Kor. J. Appl. Microbiol. Bioeng.
Vol. 13, No. 3, 191 - 198 (1985)

Lactic Acid Fermentation of Lupinseed Milk

Ouk Han, Won Taik Tae, Young Wook Kim, Joon Kyoung Lee and Cherl Ho Lee

Department of Food Technology, Korea University, Seoul, Korea
(Received June 10, 1985)

루우핀 豆乳의 乳酸醱酵에 関한 研究

韓 億・太源澤・金永旭・李濬京・李哲鎬

高麗大学校 食品工学科
(1985년 6월 10일 수리)

Seven different strains of lactic bacteria and 13 combinations of these microorganisms were tested for their acid forming capacity on a vegetable milk made from lupinseed protein concentrate (LPC). *L. acidophilus, L. casei, S. lactis, L. mesenteroides*, mixed *culture* of L. acidophilus and *S. thermophilus*, and mixed culture of *S. lactis* and L. mesenteroides were selected and further tested for their growth pattern and acid forming property on lupinseed milk both untreated and partly hydrolized one with carbohydrate decomposing enzymes. The enzyme hydrolized lupinseed milk had 1.5 folds of total free sugar; 8.2 folds of fructose, 3 folds glucose, 2.3 folds maltose, compared to the untreated lupinseed milk. For the untreated lupinseed milk, *L. mesenteroides* was appeared to be most suitable microorganism having the maximum cell concentration of 1.0×10^9/ml and the final pH 4.40 with the acidity 0.46%. For the enzyme treated lupinseed milk, mixed culture of *L. acidophilus* and *S. thermophilus* showed the best performance having 1.9×10^9 /ml maximum cell number and the final pH and acidity were 3.69 and 1.13%, respectively. Lactic acid fermentation altered the physical property of lupinseed milk; by fermentation the viscosity generally increased with untreated lupinseed milk, but decreased with enzyme hydrolized one. The viscosity change and sedimentation rate of fermented milk varied with the type of lactic bacteria. The results of sensory evaluation indicated that *S. lactis, L. casei*, mixed culture of *S. lactis* and *L. mesenteroides*, and mixed culture of *L. acidophilus* and *S. thermophilus*, grown on enzyme hydrolized lupinseed milk, could produce acceptable lactic acid beverage.

The consumption of yogurt type lactic beverages is growing fast in Korea. The history of the use of yogurt in Korea is not longer than 15 years. It has been introduced as a liquid type drink and become a popular fermented milk drink. The annual production of yogurt in Korea was 116,200 M/T in 1983, and comparing to the total production of city milk, 448,200 M/T, in the same year, the consumption of yogurt type product is very significant.[1]

Recently, production of yogurt type beverages from other substrates than milk has been widely attempted. The most studied substrate was soybean milk.[2,3] However, the acceptability of lactic fermented soybean milk is generally very low, because of the objectional flavor inherent to the bean. Lupinseed protein concentrate (LPC), which is made to eliminate objectionable flavor, color and fat soluable fractions from lupinseed kernel, can be a good substrate for this purpose.[4] It has another advantage over soybean that more abundant saccharides are available for the growth and acid formation of lactic bacteria.

In the present study, several strains of lactic bacteria and their combinations were tested for their ability to produce acceptable lactic acid beverage from LPC based milk.

Materials and Methods

Microorganisms

Seven strains of lactic bacteria, as listed below, were obtained from the Korean Federation of Culture Collections of microorganisms (KFCC).

1) *Lactobacillus acidophilus* IAM 1043
2) *Lactobacillus bulgaricus* IFO 13953
3) *Lactobacillus casei* IFO 3425
4) *Lactobacillus fermenti* IFO 3071
5) *Streptococcus lactis* ATCC 11454
6) *Streptococcus thermophilus* IFO 13957
7) *Leuconostoc mesenteroides* IFO 12060

The culture was activated in MRS broth medium and then maintained in MRS (de Man, Rogosa and Sharpe) agar slant culture.

Preparation of Lupinseed Milk

Lupinseed protein concentrate was prepared by the method of Lee et al.[4] The extraction condition was with solvent composition (hexane: alcohol: water) 10:2.5:2.5 and solvent-flour ratio 7.5:1. Lupinseed milk was prepared by using a modified method of Lee et al.[4] (Figure 1). In order to increase free sugar content of the substrate for lactic fermention, lupinseed milk was treated with a carbohydrate decomposing enzyme SP249 (Novo Co., Denmark).[5] The pH of the milk was adjusted to 5.5 and the substrate-enzyme ratio was 100:1. The mixture was incubated at 50°C for 6 hrs, and the treatment was terminated by heating in boiling water for 5 min.

LPC 7 parts + Water 93 parts
↓
Mixing (Waring blendor)
6,000 rpm, 2 min.
12,000 rpm, 1 min.
↓
Heating treatment (95℃, 5 min.)
↓
Mixing (Waring blender)
6,000 rpm, 2 min.
12,000 rpm, 1 min.
↓
Homogenizing (Gaulin homogenizer)
3,500/500 psi, 2 pass
↓
Lupinseed Milk

Fig. 1. Schematic diagram for the preparation of lupinseed milk from LPC.

Table 1. Operating condition of HPLC for free sugar analysis.

Instrument;	Gradient HPLC334 (Beckman, U. S. A.)
Column;	Lichrosorb NH_2 (E. Merk)
Mobile phase;	Acetonile: Water = 80 : 20 (V/V)
Flow rate;	1.5ml/min
Chart speed;	1.0cm/min
Detector;	RI detector (Shodex, Japan)
Attenuator;	4 X
Sample load;	20μl/injection

Fermentation

Both untreated and enzyme treated lupinseed milks were inoculated with 3%(v/v) of seed culture grown in 10% skim milk medium. The lactic fermentation was performed at the optimum growth temperatures of each strain; *L. acidophilus*, *L. casei* and mixed culture of *L. acidophilus* and *S. thermophilus* were at 37°C, *S. lactis*, *L. mesenteroides* and mixed culture of *S. lactis* and *L. mesenteroides* at 30°C. The changes in pH and acidity of lupinseed milk during fermentation were measured. The acidity was calculated as the concentration of lactic acid in the milk. The number of cells in the culture was monitored by the viable cell counts grown on the tomato juice agar plate.

HPLC Analysis

The concentrations of free sugars in the untreated and enzyme treated lupinseed milk were measured by high pressure liquid chromatography (HPLC). The free sugars were identified by using rhamnose, xylose, fructose, glucose, sucrose and maltose as the standard sugars. Table 1 shows the conditions for free sugar analysis by HPLC.

Physical Measurements

The changes in viscosity of lupinseed milk were measured by cylindrical viscometer (Brookfield syncrolectric viscometer, LVT with UL adapter). The changes in the torque with various spindle rotational speed were measured. The flow behavior was analysized by determining the consistency index and flow behavior index with Herschel-Bulkley equation and yield stress with Casson equation.

For the determination of sedimentation property, lupinseed milk samples were mixed with same amount of distilled water, homogenized and settled in 100 ml mess cylinder for 10 hrs. The sedimentation curve was used as a parameter for the dispersion stability of the milk.

Table 2. Proximate chemical composition of LPC. (Dry basis)

Moisture	6.28 (%)
Crude protein	50.22
Crude fat	2.40
Crude ash	3.04
Carbohydrate (by difference)	38.06

Sensory Evaluation

The acceptability of lupinseed lactic beverages fermented for 24 hrs was tested by a trained sensory panel. The panel was made by 7 graduate students of the Department of Food Technology, Korea University. The overall flavor acceptability and color were evaluated by ranking test. Sourness, bitterness, beany flavor and sweetness were compared by descriptive profile test.

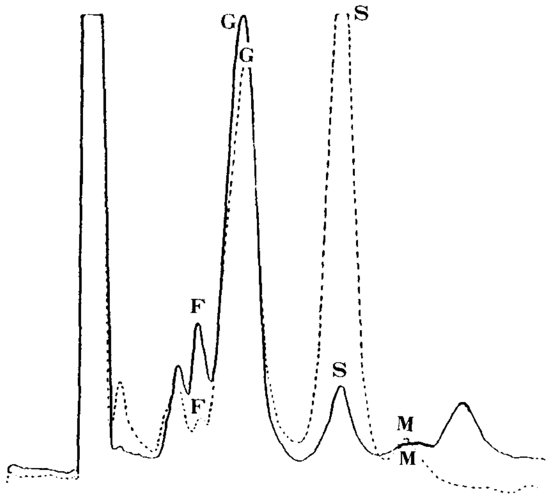

Fig. 2. HPLC chromatogram of free sugars in lupinseed milk.
F: Fructose, S: Sucrose, G: Glucose and M: Maltose
Enzyme treatment ———, Control ------

Results and Discussion

Chemical Composition of LPC

The proximate chemical composition of LPC used in this study is shown in table 2. It contained 50% protein, 38% carbohydrate and 2.4% crude fat. The total free sugar content of lupinseed milk made by 7% LPC was 3.56 mg/ml. It increased to 5.53 mg/ml by SP249 enzyme treatment, as shown in table 3. By enzyme treatment, the concentration of fructose increased 8.2 folds, glucose 3 folds and maltose 2.3 folds, compared to those of untreated lupinseed milk. Figure 2 shows the HPLC chromatogram for free sugars in lupinseed milk samples.

Selection of Useful Microorganisms

A total of 7 single strains and 13 combinations of these microorganisms were tested for their acid forming capacity. For the rapid selection, only pH change was measured for 24 hrs of fermentation. Table 4 shows the reduction of pH by fermentation with different microorganisms in lupinseed milk. In general, the enzyme treatment of lupinseed milk accelerated the reduction of pH. From these results, 5 strains of lactic bacteria were selected, and made into six test group of microorganisms as below.

(1) *L. acidophilus*
(2) *L. casei*
(3) *S. lactis*
(4) *L. mesenteroides*
(5) *L. acidophilus and S. thermophilus*
(6) *S. lactis and L. mesenteroides*

Growth Pattern of Microorganisms in Lupinseed Milk

Fig. 3-5 show the typical growth pattern of selected microorganism in lupinseed milk, both untreated and enzyme hydrolized ones. In general, *lactobacillus* species and *S. lactis* grow faster in enzyme treated lupinseed milk than untreated one. However, *Leuconostoc mesenteroides* increased better in untreated lupinseed milk. When mixture of *S. lactis* and *L. mesenteroides* was inoculated, the initial growth rate was similar in both untreated and enzyme treated samples. But at the later stage, a rapid increase in cell number was observed in enzyme treated sample. This may

Table 3. Free sugar contents of lupinseed milk.

Samples	Free sugar content (mg/ml)					
	Rhamnose	Fructose	Glucose	Sucrose	Maltose	Total
Control	–	0.05	1.49	1.55	0.07	3.56
Enzyme treatment	trace	0.41	4.42	0.54	0.16	5.53

Table 4. pH changes in lupinseed milk by various combinations of lactic acid bacteria.

Combinations	Control 0	Control 12 (hrs.)	Control 24	Enzyme treatment 0	Enzyme treatment 12 (hrs.)	Enzyme treatment 24
1) L. a	5.61	4.70	4.70	5.40	4.48	3.77
2) L. b	5.61	5.30	4.80	5.40	5.02	4.91
3) L. c	5.61	5.04	5.04	5.40	3.95	3.71
4) L. f	5.61	5.08	4.63	5.40	4.24	4.24
5) S. l	5.61	5.54	4.20	5.40	5.21	4.19
6) S. t	5.61	5.04	4.77	5.40	4.60	3.89
7) Le. m	5.61	5.30	4.60	5.40	5.10	4.70
8) L. a+S. t	5.61	4.85	4.80	5.40	4.20	3.75
9) L. b+S. t	5.61	4.95	4.69	5.40	4.70	3.77
10) L. b+L. c	5.61	4.98	4.90	5.40	4.85	4.85
11) L. b+S. l	5.61	5.21	5.21	5.40	5.16	5.16
12) L. c+Le. m	5.61	5.28	5.21	5.40	5.04	5.04
13) L. f+S. t	5.61	4.61	4.53	5.40	4.22	4.18
14) L. f+Le. m	5.61	4.80	4.66	5.40	4.30	4.25
15) S. l+S. t	5.61	5.39	4.82	5.40	5.00	5.00
16) S. l+Le. m	5.61	5.40	4.50	5.40	5.16	4.40
17) S. t+Le. m	5.61	4.98	4.93	5.40	4.41	3.85
18) L. b+S. l+Le. m	5.61	5.31	5.08	5.40	5.19	4.68
19) L. f+S. l+Le. m	5.61	5.05	4.60	5.40	5.17	4.65
20) L. f+S. t+Le. m	5.61	4.75	4.75	5.40	4.10	4.10

* L. a : *Lactobacillus acidophius* L. b : *Lactobacillus bulgaricus* L. c : *Lactobacillus casei*
L. f : *Lactobacillus fermenti* S. l : *Streptococcus lactis*
S. t. : *Streptococcus thermophilus* Le. m : *Leuconostoc mesenteroides*

Table 5. Growth and acid production by lactic acid organisms in lupinseed milk.

Organism	Control pH*	Control Total acid* %	Control Viable counts/ml**	Enzyme treatment pH*	Enzyme treatment Total acid* %	Enzyme treatment Viable counts/ml**
Intitial range	5.51	0.19	—	5.42	0.29	—
L. acidophilus	4.50	0.38	5.60×10^8	3.70	1.00	1.70×10^9
L. casei	4.60	0.37	4.10×10^8	3.70	1.01	7.00×10^8
S. lactis	4.45	0.36	1.50×10^8	4.15	0.57	4.00×10^8
L. mesenteroides	4.40	0.46	1.00×10^9	4.30	0.48	2.30×10^8
L. acidophilus+S. thermophilus	4.43	0.41	8.00×10^8	3.69	1.13	1.90×10^9
S. lactis+L. mesenteroides	4.40	0.37	5.40×10^8	4.25	0.65	8.30×10^9

* pH and titratable acidity were determined after 24hr.
** The viable counts represent the maximum attained during the 24-hr incubation period.

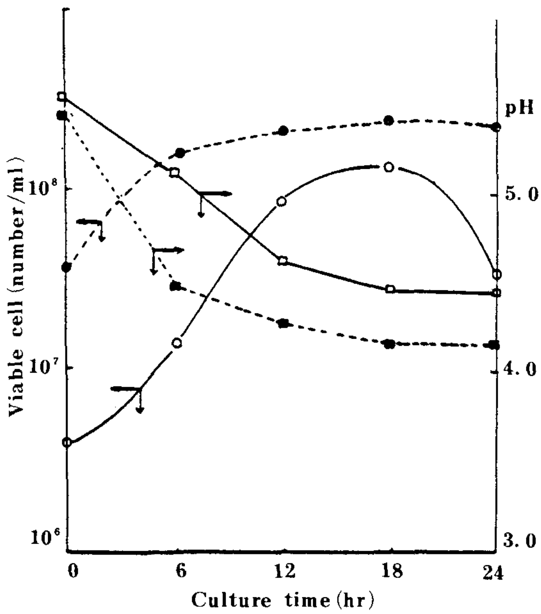

Fig. 3. Changes in bacterial numbers and pH during fermentation of lupinseed milk with *S. lactis*.

——— Control, -------- Enzyme treatment

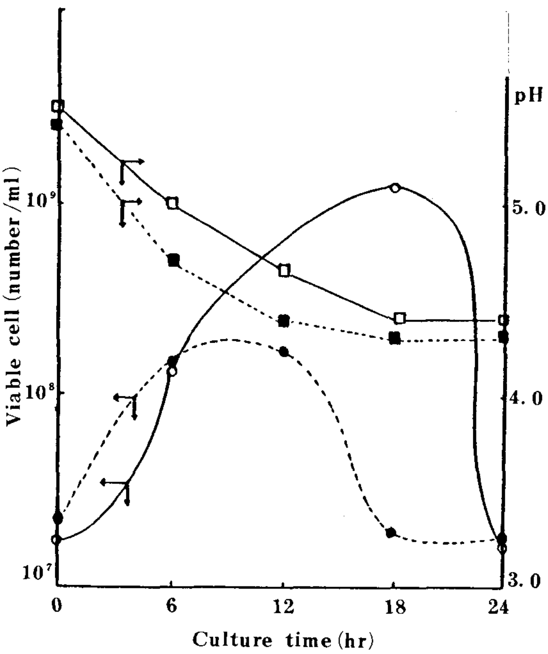

Fig. 4. Changes in bacterial numbers and pH during fermentation of lupinseed milk with *L. mesenteroides*.

——— Control, -------- Enzyme treatment

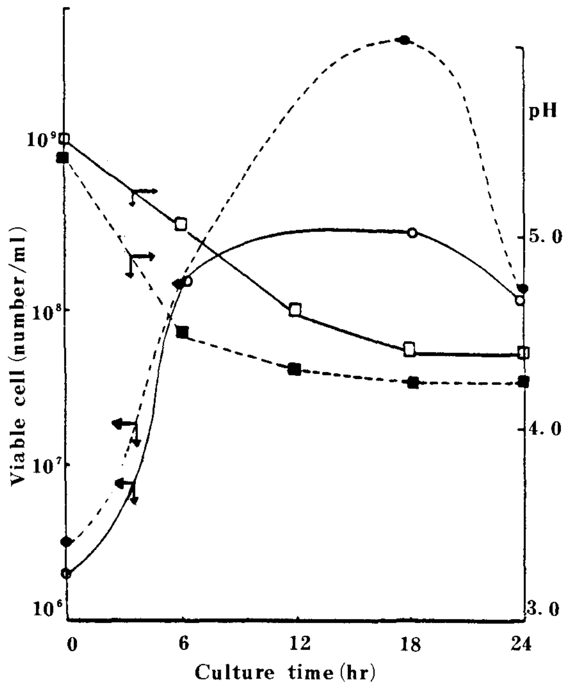

Fig. 5. Changes in bacterial numbers and pH during fermentation of lupinseed milk with mixed culture of *S. lactis* and *L. mesenteroides*.

——— Control, -------- Enzyme treatment

indicate that the initial growth of *L. mesenteroides* accelerates the growth of *S. lactis*. The reduction of pH coincided with the growth of microorganisms in lupinseed milk, and also with the increase in acidity, as shown in Table 5.

For untreated lupinseed milk, *L. mesenteroides* recorded the lowest pH, highest acidity and the largest number of viable cells, 1.0×10^9/ml. Enzyme treatment of lupinseed milk did not improve the performance of this organism. This can be explained by the fact that *L. mesenteroides* is able to utilize arabinose, maltose, raffinose, ribose and sucrose, which may be contained more in untreated lupinseed milk and are not well utilized by *lactobacillus and S. lactis*.[6,7] Since *L. mesenteroides* is heterofermentative organism, the reduction of pH and the increase in acidity may not be entirely due to the lactic acid formation. It is also interesting to note that *L. mesenteroides* plays a major role in the fermentation of Kimchi; the Korean traditional fermented vegetable pickle. When lupinseed milk was partly hydrolized by enzyme treatment prior to lactic fermentation, the mixed culture of *L. acidophilus* and *S. thermophilus* recorded the best performance, in which the pH reached to 3.69, acidity to 1.13% and the mximum number of viable cell reached to 1.9×10^9/ml. The mixed culture of *S. lactis* and *L. mesenteroides*

Table 6. Changes in yield stress, consistency index, and flow behavior index of lupinseed milk before and after 24 hr incubation with various lactic acid bacteria.

Organism	c	b	s
Control	597.00	112.38	0.9017
L. acidophilus	982.20	827.94	0.5693
L. casei	720.95	840.41	0.4543
S. lactis	921.00	1051.96	1.0090
L. mesenteroides	802.29	676.71	0.4425
L. a+S. ther.	404.23	234.53	0.6865
S. l. +L. mes.	971.08	444.63	0.9644
Enzyme treated	355.94	133.08	0.8915
L. acidophilus	148.61	253.22	0.5808
L. casei	175.03	293.56	0.5053
S. lactis	6.00	10.64	1.0520
L. mesenteroides	6.64	12.53	0.7797
L. a+S. ther.	399.08	447.10	0.4758
S. l. +L. mes.	329.68	207.25	0.6922

c: yield stress ($dyne/cm^2$)
b: consistency index ($dyne \cdot sec/cm^2$)
s: flow behavior index (dimensionless)

showed the greatest number of viable cell, 8.30×10^9, although the acid formation was not so high.

Physical Property of Lactic Fermented Lupinseed Milk

Both untreated and enzyme treated lupinseed milk exhibited Bingham-Pseudoplastic flow behavior. In the case of untreated lupinseed milk, lactic fermentation generally increased the yield stress and consistency index. On the other hand, in the case of enzyme treated lupinseed milk, lactic fermentation decreased the yield stress, as shown in Table 6.

Lactic fermentation increased the apparent viscosity of untreated milk, but decreased that of enzyme treated one. Especially *S. lactis* and *L. mesenteroides* decreased sharply the apparent viscosity, yield stress and consistency index of enzyme treated lupinseed milk. The explanation of this phenomena needs further investigation.

Fig.6 shows the sedimentation curve of lactic fermented lupinseed milks. In general, enzyme treated samples exhibited rapid sedimentation. This may be due to the reduction of viscosity and partial solubilization of insoluble particles by enzyme hydrolysis and lactic fermentation. The changes of pH will influence the sedimentation property,

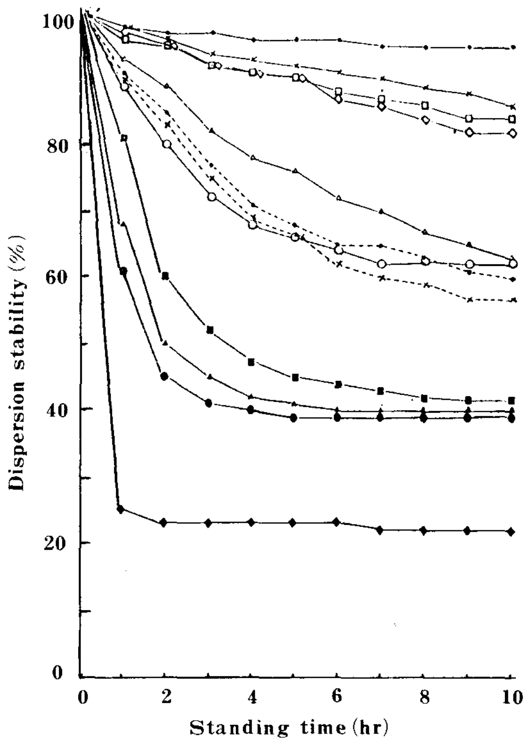

Fig. 6. Changes in dispersion stability of fermented lupinseed milk with various lactic acid bacteria.

Control	Enzyme treatment	
○—○	●—●	*L. acidophilus*
△—△	▲—▲	*L. casei*
□—□	■—■	*S. lactis*
◇—◇	◆—◆	*L. mesenteroides*
•—•	•···•	*L. acidophlus+ S. thermophilus*
×—×	×···×	*S. lactis + L. mesenteroides*

because the isoelectric point of lupinseed protein is in the vicinity of pH 4.5. The amount of soluble material and sedimented solid must be determined later in order to explain clearly the dispersion stability of the samples.

Sensory Quality

The ranking test for the overall flavor acceptability showed that *S. lactis* grown in enzyme treated lupinseed milk marked the first rank. For untreated lupinseed milk mixed strain of *S. lactis* and *L. mesenteroides* yielded the most acceptable product, as analyzed by Duncan's multiple range test shown in Table 7-1.

In general untreated lupinseed milk yielded poor quality of lactic beverage except for the one fermented with mixed culture of *S. lactis* and *L. mesenteroides*. No significant dif-

Table 7. Results of Duncan's multiple range test for the flavor of lupinseed milk fermented with different lactic bacteria.

7 − 1. Overall flavor acceptability by ranking test

S. lactis	*L. casei*	*S. lactis* + *L. mesen*	*S. lactis* + *L. mesen*	*L. acido.* + *S. thermo.*	*L. mesen.*	*L. acido.*	*S. lactis*	*L. mesen.*	*L. acido.*	*L. acido.* + *S. thermo.*	*L. casei*
(E)	(E)	(C)	(E)	(E)	(E)	(E)	(C)	(C)	(C)	(C)	(C)

level of significance P <0.01

7 − 2. Sour taste by profile test

L. acido. + *S. thermo.*	*L. casei*	*L. acido.*	*L. casei*	*L. acido.*	*S. lactis* + *L. mesen.*	*L. mesen.*	*L. mesen.*	*L. acido* + *S. thermo.*	*S. lactis* + *L. mesen.*	*S. lactis*	*S. lactis*
(E)	(E)	(E)	(C)	(C)	(E)	(E)	(C)	(C)	(C)	(E)	(C)

(level of significance P< 0.01

7 − 3. Bitter taste by profile test

L. acido + *S. thermo.*	*S. lactis*	*S. lactis* + *L. mesen.*	*L. acido.*	*L. casei*	*L. mesen.*	*L. acido.* + *S. thermo.*	*S. lactis* + *L. mesen.*	*S. lactis*	*L. acido.*	*L. casei*	*L. mesen.*
(E)	(E)	(E)	(E)	(E)	(E)	(C)	(C)	(C)	(C)	(C)	(C)

(level of significance P< 0.01

* C: No enzyme treatment
E: Enzyme treated lupinseed milk

ference was found in the color of fermented samples.

Significant differences was found in sour taste and bitter taste between samples, but no significant difference was noticed in beany taste and sweet taste. The mixed culture of *L. acidophilus* and *S. thermophilus*, *L. casei* and *L. acidophilus* in enzyme treated lupinseed milk yielded very strong sour taste, as shown in Table 7-2. The beany flavor of the samples were in general very weak. The mixed culture of *L. acidophilus* and *S. thermophilus*, *S. lactis* and the mixed culture of *S. lactis* and *L. mesenteroides* in enzyme treated sample recorded slight bitterness. (Table 7-3) However, this might partly be influenced by the strong sour taste of the samples. The panel hardly recognized sweet taste in the samples.

Conclusion

Lupinseed milk made from LPC can be utilized as the substrate for lactic fermentation to produce vegetable yogurt type beverages. The enzyme hydrolysis of lupinseed milk prior to lactic fermentation accelerates the growth and acid formation of lactic bacteria. It also improve the physical property and sensory acceptability of the product. *S. lactis*, *L. casei*, the mixed culture of *S. lactis* and *L. mesenteroides* and the mixed culture of *L. acidophilus* and *S. thermophilus* gave promising results to produce lactic beverages from enzyme treated lupinseed milk. *L. mesenteroides* showed interesting characteristics to utilize untreated lupinseed milk for lactic acid production. Further study with these microorganisms on their product acceptability and physical stability is needed.

요 약

루우핀콩 蛋白質 濃縮物(LPC)을 利用한 豆乳에 7 가지 乳酸菌과 13가지 乳酸菌 組合을 接種하여 酸生成能力을 檢討하고 루우핀 豆乳에 炭水化物 分解 酵素로 処理한 것과 하지 않은 것의 差異를 살펴보았다. 그 結果, ***L. acidophilus*, *L. casei*, *S. lactis*, *L. mesenteroides*, *L. acidophilus*와 *S. thermophilus*의 混合菌과 *S. lactis*와 *L. mesenteroides*** 의 混合菌을 選定하여 炭水化物 分解酵素로 処

理한 루우핀 豆乳와 処理하지 않은 루우핀 豆乳에서의 乳酸菌의 生育과 酸生成 能力을 調査하였다. 酵素処理에 依한 루우핀 豆乳의 糖組成은 処理하지 않은 루우핀 豆乳에 比하여 Fructose가 8.2倍, Glucose가 3.0倍 그리고 maltose가 2.3倍였으며 總遊離糖 含量은 1.5倍였다.

酵素処理하지 않은 루우핀 豆乳에서는 *L. mesenteroides*가 最大 生菌数 1.0×10⁹/ml, 最終 pH 4.40 및 最終 酸度 0.46%를 나타내어 가장 優秀하였으며 酵素 処理한 루우핀 豆乳에서는 *L. acidophilus*와 *S. thermophilus*의 混合菌이 最大 生菌数 1.9×10⁹/ml, 最終 pH 3.69 및 最終 酸度 1.13 %를 보여주어 가장 뛰어난 乳酸菌 組合임을 나타내었다.

乳酸 醱酵를 通한 루우핀 豆乳의 物理的 性質의 変化를 살펴보면 大体로 醱酵가 進行됨에 따라 酵素 処理하지 않은 루우핀 豆乳 基質에서는 粘度가 增加하였고 酵素処理한 基質에서는 減少하는 傾向을 나타내었다. 또한 醱酵된 루우핀 豆乳의 粘度 変化와 沈澱速度는 乳酸菌에 따라 多樣함을 보여주었다.

官能 検査 結果에서 酵素 処理한 루우핀 豆乳를 *S. lactis* 및 *L. casei*로 倍養한 것과 *S. lactis*와 *L. mesenteroides* 및 *L. acidophilus*와 *S. thermophilus*의 混合菌으로 倍養한 것이 비교적 優秀한 風味를 지녔으며 乳酸菌 飮料로서의 使用 可能性이 確認되었다.

References

1. Lee, C.H.; Present status and development of lactic acid fermented foods in Korea. UNIDO Symposium on Lactic Acid Fermentation. Nov. 26-27, Mexico City, Mexico. (1984)
2. Angels, A.G. and E.H. Marth; Growth and activity of lactic-acid bacteria in soymilk, 1. Growth and acid production, J. Milk Food Technol., **34**,30 (1971)
3. Mital, B.K. and K.H. Steinkraus; Utilization of oligosaccharides by lactic acid bacteria during fermentation of soy milk. J. Food Sci., **40**,114 (1975)
4. Lee, C.H., C.S. Kim and S.H. Oh; Utilization of Australian lupinseed in Korean food system, Third half-yearly progress report, The Grain Pool of Western Australia (1983)
5. Lee, C.H., C.S. Kim and S.H. Oh; Utilization of Australian lupinseed in Korean food system, Fourth half-yearly progress report, The Grain Pool of Western Australia (1984)
6. Bergey's Manual of Determinative Bacteriology, 8th ed. The William & Wilkins Co., altimore (1974)
7. Mitsuoka, T.; Medical effect lactic acid and a new field of utilization. Nippon Shokuhin Kogyo Gakkaishi, **31**,285 (1985)