Changes of raffinose and stachyose in soy milk fermentation by lactic acid bacteria from local fermented foods of Indonesian

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ABSTRACT

The objective of this study was to evaluate the fermentative characteristics of lactic acid bacteria isolated from local fermented foods and consume raffinose and stachyose during fermentation soymilk. *Lactobacillus plantarum pentosus SMN, 01, Lactobacillus casei subsp rhamnosus FNCC, 098*, *Lactobacillus casei subsp rhamnosus FNCC, 099, Streptococcus thermofilus, 001, Lactobacillus delbrueckii subsp. bulgaricus FNCC, 0045, Lactobacillus plantarum SMN, 25, and Lactobacillus plantarum pentosus FNCC, 235* exhibited variable α-galactosidase activity with *Lactobacillus plantarum SMN, 25,* showing the highest activity in MRS supplemented media. However, all organisms reached the desired therapeutic level (10^8 cfu/mL) likely due to their ability to metabolize oligosaccharides during fermentation in soymilk at 41 °C. The oligosaccharide metabolism depended on α-galactosidase activity. *Lactobacillus plantarum SMN, 25,* L. *plantarum pentosus SMN, 01* and *Lactobacillus plantarum pentosus FNCC, 235* reduced raffinose and stachyose by 81.5, 73.0, 67.0 %, and 78.0, 72.5, 66.0 % respectively in soymilk.

Keywords: Lactobacillus sp, fermentation soymilk, α-galactosidase, *raffinose, stachyose*
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INTRODUCTION

Soybeans (G*lycine max. L. Merr*) are a rich source of protein and economical protein food. Soy-based foods may provide a range of health benefits to consumers due to their hypolipidemic, anticholesterolemic and antiatherogenic properties as well as due to reduced allergenicity (Trindade *et al.*, 2001). It also contains isoflavones, which have been linked to reduced risk of most hormone-associated health disorders (Kurzer, 2000). However, consumption of soymilk is hindered due to the presence of unpleasant off-flavors carried over from soy beans. These characteristic flavors are caused by *n*-hexanal and pentanal, which occur in beans as a product of breakdown of unsaturated fatty acids (Arai *et al.*, 1996; Scalabrini *et al.*, 1998). In addition to these aldehydes, soymilk contains various oligosaccharides including raffinose and stachyose that may cause a gastrointestinal discomfort to consumers (Scalabrini *et al.*, 1998; Shin *et al.*, 2000). Raffinose and stachyose are α-galactosides of sucrose comprising three and four monomeric units respectively and are non-digestible in the gut due to the absence of α-galactosidase in the human intestinal mucosa. Consequently, intact oligosaccharides pass directly into the lower intestine where they are metabolized by bacteria that possess this enzyme, resulting in the production of gases (Tsangalis and Shah, 2004). This problem could be alleviated by using a specific enzyme, α-galactosidase or an organism that possesses high α-galactosidase activity to minimize the content of flatulence-causing oligosaccharides in the product (Scalabrini *et al.*, 1998). There has been an

alternative approach that utilizes lactic acid bacteria processing ά-galactosidase activity which can hydrolysed the raffinose and stachyose during fermentation (Garro *et al.*, 2005). This would provide simple sugar as substrate for the lactic acid bacteria as well as reduce the level of raffinose and stachyose. Some researchers (Bordignon *et al.*, 2004) have reported the lactic acid bacteria fermentation would be a promising way, because some lactic acid bacteria are effective to reduce the off-flavour. Others researchers (Mital and Steinkraus, 1975; Pinthong *et al.*, 1980; Garro *et al.*, 1983) have reported the reduction of raffinose and stachyose during fermentation of soymilk using pure or mixed culture of following bacteria: *Lactobacillus cellobiosis, L. plantarum, L. fermentum, L. delbrueckii, L. fermenti, L. pentosaceus and L. bulgaricus*, *Streptococcus thermofilus.* Several *Bifidobacterium* strains have been reported to produce varying levels of α-galactosidase, which metabolized α-galactosyl oligosaccharides in soymilk (Scalabrini *et al.*, 1998). Soymilk is a good medium for growing *Bifidobacterium* because it contains oligosaccharides that are fermented by most of the strains belonging to this genus (Liu, 1997; Scalabrini *et al.*, 1998). *Bifidobacterium* sp., *Lactobacillus acidophilus*, and *L. casei* have been associated with health-promoting effects and are classified as probiotic organisms since they are thought to improve the microbial balance in the human gastrointestinal tract (Schrezenmeir and De Vrese, 2001). Health benefits attributed to probiotics include antimicrobial, antimutagenic, anticarcinogenic and antihypertensive properties (Lourens-Hattingh and Viljoen, 2001). The ability of lactic acid bacteria to ferment the

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available carbohydrates in a growing medium varies with strains. Matsuoka *et al.* (1968) found that *S. thermophilus* produced a greater amount of acid in soymilk than *Lactococcus lactis* and *L. delbrueckii* ssp. *bulgaricus.* Mital *et al.* (1974) also reported that certain organisms such as *S. thermophilus*, *L. acidophilus, L. cellobiosis* and *L. plantarum* which utilize sucrose, exhibited significant growth and produced substantial amounts of acid in soymilk. Others such as *L. delbrueckii* ssp. *bulgaricus* grew poorly in soymilk because of their inability to ferment sucrose and other carbohydrates in soymilk. The use of lactic acid bacteria in preparing fermented soy products has received much attention (Lee *et al.*, 1990; Cheng *et al.*, 1990; Karleskind *et al.*, 1991; Sherkat *et al.*, 2001). Several studies on α-galactosidase activity and metabolism of α-galactosyl oligosaccharides by *Bifidobacterium* strains in soymilk have been reported but there is a lack of detailed information in the literature about the behavior of probiotic organisms (*L. acidophilus* and *L. casei*) and their importance as a part of the starter cultures for making fermented soy products. The objective of this study was to evaluate the fermentative characteristics of lactic acid bacteria isolated from local fermented foods and consume raffinose and stachyose during fermentation soymilk

MATERIAL AND METHODS

Soybeans (G*lycine max. L. Merr*) of cultivar wilis were obtained from Gading, Playen, Gunungkidul in Yogyakarta. High-performance liquid chromatography (HPLC, Hitachi, Japan). Raffinose and stachyose standards were purchased from Sigma (Merck, oxoid). All other chemicals and reagents were of analytical grade.

Strains and culture conditions

Seven strains of lactic acid bacteria were isolated from local fermented foods such as cassava fermented (gatot), bamboo shoot, moroni ketchup, yoghurt. *Lactobacillus plantarum pentosus SMN, 01, L. casei subsp rhamnosus FNCC, 098*, *L. casei subsp rhamnosus FNCC, 099, Streptococcus thermofilus,* 001*, Lactobacillus delbrueckii subsp. bulgaricus FNCC, 0045, L. plantarum SMN, 25, and L. plantarum pentosus FNCC, 235* were obtained from food and nutrition culture collection of Centre for Food and Nutrition studies, Gadjah Mada University. Culture stock were kept in 10 % glycerol and 10 % skim milk with the ratio 1 : 1, kept in sterile 1,5 ml polyethylene srew cap tubles, at – 40 °C. The strains were rejuvenated in MRS broth (Merck, Oxoid) at 37 °C for 24 h.

Soymilk preparation

Soybean grains (500 g) were soaked in cold water for 12 h at room temperature (27 °C). After soaking, water was discarded and the grains were then ground in a blender, for three minutes, in 4000 mL of hot water at 60 °C. Soymilk was extracted by filtration through a cotton cloth.

The slurry was cooked for ten minutes, filtered and boiled at 70 °C for 30 minute.

Extraction of crude α-galactosidase

One of the requirements for good growth of cultures in soymilk media is the activity of α-galactosidase. All organisms (*L. plantarum pentosus SMN, 01, L. casei subsp rhamnosus FNCC, 098 L. casei subsp rhamnosus FNCC, 099, Streptococcus thermofilus, 001, L. delbrueckii subsp. bulgaricus FNCC, 0045, L. plantarum SMN, 25, and L. plantarum pentosus FNCC, 235*) were assessed for α-galactosidase activity according to the methods of Scalabrini *et al.* (1998) and Tsangalis and Shah (2004). In order to ascertain the metabolic characteristics of *L. plantarum pentosus SMN, 01, L. casei subsp rhamnosus FNCC, 098 L. casei subsp rhamnosus FNCC, 099, Streptococcus thermofilus, 001, L. delbrueckii subsp. bulgaricus FNCC, 0045, L. plantarum SMN, 25, and L. plantarum pentosus FNCC, 235* in soymilk, α–galactosidase activity was examined in MRS basal broth supplemented with 2% (w/v) glucose, 2% (w/v) raffinose or a mixture of 1% (w/v) each of raffinose and glucose. During fermentation, 50 mL aliquots were withdrawn aseptically at 6, 12, 18, 24, and 30 h and stored at 4 °C. Bacterial cells were harvested by centrifuging at 4000 *x g* for 10 min at 4 °C using a refrigerated centrifuge. The cell pellet was washed in 20 mL cold 50 mM sodium citrate buffer (pH 5.5) and centrifuged at 4000 *x g* for 10 min and this was repeated twice. Finally cells were resuspended in 10 mL of the same buffer, placed in an ice bath and sonicated three times for 5 min. The cell debris was removed by centrifugation at 10,000 *x g* for 30 min at 4 °C. The supernatant was used as a crude enzyme extract.

Assay for α-galactosidase

Crude enzyme extracts from the organisms were assayed for α-galactosidase activity according to the method of Scalabrini *et al.* (1998). Briefly, 250 µL of crude enzyme extract was mixed with 500 µL of 5 mM p-nitrophenyl-α-D-galactopyranoside (*p*NPG) and incubated at 37 ^{0}C for 30 min. The reaction was stopped by addition of 500 µL of cold 0.2 M sodium carbonate. The α-galactosidase activity was determined by the rate of hydrolysis of *p*NPG. The amount of p-nitrophenol released was measured with a spectrophotometer at 420 nm. One unit of enzyme activity was defined as the amount of enzyme that released 1 µmol of *p*-nitrophenol from *p*NPG per millilitre per min under assay conditions. The specific activity was expressed as units (U) of α-galactosidase activity per milligram of protein.

Fermentation of soymilk

Eight batches of 250 mL of soymilk were heat treated (100 °C for 30 min) and aseptically inoculated with 1% (v/v) of each culture and incubated at 41 °C for 48 h. All fermentations were performed in triplicate. A control

consisted of uninoculated soymilk. During fermentation, aliquots from each batch were taken at 0, 6, 12, 18, 24, and 30 h to monitor cell growth, pH changes, organic acids production, and metabolism of oligosaccharides activities.

Cell Growth

The cell growth of each organism was assessed by enumerating bacterial population during 30 h of fermentation in soymilk as described in the procedure determination of viability The colony counts of *L. plantarum pentosus SMN, 01, L. casei subsp rhamnosus FNCC, 098, L. casei subsp rhamnosus FNCC, 099, Streptococcus thermofilus, 001, Lactobacillus delbrueckii subsp. bulgaricus FNCC, 0045, L. plantarum SMN, 25, and L. plantarum pentosus FNCC, 235* were determined as described previously (Dave and Shah, 1996; Tharmaraj and Shah, 2003). The media that were selected as suitable for the viability studies included MRS agar (Sigma, Jakarta, Indonesia) and aerobic incubation at 41 °C for 30 h for strains *L. plantarum pentosus SMN, 01, L. casei subsp rhamnosus FNCC, 098, L. casei subsp rhamnosus FNCC, 099, Streptococcus thermofilus, 001, L. delbrueckii subsp. bulgaricus FNCC, 0045*, *L. plantarum SMN, 25, and L. plantarum pentosus FNCC, 235*. The incubation was performed at 41 °C anaerobically for 30 h for all cultures (Laroia and Martin, 1991; Dave and Shah, 1996; Ravula and Shah 1998; Tharmaraj and Shah, 2003).

pH changes and production of organic acids

During culture growth, the main metabolic products are organic acids, particularly lactic and acetic acids. The pH changes in batches of soymilk were monitored during fermentation at 0, 6, 12, 18, 24 and 30 h using a pH meter (HANNA instruments 8417, Singapore).

Determination of organic acids

Determination of organic acids was carried out according to Shah and Ravula (2000). Briefly, 3 mL yoghurt samples were mixed with 50 µL of 15.5 M nitric and 1.0 mL of 0.01 M sulfuric acids. The resulting mixture was centrifuged at 14,000 *x g* for 30 min using an Eppendorf 5415C centrifuge for removal of proteins. The supernatant was filtered through a 0.20 µm membrane filter into an HPLC vial. The separation of organic acids was achieved using a Varian HPLC fitted with an Aminex HPX - 87H, 300 x 7.8 mm ion exchange column (Biorad Life Science Group, Hercules, USA) and a guard column maintained at 65 °C. The mobile phase was 0.01M H_2SO_4 with a flow rate of 0.6 mL/min. Quantification of acetic and lactic acids were performed from the standard curves obtained using solutions of pre-determined concentrations.

Determination of oligosaccharides

The extraction of sugars from fermented and unfermented soymilk samples was performed using the method described previously by Scalabrini *et al.* (1998) with some modifications. Briefly, 3 mL aliquots were centrifuged at 14,000 *x g* for 30 min for protein removal, followed by filtration using a 0.20 µm membrane filter. The concentration of raffinose, stachyose and sucrose was determined with a Varian HPLC fitted with an Alltima amino column (250 x 4.6 mm x 5 µm 100 Å) and corresponding guard column maintained at 30 °C and an RI detector (ERC-7515A, ERMA Cr. Inc., Kawaguchi City, Japan). The mobile phase consisted of 75% acetonitrile and 25% distilled water and was maintained at a flow rate of 1 mL/min isocratically. A 20 µL injection volume was used for both samples and standards. The retention times of the standards for raffinose, stachyose and sucrose (Sigma) were at 11.4, 19.1 and 7.8 min, respectively. Standard stock solutions of raffinose (2.53 g/100 mL), stachyose (2.03 g/100 mL) and sucrose (2.53 g/100 mL) were used for preparation of standard calibration curve. The concentration of oligosaccharides was derived from the standard curve and was expressed as milligram of sugar per 100 mL of soymilk.

Statistical analysis

All results obtained were analysed as a split plot in time design using general linear model procedure of the SAS System (SAS, 1996). The univariate ANOVA test was validated by fulfilling Huynh-Feldt (H-F) condition (Littell *et al.*, 1998). Where appropriate, one-way ANOVA and correlational analysis were employed using Microsoft Excel (Albright *et al.*, 1999) and the multicomparison of means was assessed by Tukey's test. The statistical level of significance was presen at 0.05.

RESULTS AND DISCUSSION

Proximate composition and total monosaccharides and oligosaccharides contents of cultivar wilis soybean and soymilk

Results of analysis proximate composition and total monosaccharides and oligosaccharides contents are given in Table 1. Chromatograms profiles of monosaccharides and oligosaccharides detected using HPLC method from soymilk is shown in Figure 1.

Table 1. Proximate composition wilis soybean (mg/100g dry weight)[a] and soymilk (mg/100L)[a]

Composition	Will soybeans	soymilk
moisture	11.0 ± 0.4	88.90 ± 1.8
protein	34.0 ± 1.0	3.6 ± 0.1
fat	21.6 ± 0.2	1.3 ± 0.03
carbohydrates	28.1 ± 1.2	2.08 ± 0.01
raffinose (mg)	5.87 ± 2.8	3.79 ± 0.64
stachyose (mg)	8.75 ± 1.5	6.17 ± 0.78
ash	2.97 ± 0.1	0.46± 0.01

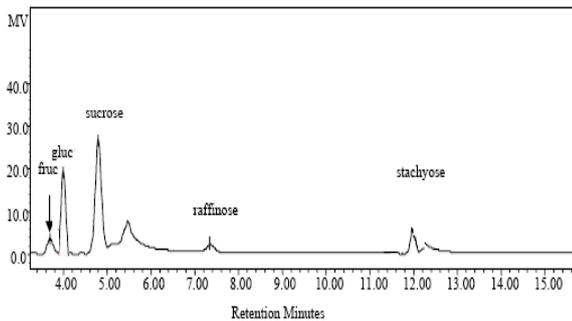[a] From means standard deviation of three duplicates for each sample

Figure 1. Chromatograms profiles of monosaccharides and oligosaccharides detected using HPLC method from soymilk

α–Galactosidase specific activity of strains lactic acid Bacteria isolated from local fermented foods

The α-galactosidase activity of *strains lactic acid Bacteria* isolated from local fermented foods is shown in Table 2. The organisms exhibited α-galactosidase activity at varying degrees, and fermentation time was a significant ($P<0.05$) factor for α-galactosidase activity during the 30 h incubation at 41 °C. This might be due to growth differences in the respective media which likely determined the amount of enzymes produced during fermentation. Interestingly, α-galactosidase activity in the medium containing raffinose declined substantially during 12 to 24 h of fermentation for some organisms but increased significantly ($P < 0.05$) for all microorganisms at 30 h. This followed the growth pattern of the organisms in the medium during the fermentation period. However, *L. plantarum SMN, 25,* grew better than the other organisms in that medium as α-galactosidase activity increased significantly ($P < 0.05$) until the end of fermentation. This suggests that *L. plantarum* SMN, 25, may have hydrolyzed raffinose mainly by producing acetate and lactate from the bifidus carbohydrate catabolism pathway. Bordignon *et al.* (2004) reported strain, *L. delbrueckii* ssp. *bulgaricus* showed the lowest α-galactosidase activity in the medium containing raffinose (compared to other organisms). Although α-galactosidase specific activity in the glucose/raffinose medium was not as high as that of medium containing raffinose, the trend of activity was similar. The presence of glucose in MRS medium, on the other hand, resulted in high α-galactosidase activity for the majority of the organisms in comparison to other media except *L. plantarum SMN, 25,* in 2% raffinose. All organisms grew well in MRS medium, which suggests that an increase in α-galactosidase is due to an increase in cell density. After reaching the log phase, α-galactosidase activity was reduced ($P < 0.05$). This reduction was most probably a result of depletion of the readily available carbon source or other factors such as accumulation of organic acids drop in pH and reduction/aging bacteria population (Table 4). The presence of 1% each of glucose and raffinose did not stimulate ($P > 0.05$) the synthesis of α-galactosidase as much as that containing 2% raffinose

did (Table 2). Mital and Steinkraus (1975) reported that 1% raffinose supplementation increased the production of α-galactosidase for lactic acid bacteria strains but with little or no effect on the α-galactosidase activity of lactic acid bacteria. Since raffinose is an α-galactoside sugar found in soymilk, these selected organisms may grow well in soymilk for manufacture of soy based yoghurt. The effect of raffinose and glucose on the production of organic acids by strains of lactic acid bacteria isolated from local fermented foods (no shows). *L. plantarum SMN, 25,* exhibited the highest ($P < 0.05$) acid lactic production in 2% raffinose medium with significant ($P < 0.05$) increases during fermentation as opposed to other organisms which produced organic acids in comparable quantities ($P >0.05$). Omogbai *et al.* (2005) reported that some *lactic acid bacteria* strains produced relatively low quantities of acetic acid in a modified medium. The production of acetic acid in 1% raffinose and 1% glucose by *L. plantarum pentosus SMN, 01, L. casei subsp rhamnosus FNCC, 098, L. casei subsp rhamnosus FNCC, 099, Streptococcus thermofilus, 001, Lactobacillus delbrueckii subsp. bulgaricus FNCC, 0045, L. plantarum SMN, 25, and L. plantarum pentosus FNCC, 235* showed a slight ($P > 0.05$) variation in concentrations to that produced in the 2% raffinose. The mixture of glucose/raffinose stimulated higher ($P < 0.05$) production of lactic acid by all organisms studied compared to the medium containing 2% raffinose. The MRS broth which contained 2% glucose showed similar production of lactic acid to that of the mixture but low ($P < 0.05$) production of acetic acid for all the organisms. Our study showed metabolism of raffinose as the sole energy source produced substantial amount of lactic acid.

Sugars metabolism in soymilk

Tables 3 and 4 show the utilization of raffinose, stachyose and sucrose and the production of lactic acids. The utilization of raffinose and stachyose in soymilk varied and appeared to depend on the α–galactosidase activity of the organism (Table.2). *L. plantarum SMN, 25, L. plantarum pentosus SMN, 01* and *L. plantarum pentosus FNCC, 235* reduced raffinose significantly ($P < 0.05$) by 78.1%, 72.5% and 66 %, respectively, whereas the remaining organisms showed less than 66% reduction after 24 h. Bordignon *et al.* (2004) found that *lactic acid bacteria* strains metabolized raffinose in soymilk, as opposed to yoghurt cultures, which did not reduce raffinose and stachyose during growth in soymilk. Our study was in line with that reported by Bordignon *et al.* (2004), which showed that raffinose, was substantially metabolized by *lactic acid bacteria* strains. The organisms in general metabolized stachyose by over 66% after 24 h, with *L. plantarum SMN, 25,* and *L. plantarum pentosus SMN, 01* showing the highest hydrolysis of 78% and 72.5%, respectively. Our findings are in line with those of Mital and Steinkraus (1975), who reported that fermentation of soymilk with lactic cultures possessing α-galactosidase activity reduced raffinose and stachyose contents. Overall, the sucrose concentration in soymilk was significantly ($P < 0.05$)

Table 2. α-Galactosidase activity of *strains lactic acid Bacteria* isolated from local fermented foods in supplemented media at 41 °C for 30 h[(x)]

Strain	Incubation time (h)	α-galactosidase specific activity(U/mg)		
		raffinose	raff + gluc	glucose
L. casei subsp rhamnosus FNCC, 098	0	5.02 ± 1.04[a]	6.89± 1.24[a]	12.65 ± 0.44[a]
	6	5.18 ± 1.05[b]	7.01 ± 1.14[a]	12.36 ± 0.45[a]
	12	5.32 ± 1.10[c]	7.43 ± 1.04[c]	13.49 ± 0.24[b]
	18	5.57 ± 1.05[d]	7.65 ± 1.05[d]	14.37 ± 0.45[c]
	24	7.02 ± 1.11[d]	9.35 ± 1.11[d]	14.65 ± 0.34[d]
	30	6.72 ± 1.45[e]	9.27 ± 1.15[d]	14.68 ± 0.35[d]
L. plantarum SMN, 25	0	11.27 ± 0.45[a]	14.21 ± 1.23[a]	13.62 ± 0.44[a]
	6	17.20 ± 1.11[b]	16.7 ± 1.14[b]	17.69 ± 0.45[b]
	12	25.12 ± 1.04[c]	17.4 ± 1.04[c]	23.48 ± 0.24[c]
	18	65.78 ± 1.45[d]	19.53 ± 1.05[d]	36.16 ± 0.75[d]
	24	98.66 ± 2.14[e]	27.22 ± 1.16[e]	43.81 ± 0.34[e]
	30	100.87 ± 2.45[f]	28.17 ± 1.15[f]	43.89 ± 0.65[e]
L. delbrueckii subsp. Delbrueckii FNCC, 045	0	5.02 ± 0.15[a]	6.89± 1.24[a]	12.65 ± 0.44[a]
	6	5.18 ± 0.14[a]	7.21 ± 1.14[a]	12.36 ± 0.45[a]
	12	5.32 ± 0.32a	7.43 ± 1.04[a]	13.49 ± 0.24[b]
	18	5.57 ± 0.74[b]	7.65 ± 1.05[b]	14.37 ± 0.45[b]
	24	7.02 ± 0.41[c]	9.35 ± 1.11[c]	14.65 ± 0.34[c]
	30	6.72 ± 0.45[c]	9.27 ± 1.15[c]	14.68 ± 0.35[c]
Streptococcus thermofilus, 001	0	5.02 ± 0.15[a]	6.89± 1.24[a]	12.65 ± 0.44[a]
	6	11.18 ± 1.45[b]	7.21 ± 1.16[b]	16.36 ± 0.45[b]
	12	18.32 ± 1.45[c]	7.43 ± 1.04[b]	23.49 ± 0.44[c]
	18	26.21 ± 1.45[d]	17.57 ± 1.05[c]	30.68 ± 0.45[d]
	24	38.13 ± 1.45[e]	27.29 ± 1.10[e]	38.19 ± 0.34[e]
	30	40.31 ± 1.45[f]	27.74 ± 1.15[e]	38.85 ± 0.35[e]
L. plantarum pentosus SMN, 01	0	10.02 ± 1.12[a]	6.89± 1.24[a]	12.65 ± 0.44[a]
	6	16.18 ± 1.45[b]	7.25 ± 1.14[b]	15.36 ± 0.45[b]
	12	22.32 ± 1.04[c]	7.43 ± 1.04[b]	23.49 ± 0.24[c]
	18	36.21 ± 1.45[d]	17.57 ± 1.05[c]	33.68 ± 0.45[d]
	24	88.13 ± 1.14[e]	27.29 ± 1.09[d]	43.19 ± 0.74[e]
	30	90.31 ± 1.45[f]	27.74 ± 1.15[d]	43.85 ± 0.35[e]
L. plantarum pentosus FNCC, 235	0	9.02 ± 1.00[a]	6.89± 1.24[a]	12.65 ± 0.44[a]
	6	12.18 ± 1.04[b]	7.21 ± 1.14[a]	15.36 ± 0.45[b]
	12	18.32 ± 1.45[c]	7.43 ± 1.04[a]	23.49 ± 0.24[c]
	18	26.61 ± 1.05[d]	17.57 ± 1.05[b]	33.68 ± 0.45[d]
	24	38.13 ± 1.45[e]	27.29 ± 1.11[c]	44.19 ± 0.34[e]
	30	40.81 ± 1.14[f]	27.74 ± 1.15[c]	43.85 ± 0.35[e]
L. casei subsp rhamnosus FNCC, 099	0	5.02 ± 1.05[a]	6.89± 1.24[a]	12.65 ± 0.41[a]
	6	5.18 ± 1.04[a]	7.21 ± 1.14[a]	12.36 ± 0.45[a]
	12	5.32 ± 1.05[a]	7.43 ± 1.04[a]	13.49 ± 0.24[a]
	18	5.57 ± 1.14[b]	7.65 ± 1.05[b]	14.37 ± 0.45[b]
	24	7.02 ± 1.10[c]	9.35 ± 1.11[c]	14.65 ± 0.34[b]
	30	6.72 ± 1.15[c]	9.27 ± 1.15[c]	14.68 ± 0.35[b]

[(x)]From means ± standard deviation of three duplicates for each sample.
[abcdef]Means in the same column for particular strains with different small letter superscripts are significantly different.
One unit of enzyme was defined as the amount of enzyme required to release 1 µmol of p-nitrophenol from p-nitrophenyl-α-D-galactopyranoside per mL per min under assay, Significant when $P < 0.05$

Table 3 Changes in concentration of raffinose, stachyose and sucrose in soymilk during fermentation with *strains lactic acid Bacteria* isolated from local fermented foods for 0, 6, 12, 18, and 30 h at 41 °C[(x)]

Sugar	Strain	Sugar concentration, mg/ 100 mL Incubation time (h)					
		0	6	12	18	24	30
Raffinose							
	Lcr 098	3.79 ± 0.64^a	3.76 ± 0.24^a	3.20 ± 0.12^a	2.71 ± 0.12^a	2.01 ± 0.02^a	1.99 ± 1.2^a
81,5	Lp 025	3.79 ± 0.64^a	3.61 ± 0.16^b	2.51 ± 0.15^b	1.60 ± 0.15^b	0.70 ± 0.05^b	1.00 ± 1.5^b
	Ldb 045	3.79 ± 0.64^a	3.75 ± 0.26^a	3.25 ± 0.17^a	2.74 ± 0.11^c	2.04 ± 0.07^c	2.03 ± 2.7^c
	St 001	3.79 ± 0.64^a	3.75 ± 0.24^a	3.13 ± 0.12^c	2.53 ± 0.12^d	1.59 ± 0.02^d	1.57 ± 2.9^d
73	Lpp 01	3.79 ± 0.64^a	3.74 ± 0.14^a	2.92 ± 0.13^c	1.83 ± 0.13^e	1.03 ± 0.03^e	1.52 ± 3.8^d
67	Lpp235	3.79 ± 0.64^a	3.76 ± 0.26^a	3.17 ± 0.16^a	2.13 ± 0.11^f	1.25 ± 0.16^f	1.91 ± 1.6^a
	Lcr 099	3.79 ± 0.64^a	3.76 ± 0.24^a	3.25 ± 0.13^a	2.85 ± 0.08^h	2.16 ± 0.13^e	2.14 ± 1.3^e
Stachyose							
	Lcr 098	6.17 ± 0.78^a	6.15 ± 0.32^a	5.75 ± 0.23^a	5.14 ± 0.03^a	4.47 ± 0.05^{bb}	4.45 ± 0.03^{bb}
78	Lp 025	6.17 ± 0.78^a	6.01 ± 0.35^b	4.62 ± 0.15^b	2.78 ± 0.01^b	1.36 ± 0.01^{bb}	0.79 ± 0.01^{bb}
	Ldb 045	6.17 ± 0.78^a	6.14 ± 0.46^a	5.83 ± 0.14^a	5.15 ± 0.12^a	4.45 ± 0.12^{bf}	4.25 ± 0.17^{bf}
	St 001	6.17 ± 0.78^a	6.14 ± 0.37^a	5.13 ± 0.13^c	4.12 ± 0.26^{bg}	2.16 ± 0.26^{bg}	2.12 ± 0.26^{bg}
72.5	Lpp 01	6.17 ± 0.78^a	6.13 ± 0.27^a	4.93 ± 0.17^c	3.61 ± 0.21^{ba}	1.69 ± 0.21^{ba}	1.56 ± 0.21^{ba}
66	Lpp235	6.17 ± 0.78^a	6.12 ± 0.26^a	5.09 ± 0.13^c	4.01 ± 0.28^{bc}	2.11 ± 0.28^{bc}	2.01 ± 0.28^{bc}
	Lcr 099	6.17 ± 0.78^a	6.14 ± 0.33^a	5.83 ± 0.14^a	5.76 ± 0.15^{bd}	4.69 ± 0.13^{bd}	4.56 ± 0.12^{bd}
Glucose							
	Lcr 098	20.1 ± 1.45^a	17.2 ± 1.24^a	13.6 ± 0.44^a	8.1 ± 0.24^a	3.42 ± 0.03^a	3.42 ± 0.06^a
	Lp 025	20.1 ± 1.45^a	16.1 ± 1.14^b	11.6 ± 0.45^b	6.2 ± 0.23^b	1.82 ± 0.05^a	1.82 ± 0.04^b
	Ldb 045	20.1 ± 1.45^a	17.4 ± 1.04^a	13.4 ± 0.24^a	8.6 ± 0.14^c	3.82 ± 0.11^c	3.82 ± 0.09^c
	St 001	20.1 ± 1.45^a	17.5 ± 1.05^a	13.6 ± 0.45^a	9.2 ± 0.12^d	$4.42 \pm 0.12^{d.}$	4.42 ± 0.11^d
	Lpp 01	20.1 ± 1.45^a	17.2 ± 1.11^a	13.2 ± 0.34^a	8.5 ± 0.13^c	3.62 ± 0.14^a	3.62 ± 0.04^a
	Lpp235	20.1 ± 1.45^a	17.7 ± 1.15^a	13.8 ± 0.35^a	9.6 ± 0.13^d	5.03 ± 0.05^e	5.03 ± 0.15^e
	Lcr 099	20.1 ± 1.45^a	17.4 ± 1.12^a	13.4 ± 0.33^a	8.7 ± 0.05^c	3.82 ± 0.06^c	3.82 ± 0.10^c

Lp025=Lactobacillus plantarum pentosus SMN, 01, Lcr098=Lactobacillus casei subsp rhamnosus FNCC, 098, Lcr099= Lactobacillus casei subsp rhamnosus FNCC, 099, Ldb045=Lactobacillus delbrueckii subsp. bulgaricusi FNCC, 0045, Lp025=Lactobacillus plantarum SMN, 25, and Lpp235=Lactobacillus plantarum pentosus FNCC, 235, St001= Streptococcus thermofilus, 001
[(x)]From means ± standard deviation of three duplicates for each sample.
[abcdef]Means in the same column for particular strains with different small letter superscripts are significantly different. Significant when $P < 0.05$

Table 4. Metabolic activity of investigated cultures during growth in soymilk for 0, 6, 12, 18, and 30 h at 41 °C[(x)]

Strain	Incubation time	pH	Cell counts (cfu/mL)	Lactic acid (mg/mL)
L. casei subsp rhamnosus FNCC, 098	0	6.4 ± 0.14^a	6.8 ± 0.02^a	0.2 ± 0.01^a
	6	6.2 ± 0.11^b	7.0 ± 0.09^b	0.3 ± 0.01^b
	12	5.8 ± 0.13^c	7.6 ± 0.11^c	0.5 ± 0.03^c
	18	5.6 ± 0.09^d	8.0 ± 0.05^d	1.0 ± 0.02^d
	24	4.8 ± 0.09^e	8.2 ± 0.08^e	1.2 ± 0.01^e
	30	4.8 ± 0.10^e	8.1 ± 0.06^e	1.2 ± 0.02^e
L. delbrueckii subsp. Delbrueckii FNCC, 045	0	6.4 ± 0.14^a	6.8 ± 0.02^a	0.2 ± 0.01^a
	6	6.1 ± 0.09^b	6.9 ± 0.08^a	0.4 ± 0.02^b
	12	5.6 ± 0.12^c	7.1 ± 0.11^b	0.9 ± 0.01^c
	18	4.4 ± 0.08^d	7.6 ± 0.09^c	1.4 ± 0.02^d
	24	4.0 ± 0.08^e	8.2 ± 0.08^d	1.8 ± 0.03^e
	30	4.2 ± 0.09^d	7.6 ± 0.08^c	1.5 ± 0.01^d
L. plantarum SMN, 25	0	6.4 ± 0.14^a	6.8 ± 0.02^a	0.2 ± 0.01^a
	6	6.2 ± 0.13^b	7.0 ± 0.11^b	0.3 ± 0.03^b
	12	6.0 ± 0.09^c	7.5 ± 0.10^c	0.6 ± 0.02^c
	18	5.7 ± 0.11^d	7.8 ± 0.08^d	1.1 ± 0.01^d
	24	5.2 ± 0.13^e	8.1 ± 0.05^e	1.2 ± 0.02^e
	30	5.0 ± 0.24^e	8.1 ± 0.04^e	1.2 ± 0.01^e
Streptococcus thermofilus, 001	0	6.4 ± 0.14^a	6.8 ± 0.02^a	0.2 ± 0.01^a
	6	6.2 ± 0.13^b	6.9 ± 0.12^a	0.3 ± 0.03^b
	12	6.0 ± 0.09^c	7.3 ± 0.06^b	0.5 ± 0.07^c
	18	5.6 ± 0.11^d	7.8 ± 0.07^c	1.0 ± 0.02^d
	24	4.8 ± 0.13^e	8.3 ± 0.06^d	1.4 ± 0.03^e
	30	4.7 ± 0.14^e	7.8 ± 0.04^e	1.3 ± 0.11^e
L. plantarum pentosus SMN, 01	0	6.4 ± 0.14^a	6.8 ± 0.02^a	0.2 ± 0.01^a
	6	6.3 ± 0.13^a	7.4 ± 0.01^b	0.3 ± 0.03^a
	12	5.8 ± 0.10^b	7.9 ± 0.08^c	0.6 ± 0.02^b
	18	5.2 ± 0.09^c	8.2 ± 0.01^d	1.4 ± 0.02^c
	24	4.6 ± 0.07^d	8.8 ± 0.05^e	1.6 ± 0.01^d
	30	4.5 ± 0.09^d	8.6 ± 0.04^e	1.6 ± 0.02^d
L. plantarum pentosus FNCC, 235	0	6.4 ± 0.14^a	6.8 ± 0.02^a	0.2 ± 0.01^a
	6	6.2 ± 0.13^b	7.0 ± 0.11^b	0.3 ± 0.03^a
	12	6.0 ± 0.09^c	7.5 ± 0.10^c	0.6 ± 0.02^b
	18	5.7 ± 0.11^d	7.8 ± 0.08^d	1.1 ± 0.01^c
	24	5.2 ± 0.13^e	8.1 ± 0.05^e	1.2 ± 0.02^d
	30	4.9 ± 0.11^f	8.1 ± 0.04^e	1.2 ± 0.01^d
L. casei subsp rhamnosus FNCC, 099	0	6.4 ± 0.14^a	6.8 ± 0.02^a	0.2 ± 0.01^a
	6	6.2 ± 0.11^b	7.0 ± 0.09^b	0.3 ± 0.01^a
	12	5.8 ± 0.13^c	7.4 ± 0.11^c	0.5 ± 0.03^b
	18	5.6 ± 0.09^d	7.8 ± 0.05^d	1.0 ± 0.02^c
	24	4.8 ± 0.09^e	8.0 ± 0.08^d	1.2 ± 0.01^d
	30	4.8 ± 0.10^e	8.1 ± 0.06	1.2 ± 0.02^d

[(x)]From means ± standard deviation of three duplicates for each sample. [abcdef]Means in the same column for particular strains with different small letter superscripts are significantly different. Significant when $P < 0.05$

reduced by *Lactobacillus plantarum SMN, 25, L. plantarum pentosus SMN, 01* and *L. plantarum pentosus FNCC, 235* after 24 h (Table 4).

Viability of selected microorganisms and organic acids production in fermented soymilk

In general, *strains lactic acid Bacteria* isolated from local fermented foods achieved the desired therapeutic level (10^8 cfu/mL) during growth by each organism in soymilk after 24 h of fermentation as shown in Table 3. Organic acid production, pH decline and other metabolic activities occurred during the first 12 to 24 h of incubation, which corresponded to the exponential phase of the growth. *L. plantarum pentosus SMN, 01, L. plantarum SMN, 25,* and *L. plantarum pentosus FNCC, 235 exhibited* better growth ($P < 0.05$) throughout the cultivation compared to other microorganisms. On the other hand, *L. casei subsp rhamnosus FNCC, 098 L. casei subsp rhamnosus FNCC, 099, S. thermofilus, 001,* and *L. delbrueckii subsp. bulgaricus FNCC, 0045*, showed slow growth by one log cycle lower ($P < 0.05$) than the rest of the organisms throughout the incubation. *S. thermofilus, 001* grew better than *L. casei subsp rhamnosus FNCC, 098 L. casei subsp rhamnosus FNCC, 099,* and *L. delbrueckii subsp. bulgaricus FNCC, 0045* and produced higher ($P < 0.05$) organic acid than the latter during fermentation. Wang *et al.* (1974) reported that *L. delbrueckii* ssp. *bulgaricus* grew poorly in soymilk because of their inability to ferment sucrose and other soy carbohydrates. Mital *et al.* (1974) also reported that certain organisms such as strains *S. thermophilus*, *L. acidophilus, L. cellobiosis* and *L. plantarum,* which utilized sucrose, grew well and produced large amounts of acid in soymilk. However contrary to our finding, the selected organisms used in this study produced lower amounts of organic acids in soymilk even though they grew well. Liu (1997) also reported that lactic acid bacteria grew well in soymilk but produce less organic acids. The low levels of organic acid concentrations in fermenting soymilk presumably encouraged cell growth and in other studies (Angeles and Marth, 1971; Kamaly, 1997; Liu, 1997). Furthermore, these selected strains possess α-galactosidase (Table.2) and can utilize sucrose and other soy carbohydrates in soymilk as sources of energy, which enhanced better cell growth during 30 h fermentation at 42 °C (Mital *et al.*, 1974; Liu, 1997). Although the organisms in our study showed a consistent increase in cell concentration from the start of incubation until 24 h, the required pH of 4.5 was not reached within this time frame in comparison to the MRS medium with the exception of *L. plantarum pentosus SMN, 01, L. plantarum SMN, 25,* and *L. plantarum pentosus FNCC 235* that showed a pH decline of 4.12. However, after a prolonged incubation for 30 h, the pH of all batches declined to below 4.5. In general, the production of organic acids by all organisms in MRS was significantly higher ($P < 0.05$) than those produced in the fermented soymilk from 0 to 30 h (Table 4), thus making soymilk a potential medium for bacterial growth. Soymilk was reported previously as an appropriate

growth medium for some lactic acid bacteria (Angeles and Marth, 1971; Kamaly, 1997; Liu, 1997)**.**

CONCLUSIONS

Seven strains of lactic acid bacteria isolated from local fermented foods and added into soymilk during processing can degradation rafiinose and stachyose. Strains *Lactobacillus plantarum pentosus SMN, 01, L. casei subsp rhamnosus FNCC, 098 L. casei subsp rhamnosus FNCC, 099, Streptococcus thermofilus, 001, Lactobacillus delbrueckii subsp. bulgaricus FNCC, 0045*, *L. plantarum SMN, 25,* and *L. plantarum pentosus FNCC 235* exhibited variable α-galactosidase activity with *L. plantarum SMN, 25* showing the highest activity in MRS supplemented media. However, all organisms reached the desired therapeutic level (10^8 cfu/mL) likely due to their ability to metabolize oligosaccharides during fermentation in soymilk at 41°C. The oligosaccharide metabolism depended on α-galactosidase activity. *L. plantarum SMN, 25, L. plantarum pentosus SMN, 01* and *L. plantarum pentosus FNCC 235* reduced raffinose and stachyose by 81.5, 73.0, 67.0 %, and 78.0, 72.5, 66.0 % respectively in soymilk.

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