Stimulatory Effect of Shiitake Mushroom Extract on Curd Formation of Acid Milk by Lactic Acid Fermentation

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Abstract Shiitake mushroom extract, obtained from industrial wastes of dry shiitake, was found to reduce the coagulation time of skim milk culture media by stimulating the bacterial growth of *Lactobacillus delbrueckii* subsp. *bulgaricus* B-5 b incubated at temperatures from 25 to 45°C. It was observed that the stimulatory effect depended on the incubation temperature, being this effect stronger at 35°C. The faster acidification of the culture media led to a rapid aggregation of casein with the formation of coarser gels. These samples showed great propensity to spontaneous syneresis, and the effect was intensified as incubation temperature increased.

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Key words : Shiitake mushroom extract, Acid milk curd, Coagulation time, Syneresis

Milk gel formation, as a result of fermentation or acidification, is very important in the manufacture of dairy products, and factors affecting the quality of the acid milk curd used as a raw material for products such as fresh cheese or fermented milk need to be considered in order to manufacture dairy foods of a higher quality^{2,4)}.

On the other hand, one of the most popular mushrooms found in the Japanese diet is shiitake mushroom, *Lentinus edodes*, which is widely produced in Miyazaki Prefecture. However, only product of high quality is accepted from the plantation sites, and the large amount of dry mushroom flakes discarded represents a considerable source of contamination for the environment. Recently, some valuable cooked foods have been manufactured using a

lower quality shiitake mushroom and on the process, a water extract is obtained from the rehydration step of the dry mushroom¹⁾. Although this extract has been used as a flavor additive, it apparently contains many physiologically active substances that might find practical application in other fields⁷⁾. However, little has been reported in such respect.

In the present paper, our objective was to observe the stimulatory effect of shiitake mushroom extract on the curdling and syneresis of skim milk culture media incubated with *Lactobacillus delbrueckii* subsp. *bulgaricus* B-5 b at different temperatures.

乳酸発酵によるミルク酸カード形成に対する椎茸エキスの促進効果：バルガス マイノル・大橋登美男（宮崎大学農学部，宮崎市 889-21）

Materials and Methods

Preparation of shiitake mushroom extract

One volume of dry shiitake mushroom graded low-quality was soaked in about 10 volumes of water at room temperature (15–20°C) during 13 to 15 hr. The supernatant obtained was filtered, concentrated and pasteurized by heating to 95°C. The general composition of the shiitake mushroom extract was determined in the conventional way^{6,10}. The extract contained 57.7% water, 2.52% total N, 9.60% total sugars, 6.76% ash, 450 mg % P and 31 mg % Ca.

Preparation of milk curd samples

Portions of 1% shiitake mushroom extract were added to 20% reconstituted skim milk media, previously inoculated with 2% of a fresh culture starter of *Lactobacillus delbrueckii* subsp. *bulgaricus* B-5 b, and kept at 5°C before use within 1 hr. Control samples were run parallel without the addition of the extract and acidities of the acid milk samples were determined every 3 hr during 24 hr, as described elsewhere¹¹. The general composition of the skim milk culture was determined according to the normal procedures^{6,11}. The contents of moisture, whole protein, lactose, ash, P and Ca were 80.8%, 7.27%, 7.30%, 1.45%, 226 mg % and 261 mg %, respectively.

Measurement of clotting times and syneresis

Clotting times were determined visually by observing the changes of the substrate in the flask, i.e., the appearance of a heterogeneous flow and of first traces of coagulum on the inner surface when the flask was gently tipped⁹.

On the measurement of syneresis, the curd formed in a beaker was retained by means of a wire grid placed on top of it. This enabled the whey to be poured off easily at time intervals for measurement of its volume⁸.

Results and Discussion

Fig. 1 shows the effect of the incubation temperature and incubation time on the formation of acid milk curd in 20% skim milk culture media, with and without the addition of shiitake mushroom extract.

Clotting times were decreased by increased temperature and the effect of the addition of the extract was considerable. At 15°C, the activity of the lactic acid bacteria was apparently too low for the samples to curdle, even in the presence of the extract. This effect was first noticed at 25°C, when a 7-hr difference in the coagulation time was observed. At higher incubation temperatures, this effect was less pronounced; 4 and 3-hr differences for samples incubated at 35 and 45°C, respectively.

The use of a rather high concentration of skim milk in the culture media seems to have been responsible for the absence of syneresis in the not-supplemented samples, even after an incubation period of 24 hr. However, when shiitake mushroom extract was added, syneresis took place at 15 and 12 hr, in samples incubated at 35 and 45°C, respectively, and around 15 and 50% of whey had separated from the curd after 24 hr of incubation, respectively.

Changes in acidity during the fermentation process at the different temperatures are shown in Fig. 2. According to Morichi⁹, and Smith *et al.*¹², these values can be used as an indirect measure of the growth of lactic acid bacteria in milk. In the absence of the extract, the lactic acid bacteria grew better at 45 than at 35°C. However, the stimulatory effect of the extract was apparently not the same at different temperatures; the growth stimulation became stronger as temperature increased from 15 to 35°C, but was less pronounced at 45°C.

By comparing Fig. 1 and Fig. 2, it could be deduced that at different incubation temperatures, clotting times not only were shortened by the presence of the extract, but also that for

Fig. 1. Effect of incubation temperature on curd formation and syneresis of skim milk samples.

□ not curdled □ curdled ■ syneresis

a given temperature, increasing the rate of gelation resulted in the onset of gelation at a slightly lower acidity value. At 35 and 45°C, not-supplemented samples clotted when acidity was approximately 0.58 and 0.63, but in its presence these values were about 0.53 and 0.45, respectively. In the not-supplemented samples the acidification of skim milk media took place more slowly, what probably gave more time to the casein network to form a firmer and more continuous gel with less tendency to undergo subsequent syneresis⁴⁾. In supplemented samples, the first signs of syneresis were detected with acidity values of 1.73 and 0.95, respectively, in contrast to the not-supplemented samples showing similar acidities.

Syneresis of curd results from mainly hydrophobic interactions within the casein

network, what makes the curd shrink and exert a pressure on the entrapped moisture forcing it out through the matrix pores, and any external factor that can reduce the hydrophobicity of the casein micelles, such as a temperature increase, would lead to a reduction in clotting times, and consequently to a rapid aggregation of casein with the formation of large dense aggregates which precipitate rather than form a gel^{2,4)}. This accorded with the observation that syneresis increased by raising the temperature, what increased the energy available for hydrophobic interactions, i.e., a reduction in repulsive forces among the casein micelles. Besides, a faster lactic fermentation promoted by the presence of the extract contributed to form a coarser network with a greater propensity to spontaneous syneresis.

Lactic Acid Fermentation by Shiitake Extract

Fig. 2. Effect of the addition of 1% shiitake mushroom extract on the lactic acid fermentation at various temperatures, expressed as the variation of acidity during a 24-hr incubation period.

(○), control samples ; (●), extract-added samples.

In this experiment, syneresis was strongly stimulated by the extract addition around the optimal incubation temperature range of *Lactobacillus delbrueckii* subsp. *bulgaricus* B-5 b⁹⁾. Depending on the final utilization of the curd obtained, separation of whey might become an undesirable property, and since higher incubation temperatures of fermented milks, all other factors being equal and provided starter

growth is not inhibited, promote coarser gel networks, it should be considered to incubate samples at lower temperatures and/or the use of a higher milk solids concentration¹³⁾. Many other factors may have influenced not only the final gel structure but also the gelation rate, the degree of structure at different pH values, and the pH at which gelation is initiated. So, a more detailed investigation should be done on

this respect.

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