Survivability and Growth Effects of Three *Lactobacillus Cultures* on Texture and Flavor Aspects of Soymilk

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Abstract: Survivability and growth effects of three lactic acid bacteria, *Lactobacillus casei* (LC), *Lactobacillus helveticus* (LH) and *Lactobacillus rahmnosus* (*Lactobacillus casei subsp. rahmnosus 6013*) (LR) were studied in soymilk to determine their suitability for soy cheese production. After six hours of fermentation, viable count of LC, LH and LR increased to 8.74, 7.69 and 8.69 log (CFU/mL), respectively. LC, LH, LR and Glucono-δ-lactone (GDL, a commercial coagulant) showed significant increase in Titratable acidity and pH decrease in soymilk at 37 ℃. All the three *Lactobacillus* cultures were able to utilize soy sugars. The ability of the cultures to cause textural changes in soymilk, analyzed by Texture Analyser, was found competitive to GDL. Enzymes and acid produced by the cultures hydrolyzed soy protein to cause textural changes resulting into curd formation. HPLC analysis showed that all three cultures have the ability to produce and metabolize 18 free amino acids (FAA) including *Asp*, *Ile*, *Leu*, *Met*, *Phe*, *Tyr* and *Val* which are known as precursors of cheese flavoring compounds. GC-FID analysis for fatty acids (FA) indicated that all the cultures were capable of utilizing soy fatty acids and disappearance of some FA indicated their complete consumption by respective cultures in the resultant soy curd. All the three cultures were found to be suitable to cause textural and flavor precursor changes in soymilk required for soy cheese production.

Key words: soy cheese; soymilk; lactic acid bacteria; texture; amino acids; fatty acids

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乳酸菌培养物的生长特性及其对豆奶质构和风味的影响

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摘要：研究了 *Lactobacillus casei* (LC), *Lactobacillus helveticus* (LH) and *Lactobacillus rahmnosus* (*Lactobacillus casei subsp. rahmnosus 6013*) (LR)生长特性及其对大豆奶酪风味的影响。研究表明，经过 6 h 的发酵，LC、LH 和 LR 的菌落数分别增长至 8.74、7.69 和 8.69 log (CFU/mL)；37 ℃时，分别接种 LC、LH、LR 和添加有 Glucono-δ-内酯（GDL）的大豆牛奶都明显出现可滴定酸度增加、pH 减少的现象。这三种乳酸菌都能够利用大豆糖源。经质构分析发现，菌种可引起大豆牛奶质构发生变化，其凝乳的能力可与 GDL 媲美。菌种通过产酶、产酸而水解大豆蛋白，从而导致质构变化形成凝乳。这些菌种可释放 18 种游离氨基酸（FAA），其中包括可作为奶酪风味物质前体化合物的天门冬氨酸、异亮氨酸、亮氨酸、蛋氨酸、苯丙氨酸和缬氨酸。这些菌种均能利用大豆脂肪酸（FA），而在大豆凝乳中出现某些 FA 消失的现象表明所选的菌种还能分别完全消耗特定的 FA。可见，这三种乳酸菌在大豆牛奶中可引起质感及风味前体物的变化，正是生产大豆奶酪所必需的效果，故选择它们作为制备大豆奶酪的适用菌种。

关键词：大豆奶酪；豆奶；乳酸菌；质构；氨基酸；脂肪酸

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Soy cheese and milk cheese production are quite similar in terms of biochemistry, microbiology and processing technology. The final products also have similarities in appearance, texture and chemical constituents. Nutritionally, both of the products are rich in protein with good amounts of fats and minerals but deficient in carbohydrates. Both cheeses have compounds of similar nature like proteins, lipids, carbohydrates, esters, acids, alcohols, aldehydes, ketones, sulfur-containing compounds and minerals *etc*. Both have similar biochemical changes during processing *e.g.* proteolysis (cause the formation of amino acids), lipolysis (responsible for production of fatty acids) and also the production of aldehydes, alcohols, esters *etc.* Hence flavor producing cultures of milk cheese may be helpful to improve the flavor of soy cheese and probiotic cultures can provide product safety in modified soy cheese process[1].

Cheese flavor is the combined effect of many compounds. Free amino acids are important contributors to the flavor of cheese; these not only participate in overall flavor by their own flavoring effect but also act as the precursors of other flavoring compounds. Fat and fatty acids contribute in cheese flavor, hold other flavoring compounds preventing their loss and masks the bad flavor. Role of starter and non-starter cultures has been widely accepted in cheese flavor production and this provides an opportunity to improve the flavor of soy cheese. LAB (Lactic acid bacteria) cultures use free amino acids for their metabolism and produce flavoring compounds during cheese ripening process. They use *Asp*, *Ile*, *Leu*, *Met*, *Phe*, *Trp*, *Tyr* and *Val* for flavor production, so their presence in the medium can be helpful to produced flavoring compounds in soy cheese. Previously some LAB cultures have been used to produce fermented soy products and these cultures not only survived under new conditions but also produced the products with better acceptability[2~5]. Therefore, further study by focusing on the parameters important for soy cheese production and flavor improvement was needed.

Three LAB cultures *Lactobacillus casei* (LC), *Lactobacillus helveticus* (LH), *Lactobacillus casei subsp. rahmnosus 6013* (LR) were selected for this study due to the followings. LC and LH are known as good starters and flavor adjuncts in milk cheeses[6]. LH shows good proteolytic system in cheese and its extensive lysis results into a large increase of flavour precursors and some volatile compounds. LH adjunct exhibits the high rates of free amino acids formation; while LC treated cheese shows the high rate of free fatty acids release in Cheddar cheese[6]. LR and LC are proboitic cultures[7] and they can also provide product safety against pathogenic organisms.

This project was aimed to determine the growth effects of LC, LH and LR on soymilk by studying the followings. Soymilk coagulation by six hours fermentation is a common practice for the production of soy cheese; therefore, their successful growth, acid production, pH reduction and changes in soy sugars during six hours of fermentation were studied. Their ability of coagulation was studied in terms of textural changes in soymilk. Release of free amino acids and changes in fatty acid contents were also studied due to their importance in cheese flavor production.

Materials and Methods

LC and LH were purchased from Chr. Hansen's (Horsholm, Denmark) with their commercial names L. casei-01 and LH-B02, respectively. LR was obtained from China Center of Industrial Culture Collection (CICC). GDL was purchased from local market of Guangzhou.

Preparation of soymilk

ybeans were soaked overnight in distilled water at the ratio of 1:8 (soybeans:distilled water). After decanting the water, 100 g of soybean (on the basis of weight before soaking) was extracted with 600 mL of distilled water in a high-speed blender and filtered through a sieve of 180 meshes. Raw milk was autoclaved at 121 ℃ for 20 minutes and cooled to ambient temperature for further use.

Preparation of cultures

One gram of freeze dried LC and LH were added to 10 mL sterilized distilled water, thoroughly mixed and then added to sterilized MRS media at the rate of 5% (*v/v*). LR was also added at same rate and all were incubated for 24 hours at 37 ℃ and then stored at 12 ℃ as stock culture. Stock culture was inoculated in soymilk at the rate of 5% (*v/v*) and incubated at 37 ℃ for 24 hours to prepare the

mother culture. The process of making the mother culture was repeated to prepare work culture. Sterilized conditions were maintained during the whole process of culture preparation and fermentation.

Fermentation

Soymilk was inoculated with 5% (*v/v*) of work culture in laminar flow and incubated at 37 ℃ for six hours. In series of experiments, we found that 0.3% (*m/v*) GDL has the ability to produce good coagulation (data not shown) resulting in soymilk gel at 37 ℃ in 4 hours, therefore it was used as control.

Microbial analysis

Soymilk samples inoculated with LC, LH and LR were drawn at 0, 1, 2, 3, 4, 5 and 6 hours of fermentation. 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} dilutions were plated in MRS agar. Incubation was done at 37°C for 48 hours and colonies were counted to determine the viable bacterial (those capable of growth on MRS agar) count and expressed as log (CFU/mL)[6].

Physico-chemical Analysis

Titratable acidity (TA) and pH were determined at 0, 1, 2, 3, 4, 5 and 6 hours of fermentation according to the procedures described by Nielsen[8]. Acidity was expressed as percent of acetic acid.

Soy sugars estimation

HPLC method for sugar determination as described by Liu at el[6]. was applied to determine the contents of sugars in soymilk. A Waters Spherisorb NH2 column (4.6 mm×250 mm, 5 mm) was used with following conditions. Mobile phase: 70% acetonitrile in distilled deionized water, flow rate: 0.4 mL/min, column temperature: 40 ℃, a refractive index detector (model 830-RI, Jasco). External standards of sucrose, stachyose, raffinose, fructose and glucose (Sigma-Aldrich, Shanghai, China) were prepared by diluting specific amounts of sugars in deionized water. Standard curves were constructed for each sugar to determine the amounts of sugars. Soymilk samples were deproteinized and centrifuged for 20 min at 10000 rpm. Supernatants were stored at -20 ℃ until used for further analysis. Supernatants were filtered through a 0.45-mm membrane after thawing and loaded to auto-sampler of HPLC for analysis. Least squares regression analysis was used to derive equations from the values obtained for each sugar.

Textural properties

Texture of the gels obtained was analyzed on Texture Analyser model TA-XT2*i* (Stable Micro Systems, Surrey, UK). TPA fracture test was run with a cylindrical probe 0.5R 2966[9], while other settings were as follows. Parameters were, pretest speed: 2 mm/sec, test speed: 1 mm/sec, posttest speed: 5 mm/sec, distance: 10 mm, force: 100 g, time: 5 sec., count: 5. Trigger settings were, type: auto, force: 1 g, stop plot at: final; while break sensitivity was 100 g.

Free amino acids

The PICO TAG method, as described by Wang et al.[10] was used for the free amino acid profile of the coagulated soymilk. Coagulated soymilk was kept at 12 ℃ for 15 days to see the effect of cultures. 5 mL sample was homogenized in 100 mL double distilled water for three minutes and placed at refrigeration temperature over night. 1 mL from the middle portion of the solution, which was the almost clear portion of solution, was shifted to centrifuge tube and centrifuged at 10000 rpm for 10 minutes. The sample was derivatized with phenyl isothiocyanate. The HPLC running solution for gradient elution contained sodium acetate and methyl cyanide buffer at 6.4 pH. A sample size of 20 μl was injected into HPLC (Waters Systems USA) and analyzed with Water's PICO TAG amino acid analyzer. Conditions used were temperature: 38 ℃, pressure: automatic, rate of flow: 1 mL/min detector wavelength: 254 nm. External standards were used for identification and quantification.

Fatty acids

Coagulated soymilk was stored at 12 ℃ for 15 days to see the effect of cultures. Fat extraction of the samples was done with ethyl acetate, by following the method described by De Wit et al[11]. Ethyl acetate was subsequently evaporated under vacuum, lipids were dissolved in minimal volume of diethyl ether, transferred to screw-caped tubes, and dried to a constant weight in a vacuum oven over P_2O_5 at 50 ℃. 20 mg sample was taken into round bottom flask and mixed efficiently with 2 mL of 0.5 M KOH-methanol solution. The reaction mixture was heated to 70 ℃ for 10min, and then 3 mL of BF_3-methaol solution (Sigma-Aldrich, Shanghai, China) was added and kept at 70 ℃ for 5 minutes in water bath.

The reaction mixture was cooled to room temperature; fatty acid methyl esters were extracted with hexane and dried over anhydrous sodium sulfate, and concentrated by gentle stream of nitrogen.

A Hewlett-Packard 5890 gas chromatograph fitted with flame ionization detector (GC-FID) was used to analyze the fatty acids content of samples. The fatty acids were separated on Varian capillary column OV-351 (60 m ×0.32 mm) by using nitrogen as carrier gas. GC conditions were as follows: the column oven was held at 150 ℃ for 3 min, then raised to 210 ℃ at the rate of 2 ℃/min and run for 17 min. The split ratio was 1:50. The injector and the FID temperature were set as 250 ℃ and 300 ℃, respectively. Peaks in GC were identified by comparison of their retention times with those of known standards. Peak percentages and areas were calculated using Hewlett-Packard PC integration software (HP 3398A Chem Station Version A.01.01).

Statistical Analysis

Analysis of variance for the data of microbial growth (viable count), pH, acidity and sugars was performed by two-way ANOVA under factorial analysis and means were compared by Least Significant Difference Test at 0.05 level of significance by using SAS version 8.02 (SAS Institute Inc., Cary, NC, USA). FFA and texture were analyzed separately by the same method as described above but with one-way ANOVA. Duncan's Multiple Range Test to see the effects of cultures on individual free amino acid was performed. Four replicates obtained in two independent experiments for each sample in case of viable count, pH, acidity and sugars while three replicates were used for FAA, FFA and texture analysis. Microcal Origin V.7.0 was used to illustrate the results in figures.

Results

All the three LAB cultures (LC, LH and LR) showed significant increase in viable bacterial count (p<0.05) in soymilk during six hours (Figure 1). LR exhibited the best growth during first 5 hours but at 6th hour LC showed the best growth. LH showed the highest viable count at 0 hour but its growth rate was significantly low than that of other two cultures during the remaining period. The viable count of LC, LH and LR was 6.20, 6.26 and 6.31 log (CFU/mL) at 0 hour; but after six hours of fermentation it increased to 8.74, 7.69 and 8.69 log (CFU/mL), respectively.

All the three cultures showed significant decrease in pH (Figure 2) and increase in TA (Figure 3) during the 6 hours fermentation of the soymilk (p<0.05). GDL showed a significantly high change rate at 1st and 2nd hour with lowest pH and highest TA. The rate of change slowed down at 3rd and 4th hour while a negative trend was found at 5th and 6th hours in both pH and TA, resulting in significantly highest pH and lowest TA values at 6th hour (5.42 and 0.20% respectively). Among cultures, LC showed significantly highest TA and lowest pH at 6th hour but LR showed highest acidity and lowest pH at 3rd, 4th and 5th hours. LH showed significantly highest TA and lowest pH at 1st hours but lowest TA and highest pH at 3rd, 4th and 5th hour. After 6 hours of fermentation, pH values of samples with GDL, LC, LH and LR were 5.41, 5.23, 5.25 and 5.24; while TA values were 0.20, 0.24, 0.22 and 0.23 (respectively).

Sugar changes observed during six hours of fermentation are presented in Figure 4. Sucrose, stachyose and fructose were detected in soymilk fermented by all the three LAB cultures, while raffinose was not detected in all samples including soymilk. Sucrose levels decreased from 5.76 to 1.01, 5.76 to 5.12 and 5.76 to 5.042 (g/L) in the samples with LC, LH and LR, respectively. Stachyose decreased from 1.53 to 1.25 in samples with LC, 1.47 to 1.23 (g/L) with LH and 1.53 to 1.39 (g/L) in samples with LR. LH decreased fructose from 0.73 to 0.145 (g/L), while LC and LR increased it from 0.72 to 3.23 (g/L) and 0.80 to 1.24 (g/L), respectively. It was interesting to note that LC caused a noticeable decrease in sucrose contents of soymilk; a sudden decline was seen after 2 hours of fermentation while a sudden rise in fructose also occurred at the same time.

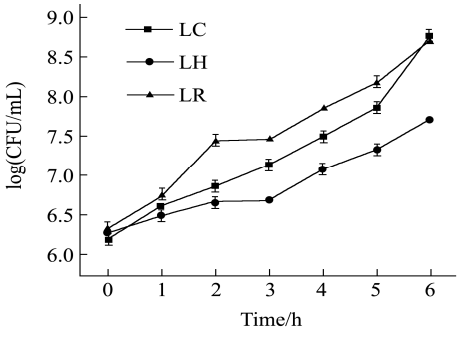

Fig.1 Changes in the viable count of Lactobacillus cultures in soymilk at 37 ℃ during six hours of fermentation

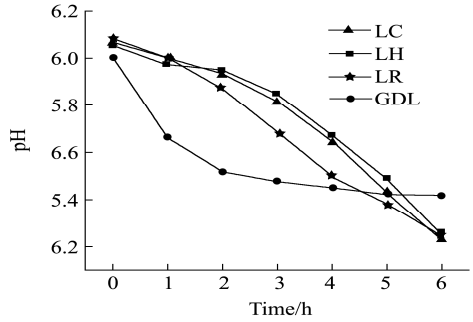

Fig.2 Changes in pH of soymilk at 37 ℃ during six hours of fermentation

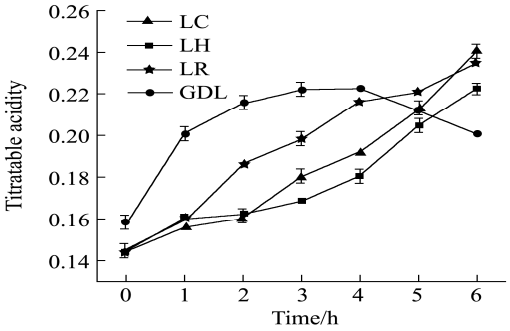

Fig.3 Titratable acidity changes in soymilk at 37 ℃ during the six hours of fermentation. Titratable acidity is expressed as percent of acetic acid in soymilk

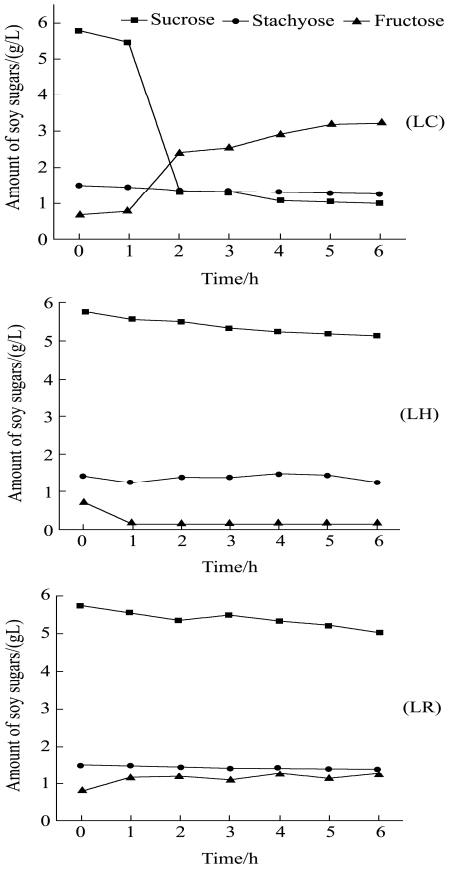

Fig.4 Soy sugar changes by LC, LH and LR during six hours fermentation of soymilk at 37 ℃

Formation of soymilk gel is an important step in curd and pehtze making. GDL was able to make soymilk gel in 4 hours, while LC and LR did the same in 6 hours but LH spent 7 hours to do the same (data not shown). We compared the gels made by selected *Lactobacillus* species on texture analyzer to study their ability of causing textural changes in soymilk. The results (Figure 5) showed that LH (1.14) caused maximum hardness followed by LR (1.135), GDL (1.017) and LC (1.017). Results of fracturability were identical to that of hardness. Springiness of the gel was the highest in the sample with LR (0.97) followed by LH (0.954), LC (0.953), and GDL (0.765). Cohesiveness was found to be the highest in samples with LR (0.566) followed by LC (0.550), LH (0.491) and GDL (0.477). LR (0.643) caused the highest gumminess followed by LH (0.560), LC (0.549), and GDL (0.485). Chewiness was also highest in samples with LR (0.624) followed by LH (0.535), LC (0.523), and GDL (0.353). LC showed the highest value for resilience followed by LR, GDL and LH with respective values of 0.22, 0.21, 0.199 and 0.197.

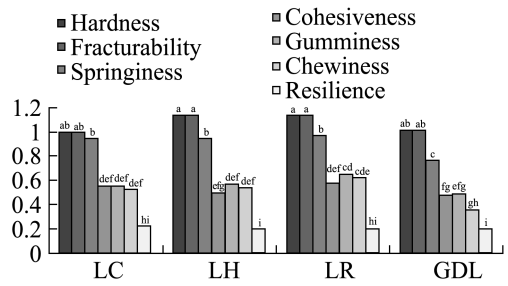

Fig.5 Textural changes produced in soymilk by LC, LH, LR and GDL at 37 °C. Values carrying different letters are significantly different (p<0.05)

FAA results (Table 1) clarify that all the treatments have the ability to cause FAA release. A total of 18 FAA were produced; LR showed the significantly highest amount of FFA followed by GDL, LC and LH (p<0.05). Within the treatments, LR showed the highest amount of *Try* followed by *Lys*, *Glu*, *Thr*, *Tyr*, *His*, *Phe* and *Ala* etc. GDL produced highest amounts of *Phe* followed by *Glu*, *Try*, *Tyr*, *Thr*, *His* and *Lys* etc. *Try* was the highest in LC inoculated soymilk while other prominent FAA were in the order of *Glu*, *Thr*, *Tyr* and *Phe* etc. LH showed highest production of *Arg* followed by *Glu*, *Thr*, *Asp* and *Ala* etc.

Table 1 Free amino acids (mg/100mL) produced by LC, LH, LR and GDL in soymilk. Values are the mean of 3 replicates. Values carrying different subscripts are significantly different (p<0.05) and subscripts present difference in individual FAA.

Free Amino Acids	LC	LH	LR	GDL
Asp	1.27^{c}	2.62^{a}	1.30^{b}	0.42^{d}
Glu	4.62^{d}	5.86^{b}	5.43^{c}	7.29^{a}
Ser	1.00^{a}	0.38^{c}	0.37^{d}	0.67^{b}
Gly	0.77^{b}	1.12^{a}	0.47^{d}	0.65^{c}
His	3.02^{c}	1.96^{d}	3.15^{b}	3.31^{a}
Arg	1.17^{d}	11.77^{a}	1.8^{b}	1.76^{c}
Thr	3.84^{b}	3.87^{a}	3.45^{d}	3.51^{c}
Ala	1.93^{c}	2.20^{b}	2.36^{a}	1.25^{d}
Pro	1.81^{a}	1.76^{b}	1.74^{c}	1.37^{d}
Tyr	2.91^{c}	1.68^{d}	3.33^{b}	3.73^{a}
Val	0.88^{c}	0.66^{d}	1.30^{a}	1.05^{b}
Met	0.54^{c}	0.59^{b}	1.24^{a}	0.50^{d}
Cys	0.05^{c}	0.20^{b}	0.45^{a}	0.05^{d}
Ile	0.36^{d}	0.46^{c}	1.13^{a}	0.87^{b}
Leu	0.84^{c}	0.41^{d}	1.52^{b}	1.56^{a}
Try	20.61^{b}	0.96^{d}	20.97^{a}	6.28^{c}
Phe	2.66^{b}	0.62^{d}	2.37^{c}	17.93^{a}
Lys	1.77^{c}	1.03^{d}	10.18^{a}	3.21^{b}
Total	50.05^{c}	38.13^{d}	62.62^{a}	55.38^{b}

Table 2 Fatty acid profiles of soy curd coagulated with LC, LH, LR and GDL. Values are mean of 3 replicates and presented as the percent amount of the esters identified by GC-FID analysis. Values carrying different subscripts are significantly different (p<0.05). nd = not detected

Free fatty acids	LC	LH	LR	GDL
C14:0	2.2654^{u}	1.9054^{v}	1.6836^{w}	0.1375^{z}
C16:0	13.8808^{i}	12.9998^{k}	13.5352^{j}	10.9056^{l}
C16:1	nd $^{/}$	nd $^{/}$	0.4631^{yz}	0.1129^{z}
C18:0	6.8105^{p}	6.1158^{r}	6.5958^{q}	4.7015^{s}
C18:1	20.7282^{f}	20.6729^{g}	20.6088^{h}	22.2074^{e}
C18:2	46.5862^{d}	50.5293^{b}	49.3149^{c}	52.6833^{a}
C18:3	6.7993^{q}	7.7768^{n}	7.2121^{o}	7.8767^{m}
C20:0	2.9295^{t}	nd $^{/}$	nd $^{/}$	0.4421^{z}
C20:1	nd $^{/}$	nd $^{/}$	nd $^{/}$	0.2243^{z}
C22:0	nd $^{/}$	nd $^{/}$	nd $^{/}$	0.5017^{y}
C22:1	nd $^{/}$	nd $^{/}$	0.5865^{x}	0.0926^{z}
C24:0	nd $^{/}$	nd $^{/}$	nd $^{/}$	0.1144^{z}

GC-FID analysis for FA resulted in the identification of 7, 6, 8 and 12 types of FA by the action of LC, LH, LR and GDL in soymilk respectively. Percent amounts of FFA as identified in ester forms are given in Table 2. All the amounts were found significantly different (p<0.05). Six FA i.e. C14:0 C16:0, C18:0, C18:1, C18:2 and C18:3 were common in all treatments. LC contained C20:0; LR contained C16:1 & C22:1 and GDL contained C16:1, C20:0, C20:1, C22:0, C22:1 & C24:0 as additional FA. C18:2 exhibited the highest percentage in all the treatments followed by C18:1, C16:0, C18:3, C18:0 and C14:0.

Discussions

The selected *Lactobacilli* showed good growth in soymilk. Many other studies also have indicated that soy is a suitable media for LAB cultures to grow[4,12]. Recent studies have shown that LAB cultures use FAA for their metabolism so their growth rate is also dependent on the liberation of FAA and a delay of 2 to 4 hours was seen due to late release of FAA[5,9]. However, other factors such as reduction of the free oxygen level in the media and production of acidity also act as growth promoters. It is clear from the results that LC and LR were able to grow more efficiently during 6 hours fermentation period because these are well-known probiotic cultures. Some studies have shown that probiotic cultures have better adaptability in soymilk possibly due to ability of soy sugar utilization[6,9], more data are needed to further explain the fact. LC used more soy sugars, which may be a reason of its better growth. On the other hand increase in TA and decrease in pH with the passage of time shows that acidity production promoted the growth in two ways. I- it positively changed the media conditions suitable for the growth of LAB cultures. II- it caused FAA release by the hydrolysis of soy proteins, which are required by LAB cultures for necessary metabolic process. This increase in acidity was principally caused by the cultures themselves through their enzyme systems and they were using soy sugars as energy source during this process. As LH used less sugars and didn't carry out this process efficiently so its growth rate was slower unless the FAA were librated later on. Generally, a good growth rate was seen in all the cultures during the later stages of fermentation due to the high acidity and liberation of FAA. GDL is a common coagulation agent to make tofu form soymilk but it is used at high temperatures. In our study, GDL showed the ability

of causing the same kind of effect at the level of 0.3% at 37 ℃ but took longer time. Therefore the findings of present study suggest that GDL can be used as coagulant along with LAB starter cultures for soy cheese production.

The basic sugars present in the soybean are sucrose, raffinose and stachyose. LC and LR exhibited similar trends in soy sugars utilization i.e. decrease in sucrose and stachyose and increase in fructose. But magnitude of sucrose reduction and fructose increment was high in LC than that of LR. LH caused decrease in sucrose and stachyose with low tempo but it utilized all of fructose in 1 hour. All of these changes were found significant at $P < 0.05$. Over all, LC caused more changes in soymilk sugar followed by LR and LH.

According to Wang et al[10]. the concentrations of soy sugars in raw soybean are: sucrose 4.30%, raffinose 0.75% and stachyose 4.13% (dry basis). While some other studies also have shown a considerable variation in the raffinose (0.3% to 0.9%) and stachyose (1.1% to 4.2%) content among genotypes of soybeans[13]. Ruiz-Teran and Owens [14] reported that soaking and cooking of soybeans remove soy sugars mainly oligosaccharides. Wang et al[10]. also reported losses of soy sugars during soaking and boiling. In our experiment, about 225 g of soybean was used to produce 1 liter of soymilk. The low level of sugars and non-detection of raffinose in the present study may be due to losses of sugars during soaking and autoclaving; Autoclaving has more potential to cause loss of soy sugars because effect of heat is multiplied in the presence of pressure. Pujola` et al[15]. not only confirmed these losses but also stressed on more losses of raffinose during soaking and cooking of beans.

LC used more sucrose than other two cultures and a considerable amount of fructose was also produced. This increasing amount of fructose confirms that sucrose was converted into fructose and glucose by the enzymatic action. As glucose was not detected, so it might be utilized by LC as energy source. However, a slight decrease in stachyose confirms that LC also used it. Data indicates that LC and LR were capable of using sucrose and stachyose but didn't show a significant usage of fructose, while LH used it significantly. Sugar utilization behavior of these cultures envisages that these cultures may be suitable to be used in combination. Because the fructose produced by LC and LR, will be utilized by LH for better growth and all will benefit from the FAA produced in the medium too.

The selected three cultures caused significant textural changes in soymilk due to their Proteolytic enzymes and production of acids during fermentation. Therefore, on the basis of textural properties of gels, we can conclude that the coagulation by these LAB cultures was efficient, competitive to commercial coagulant GDL and they can be used for soy cheese production.

Varying amounts of 18 FAA, released by LC, LH, LR and DGL, indicate the significant difference in treatments. All the treatments had the ability to produce *Asp*, *Ile*, *Leu*, *Met*, *Phe*, *Tyr* and *Val* in soymilk. These FAA are precursors of many flavoring compounds, which are produced as a result of the metabolic activity of LAB cultures during ripening of the cheese[5,16]. Among flavoring FAA, GDL showed high amounts of *Phe*, *Tyr* and *Leu*; LR released more *Val, Met and Ile* and LH exhibited high amount of *Asp*. Less amounts of total FAA in case of LH and LC were due to utilization of FAA during their metabolism activity, which resulted in the formation of certain aromatic and flavoring compounds. Therefore, after 6 hours fermentation at 37 followed by 15 days fermentation at 12 ℃, a noticeable pleasant change was observed in aroma and flavor of soy curd (data not shown). Due to key role of these amino acids in the cheese flavor production[17], a suitable combination of these treatments may be used to produce soy cheese, which may cause flavor improvement.

All the treatments have the ability to change FA content of soymilk and these changes can be helpful to produce flavoring compounds during ripening fermentation[17]. Disappearance of FA in soy curds (C16:1, C20:1, C22:0, 22:1, C24:0 in case of LC; C16:1, C20:0, C20:1, C22:0, 22:1, C24:0 in LH and C20:0, C20:1, C22:0, C24:0 in LR) after 6 hours fermentation at 37 ℃ followed by 15 days fermentation at 12 ℃, shows that these were utilized by the respective *Lactobacilli*. A noticeable pleasant change in aroma and flavor of soy curd (data not shown) supports this idea.

Conclusions

LC, LH, LR and GDL have the ability to coagulate soymilk at 37 ℃. Acidity (in case of LC, LH, LR and GDL) and protease enzymes (in case of LC, LH and LR)

hydrolyze soy proteins to cause textural changes required for coagulation; while soy sugars were utilized as energy source during this process. All of the treatments were able to produce 18 FAA including 7 flavor producing FAA. However, LC and LH showed less FAA because these were metabolized during fermentation period. LC, LH and LR were capable of utilizing FA and a significant change was observed among different treatments. Some FA disappeared from soy curd showing their complete utilization by respective *Lactobacilli*. LC, LH and LR were found suitable for soy cheese production on the basis of textural and flavor precursors producing and utilizing properties; however, LH and LC showed better overall effect.

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