotting enzyme showed little textural changes and gave smoother gel than those prepared either by lactic acid fermentation using *Streptococcus thermophilus* or by the addition of $CaSO_4$. The curds obtained by lactic acid fermentation and by the addition of inorganic salt exhibited three dimensional network structure which indicated harder gel than that prepared by soybean milk clotting enzyme.

Key words: microstructure, soybean milk curd

INTRODUCTION

For the manufacture of cheese-like products made with soybean milk, curd formation is prerequisite. There have been several studies concerning curd formation(1-7). Recently, it is well known that some proteolytic enzymes, e.g., ficin, bromelain, are able to coagulate soybean milk (8-10). Fuke and Matsuoka.(11) reported for the first time that lactic acid fermentation and addition of stem bromelain resulted in good quality curd. Later, Murata et al.(12, 13) stated that three kinds of proteinases, namely, proteinases of microbial, plant and animal origin, were capable of clotting soybean milk protein and proteinases originated from microorganisms were suitable for making soybean milk curd on an industrial scale. Also, among soybean milk curds prepared by using three kinds of proteinases(subtilisin, thermolysin and bromelain), the curd with thermolysin was more suitable than the others with respect to proteolysis and texture(smoothness)(14).

The microstructure of soybean protein curd was first observed by Saio and Watanabe.(15). Later, Lee and Rha. (16) also reported structural differences of soybean protein aggregates. From the previous studies(17-19), we reported isolation and identification of microorganisms from soil samples that produce extracellular soybean milk clotting enzyme, and also determination of suitable culture condition and medium for the production enzyme were investigated. In this paper, the microstructures of soybean milk curds, prepared either by the addition of soybean milk clotting enzyme, inorganic coagulant or by lactic acid fermentation, were compared by using scanning electron microscope.

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MATERIALS AND METHODS

Preparation of soybean milk

Soybean milk sample was prepared by soaking soybean in water at room temperature for 24h, before adding about 9 times(w/w) of water to each swollen soybean. The soybean was then homogenized for 5min by a blender. The slurry was filtrated through a gauze. The filtrate was used as the soybean milk after heating at 100°C for 10min.

Preparation of soybean milk clotting enzyme

Bacillus licheniformis strain 192, one of the soybean clotting enzyme producers reported earlier(17,18), was grown in optimum culture condition and medium. The culture broth was centrifuged at 11,000×g for 20min at 4°C to remove insoluble materials including the cells. Ammonium sulfate was added gently at 80% final saturation, precipitate was collected by centrifugation(9,000×g for 20min), and was dissolved in small amount of distilled water. Dialysis was carried(molecular weight cut off 14,000) in 0.01M potassium phosphate buffer(pH 6.1) at

4°C for 24h, and resulting dialysate was used as a crude enzyme solution.

Preparation of soybean milk curd

Soybean milk curds were prepared by three different methods described as follows :

Addition of soybean milk clotting enzyme

Ten ml of the crude enzyme solution containing 392 units was added to 300ml of soybean milk, adjusted to pH 6.1 with 1M potassium phosphate buffer(pH 4.5), and was incubated at 65°C for 15min.

Addition of calcium sulfate

Calcium sulfate was added to 500ml of soybean milk to the final concentration of 2%, and incubated at 80°C for 20min.

Lactic acid fermentation

Streptococcus thermophilus was added at 5% inoculum size to soybean milk and fermentation was carried at 35°C for 24h.

Scanning electron microscopic analysis

Freeze dried curds, obtained by three different treatments, were observed under electron microscope(DS-130, Akashi Co., Japan) at an acceleration voltage of 19KV without fixation, and the magnification ratios were 500 and 2000×, respectively.

RESULTS AND DISCUSSION

Scanning electron microscopic structures

The scanning electron microphotographs showing the microstructure of three kinds of the curds prepared by the methods of soymilk clotting enzyme addition, calcium sulfate addition and lactic acid fermentation, are presented in Fig. 1 and 2.

Among these soymilk curd, the curd prepared by the addition of enzyme shows less textural change as shown in Fig. 1, and gave smoother gel than those prepared either by lactic acid fermentation or by addition of calcium sulfate.

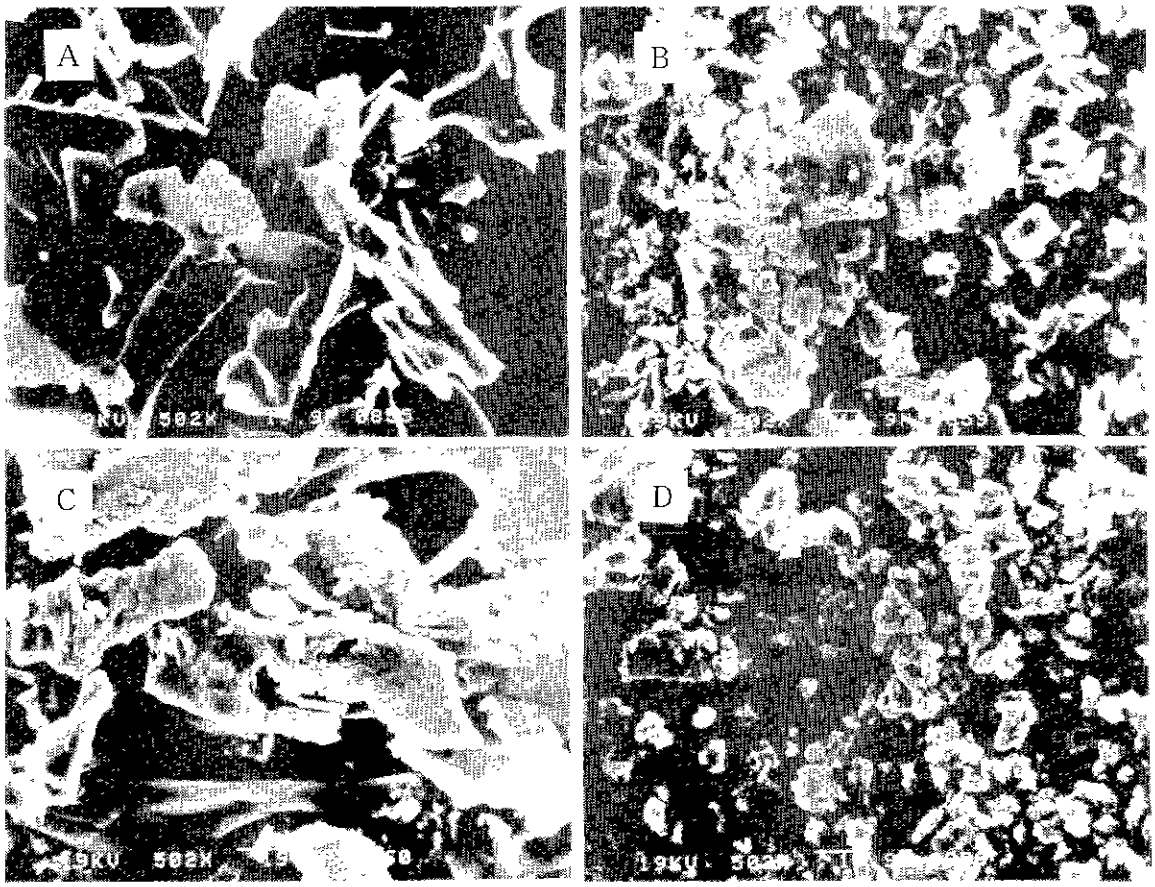

Fig. 1. Scanning electron micrographs of soybean protein curds(500×).
A, Control; B, Curd formed by soymilk clotting enzyme; C, Curd formed by lactic acid fermentation of *Streptococcus thermophilus*; D, Curd formed by inorganic salt($CaSO_4$)

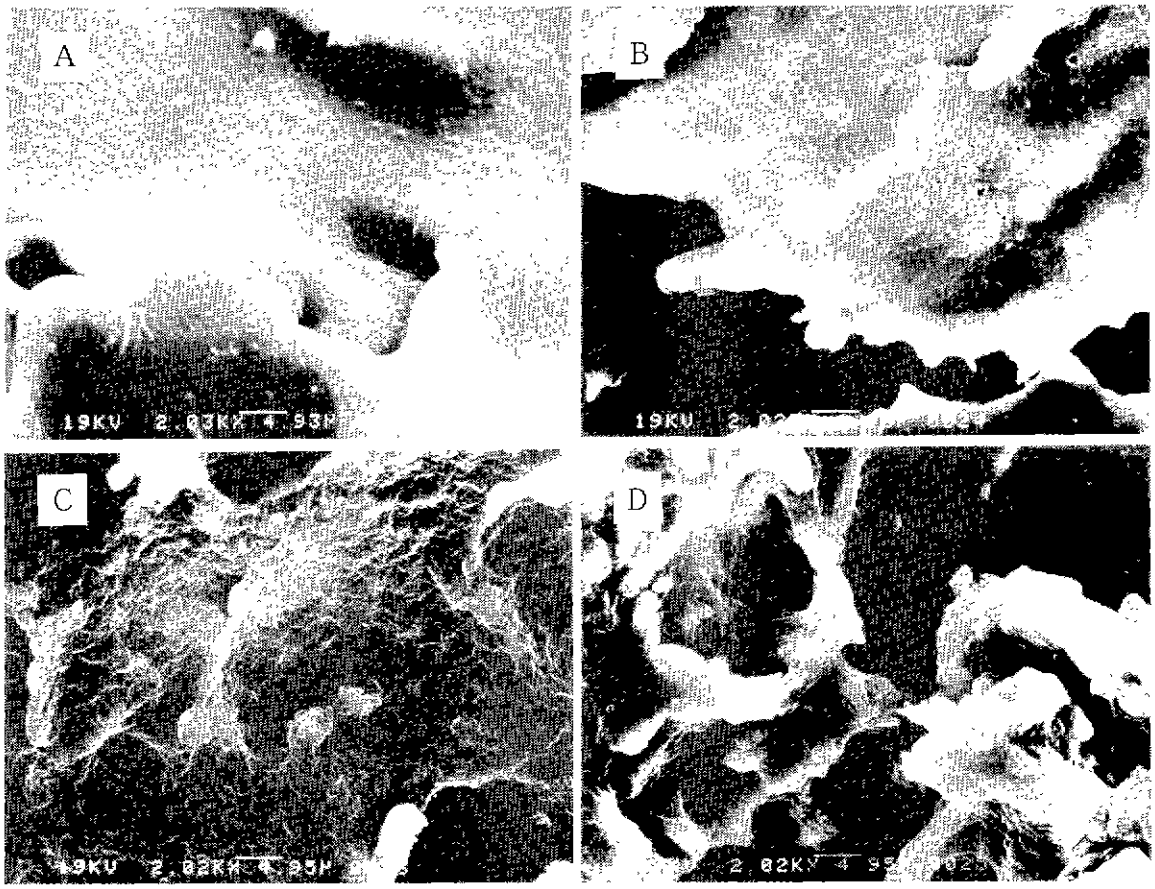

Fig. 2. Scanning electron micrographs of soybean protein curds(2,000×).
A, Control; B, Curd formed by soymilk clotting enzyme; C, Curd formed by lactic acid fermentation of *Streptococcus thermophulus*; D, Curd formed by inorganic salt($CaSO_4$)

Under a higher magnification(2,000×), the curds prepared by lactic acid fermentation and addition of calcium sulfate, show three dimensional network structures derived from destructed soymilk protein, meanwhile the curd prepared by addition of the soymilk clotting enzyme shows sheeted structure of the native soymilk protein like the untreated soymilk, as shown in Fig 2. These indicate that the gels formed by addition of calcium sulfate and lactic acid fermentation are harder than the one prepared by soymilk clotting enzyme.

The soymilk curd by lactic acid fermentation is coagulated by isoelectric precipitation. At the isoelectric point, the electrostatic repulsion force between the soymilk proteins is minimum. On the other hand, formation of soymilk protein calcium complexs by calcium addition appear to be due to the cross-linkings between soymilk proteins and calcium ions. Appurao and Narasinga(20,21) suggested that the probable calcium binding site on the protein molecules was the imidazole group of histidine.

The difference in the microstructure among these soymilk curd appear to be due to the different types of the interactions within soymilk proteins such as the disulfide bonds(22-24), hydrogen bonds, ionic bonds, covalent forces, van der Waals forces and hydrophobicity (25). Catsimpoolas and Meyer(26-28) suggested that heat-gelling of soy proteins is accompanied by hydrogen bond formation.

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