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Viscosity and Proteolysis of Yoghurt Using Ropy Strains, in the Presence of Bifidobacteria, as Compared to Those of Soyoghurt

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ABSTRACT: Yoghurt from cowmilk (YVB) and soyoghurt from soymilk (YSB) were manufactrured by innoculations of mixed cultures using 2 or 1 ropy strains, in the presence of *Bifidobacterium subsp.* The first mixed culture was consisted of *Streptococcus thermophilus* St1, *Lactobacillus delbrueckii subsp. bulgarius* Lb5, both are ropy strains, and *Bifidobacterium subsp.* B 20. The second was consisted of the strains St. 3, Lb 6 (ropy strain), and B-20. Viscosity and proteolysis of products along the time of incubation have been detected in this study. Viscosity of soyoghurt's products were higher than those of yoghurt ($P<0.01$). The peak of viscosity of products were 411 (YSB 1), 301 (YSB 2), 235 (YVB 1), and 176 mPas (YVB 2), respectively. However, proteolysis of product using TNBS method as expressed in mMol eq. Gly/mL, were higher in yoghurt than in soyoghurt. We supposed that viscosity of products using 2 ropy strains were higher than those of 1 ropy strain and influenced by the type of protein coagulated in media. The liberation of free amino acids was influenced by the type of species of bacteria growth in media, in which *L. bulgaricus*, considered as the most proteolytic among lactic acid bacteria were relatively not growth in pure culture of soymilk.

Key Words: Yoghurt, Soyoghurt, Ropy Strains, Viscosity, Proteolysis.

Introduction

Yoghurt is a milk product fermented normally by *Lactobacillus delbrueckii subsp bulgaricus* and *Streptococcus thermophilus*, and originated in the Balkans and the middle East, is considered by many to be benefical for health.

Most of adults from many etnics (monggolit, negrito, jews, black american) have suffered from lactose intolerance due to lack on lactase enzyme (lactase deficient). When such person ingest products containing milk or lactose, flatulence, abdominal pain or diarrhea often develops. Theorically, the use of yoghurt as an autodigesting source of lactose (Kollars et al., 1984), give special benefits for the people whom lacked on lactase enzyme. Althought in areas of the world where lactase deficiency is prevalent, adults seldom ingest an appreciable quantity of unmodified milk, they may ingest large quantities of yoghurt or cultured milk.

In Asia (particularly in Indonesia), consumer market for dairy products included yoghurt are very limited. They are psychologically accustomed to vegetable products, like soymilk or other related products using soybean, such as témpé or tofu (*tahu*).

Overtime, the beneficial effect of yoghurt for health has been inchanged by the presence of *Bifidobacterium subsp. or Lactobacillus acidophilus*, as the microbes living indigenously in human intestinal (Mitsuoka, 1993; Murti, 1993; 1993a; Marteau et al., 1990). Few works have covered soymilk fermented by starters of yoghurt in cowmilk and soymilk by the development of lactic acid bacteria (LAB)-type ropy strains, normally used to prepare yoghurt, in the presence of *biifidobacteria*. Fermented milk using the ropy strains are usually from Scandinavians, such as villi, filmjolk. Viscosity and proteolysis of yoghurt and other related products play an important role in consumer's acceptability. Viscosity is one of the physical measure that influenced by the structure and consistency of products. While the second

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indicate indirectly one of four basic taste known i.e. bitterness of products, because bitternes of yoghurt has attributed from proteolytic activity of *L. bulgaricus* (Renz and Puhan, 1975). These two parametres have been detected in this study.

Materials and Methods

Strains of yoghurt bacteria, i.e.: *Streptococcus thermophilus* (St 1 & 3) and *Lactobacillus delbrueckii subsp. bulgaricus* (Lb 5 & 6) as well as *Bifidobacterium subsp.* (B 20) were isolated from commercial products. Strains ST 1, Lb 5 and Lb 6 are known as the ropy strains. The strains were tyhen stored at -30°C in litmus milk with no supplement or prior incubations, except for B 20, for which yeast extract at 5g/*l* was added. Powder skim milk from (Union Laitiere Normandie, France) and Powdered soymilk (Prolait, Niort, France) were reconstituted as 10% Dry Matter and heated at 100°C for 10 min, and stored after at 4°C one night before use.

The preculture of each pure strain was prepare at 9%, and incubated at 42°C, unless for the strain B 20 at 39°C. The preculture was conducted to reach 3 g equivalent of lactic acid/kg product, and this was the source for all of mixed cultures. They were manufactured in mixed cultures as below:

1. Strains St 1, Lb 5 and B-20 in cow milk referred as YVB1 and in soymilk as YSB1.
2. STrains St 3, Lb 6 and B 20 in cowmilk as YVB2 and in soymilk as YSB2.

The mixed cultures were innoculated at 5% (final volume) and incubated at 42°C, using the formule:

$$X + Y + Z = 0.5 \times V$$

V = Volume of cowmilk/soymilk to be innoculated, mL
X = Volume of streptococci's inoculum, mL
Y = Volume of lactobacilli's inoculum, mL
Z = Fixed volume

The acidity of products was measured in 9 mL of culture after adding 0.5 mL of a 1% solution of phenolphtalaein 95% alcohol, by titrating with 0.1 N NaOH. Acidity is expressed as g equivalent of lactic acid/kg of products. Total bacterial count were enumerated by direct microscopic counting after methylene blue staining according to the method of Breed (1991) and Thompson et al., 1978 in Murti et al., (1993c). Cells of bifidobacteria are typically swollen, irregular and branched, whil lactobacilli are long rods (3 to 4 times longer than bifidobacteria), in chains. Viscosity was measured with a Haake Rotovisco RV 2 with coaxial cylindres (MK 50 rotor) at 42°C and expressed in mPa s.

Proteolysis as expressed as free amino acids groups was detected by the method of McKellar (1981) as indicated in Murti (1993a). Triplicate 2 mL samples of culture was treated (precipated) with 0.72 N Tricholoro-acetic acid (TCS) for 29 min at 25°C. The supernatan was separated from the precipitate by centrifugation at 3,000 g, 15 min (Sorval instrument-Dupont). The supernatan was then filtered through Millipore 0.45 µm; 0.2 mL of this supernatant were mixed with 2 mL of 1 M potassium borate buffer (pH 9.2) and 0.8 mL of 5 mM Trinitrobenzene sulfonic acid (TNBS-Sigma) and incubated in the dark at 25°C. After 30 min, 0.8 ml of 2 M monobasic sodium phosphat containing 18 mM sodium sulfite was added in order to stop the reaction. The measure of protelysis was at 420 nm of absorbance. Absorbance were converted to mMol of free amino group/mL of culture by a standard curve using Glycine. Proteolysis is defined as the increase in the concentration of Tricholoro-acetic acid-soluble free amino groups/mL of culture.

Results and Discussions

The changes of acidity in products show that acidity increased in YVB 1 & 2 more rapidly than in YSB 1 & 2 (Fig. 1). The soymilk cultures with 2 or 1 ropy strains produced no more than 9 g of acid/kg of product, and relatively stable after 12 h incubation. The two fermented cowmilk, on the other hand, produced up to 15 g acid/kg of products (YVB 1) and about 13 g (YVB 2). The acidity of yoghurt was significantly higher than those of soyoghurt ($P<0.01$), especially after 4 hr. We found no difference acidity between fermented beverage using 2 ropy strains and 1 ropy strain in yoghurt nor in soyoghurt. According to Murti (1993c), acidity of soymilk-yoghurt (soyoghurt) was less than yoghurt and relativelly more acceptable for the consumers since their value did not exceed 9 g of lactic acid/kg product.

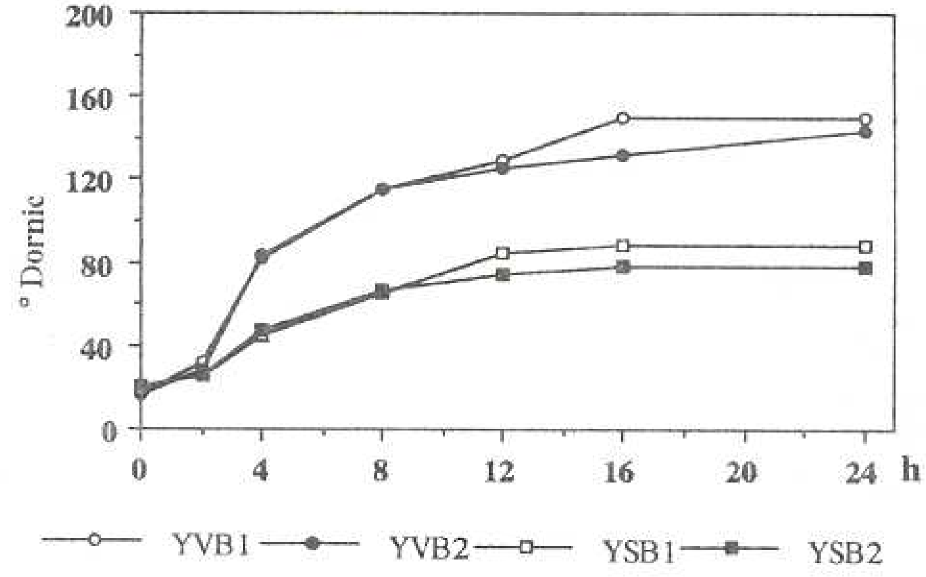

Figure 1. Activity of yoghurt (YVB 1&2) and soyoghurt (YSB 1&2)

The enumeration of yoghurt and soyobghurt with 2 ropy strains of bacteria indicated that the population of the two yoghurt's bacteria i.e., *S. thermophilus, L. delbrueckii subsp. bulgaricus* have reached maxima after 8 hr, at about 1 x 10^{10} bacteria/mL, and 1 x 10^{9} bacteria/mL respectively. (Fig. 2). In yoghurt the numbers of bifidobacteria has been stable at 5 x 10^{7} bacteria/mL. They have, however, well developed in soyoghurt, especially after 4 hr. *Lactobacillus delbrueckii subsp. bulgaricus* does not utilize sucrose and so cannot readily develop in soymilk as in pure culture, since sucrose is the major fermentable sugar (Mital and Steinkraus, 1979; Murti, 1993b). In yoghurt and soyoghurt, however, lactobacilli develop in synergy with *S. thermophilus* (buono, 1988; Murti, 1993c). For all of mixed cultures, lactobacilli were higher in yoghurt than in soyoghurt. In yoghurt, lactobacilli does not develop in competition with *Bifidobacterium subsp.*, since cow milk lack on an essential growth factor for bifidobacteria, such as N acetyl-D-glucosamine (Klaver and Kingma, 1989; Poch and Bezkorovainy, 1988) as found in soymilk (data nor published) or they cannot utilize glucose or galactose that were liberated from lactose by ß-galactosidase in two others bacteria. In soyoghurt, however, the presence of bifidobacteria give a deleterious role for lactobacilli as compared to those without the presence of bifidobacteria (Murti, 1993c). The numbers of streptococci in YVB 1 and YSB 1 were higher than those of YVB 2 and YSB 2. At the first, they were 3 times than in the second, especially after 12-16 hr, that was not the matter with lactobacilli. Bifidobacteria in YSB 1 were more numbers than in YSB 2, probably by higher release in such glucosamine or higher presence of carbon source readily use, such galactooligosaccharide (other polysaccharide), due to its capasity to hydrolyse a liasion α-1.6 (Sakai et al., 1987).

The viscosity of products using 2 ropy strains were higher than 1 ropy strain, especially after 4 hr, and in soyoghurt were higher than in yoghurt ($P<0.01$). The peak of viscosity of products were 411 (YSB 1), 301 (YSB 2), 235 (YVB 1) and 176 mPa s. (YVB 2), respectively. The values were stable after 4 hr. (Fig 4). Viscosity is one of the physical critere in consumers acceptance of this type of fermented products. The use of "ropy" strains ("epaississantes") could produce exocellulair polysaccharides that interact with casein and its cells increasing the viscosity of products (Teggats and Morris, 1990). Other than the production of exocellulair polysaccharide, the type of gelling of milk (soymilk) influence the value of viscosity. Viscosity of soyoghurt were higher than yoghurt probably due to the presence of protein globulin 7 S and 11 S in soymilk, by which the latest make a true gel with three dimensional network with covalent bonds, such as disulfide bond, rich in soymilk (Kohyama and Nishinari, 1993). While acidic coagulation of cowmilk are easy to be disrupted by stirring or flow due to its heterogenous distribution of casein (Arshad et al., 1993).

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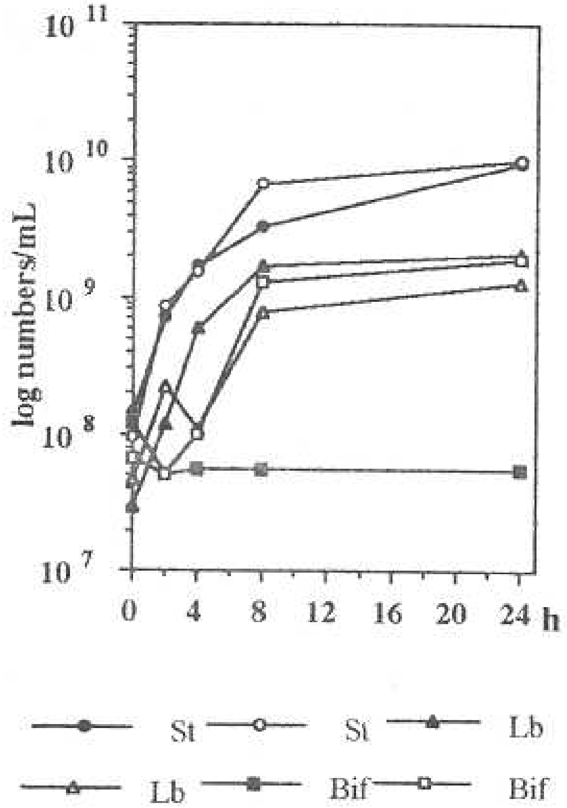

Figure 2. Bacterial counts/mL in yoghurt (blank) and soyoghurt (open) with 2 ropy strains

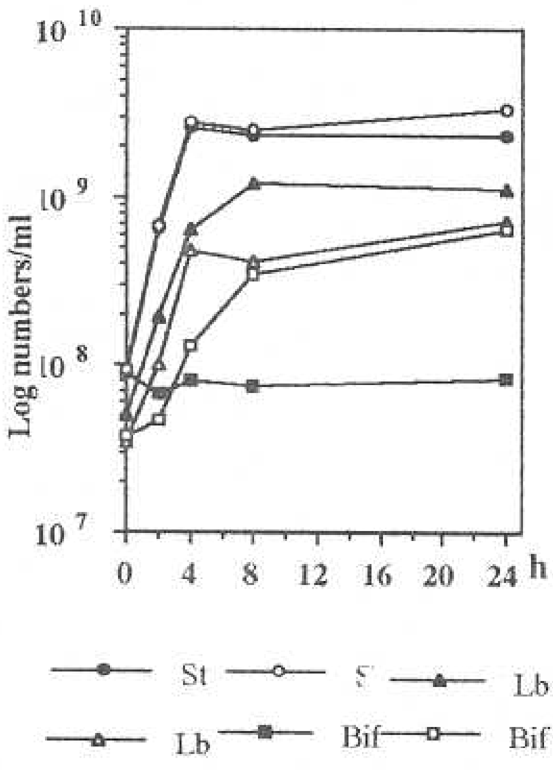

Figure 3. Bacterial counts/mL in yoghurt (blank) and soyoghurt (open) with 1 ropy strain

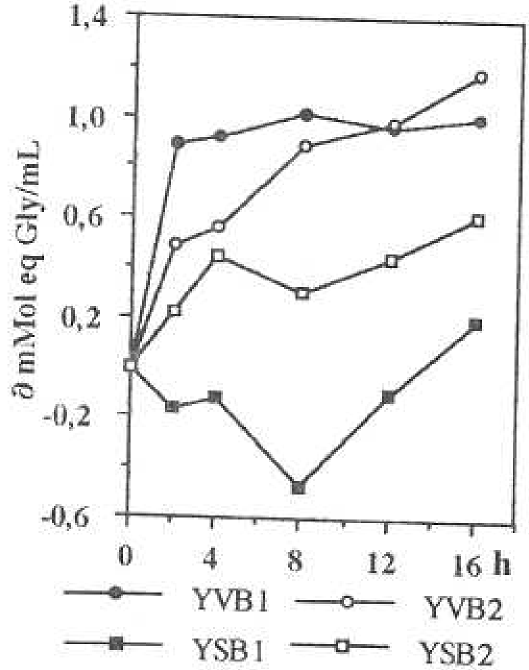

Figure 4. Viscosity of yoghurt and soyoghurt

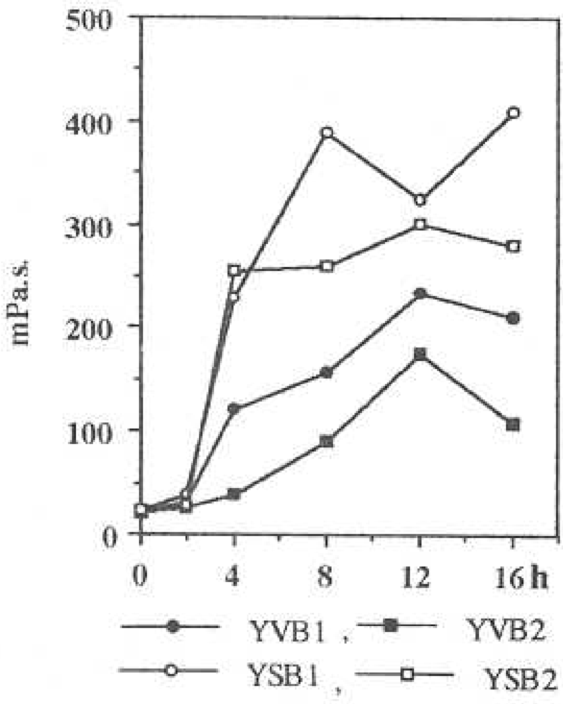

Figure 5. Proteolysis of yoghurt and soyoghurt

Proteolytic of products using the method of TNBS indicated that the release of free amino acid in yoghurt were higher than in soyoghurt ($P<0.01$) (Fig. 5). In YVB 1, the peak was earlier than YVB 2. In soyoghurt, however, product with 1 ropy strain have released free amino acids higher than that with 2 ropy strains, by which the latest has released only after 16 hr. For the two products of soyoghurt, we found proteolysis or consumption of free amino acids by the bacteria (Murti, 1993c). In mixed cultures with 3 bacteria, *S. thermophilus*, bifidobacteria and *L. bulgaricus*, we supposed that only lactobacilli have contributed to the release of free amino acid (Murti, 1993a). Rajagopal and Sandine (1990) said that in mixed cultures between *S. thermophilus* and *L. bulgaricus* have liberated more free amino acids than the sum of the individual culture. In soyoghurt with 2 ropy strains, however, the biomass increasing faster than the number of α-amino group released resulting in a decrease in proteolytic activity, probably for the profits of lactobacilli that developed faster in YSB 1 than in YSB 2, reached maxima at 1×10^9 bacteria/mL and 3×10^8 bacteria/mL, respectively.

Conclusion

Bifidobacteria could not develop in cowmilk, even in the presence of streptococci and lactobacilli, but they have well developed in soymilk giving less value of proteolytic activity. Its grows were more rapid in the presence of 2 other ropy strains than 1 ropy strain. Lactobacilli, however, the only one that have high activity of proteolytic, could growth in soymilk in the presence of other bacteria. Their growth in soymilk with 2 ropy strains were higher than with 1 ropy strain give theorically more free amino acids liberated. In fact, the lower value of proteolytic activity of soymilk with 2 ropy strains indicated that the growth other bacteria, especially bifidobacteria, were faster than the release of free amino acid. It seems that soyoghurt is more acceptable than yoghurt. But, we sould bear in mind that consummer acceptance depend also on flavor (Murti, 1993a) and the viscosity, by which fermented products with high value of viscosity probably not very good for the children, even the growth of bifidobacteria should theorically produce more L (+) lactic acid, good for children, than yoghurt.

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