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Sensory Profiles of Koumiss with added Crude Ingredients extracted from Flaxseed (*Linum usitatissimum* L.)

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Abstract

Recently, the health-promoting effects of functional foods have been shown to prevent nutrition-related diseases, improve physical and mental well-being, satisfy hunger, and provide necessary nutrients for the human body. Koumiss is a common type of fermented mare's milk that has been shown to have beneficial therapeutic effects on cardiovascular disease, tuberculosis, and diarrhea as it can nourish vessels, relieve ill mood, and improve digestion. Hence, in this study, we aimed to prepare health-promoting koumiss using different concentrations of flaxseed (*Linum usitatissimum* L.), which has the potential to control heart disease, hypertension, inflammation, and lung function. The pH was decreased to around 4.42-4.43, whereas the total anthocyanin (TA) content was increased to around 0.78~0.82% after fermentation of the koumiss premix. There were no significant differences in pH and TA among the three groups, including the control group. In sensory profile analysis, the taste, flavor, color, texture, and overall acceptability decreased in proportion to the added amount of flaxseed. These findings provided the first data describing the effects of flaxseed on koumiss fermentation, establishing a basis for commercial-scale production of koumiss containing fermented flax seed and for improving the health-promoting activity of koumiss.

Keywords

Linum usitatissimum L. (flaxseed), sensory profile, koumiss, *Lactobacillus acidophilus* ATCC43587, *Lactobacillus kefir* DH5, *Kluyveromyces marxianus* DH6

Introduction

During the last 20 years, *Linum usitatissimum* L. (flaxseed) had emerged as one of the important and attractive functional food ingredient among several food crops for developing functional foods. Since *Linum usitatissimum* L. (flaxseed) had lots of desirable nutrient composition through various reported scientific literature. In fact, the trend of today foods would be intended to satisfy hunger with providing necessary nutrients for humans, and furthermore to control nutrition-related diseases with improving physical and mental well-being (Nothlings *et al.*, 2007; Takachi *et al.*, 2008). Namely, functional foods would be recognized quiet remedy. In general, *Linum usitatissimum* L. (flaxseed) was a blue flowering rabi crop and a member of family Linaceae (Ganorkar and Jain, 2013). Proximate composition of flaxseed revealed that it was a rich source of alpha-linolenic acid (ALA, omega-3, polyunsaturated fatty acid), protein, dietary fiber and lignan (Husain, 2008; Singh and Jood, 2009). *Linum Usitatissimum* L. (flaxseed) oil or blends of flaxseed oil and sunflower oil promoted cholesterol reduction in hyper-cholesterolemic rats compared to diets formulated with hard fats (Ranhotra *et al.*, 1993). *Linum usitatissimum* L. (flaxseed) protein was effective in lowering plasma cholesterol

and triglycerides (TAG) compared to soy protein and casein protein (Bhathena *et al.*, 2002). *Linum usitatissimum* L. (flaxseed) was a rich source of dietary fiber (accounting 28%), both soluble as well as insoluble fibers (Morris, 2007). Soluble fiber and other components of *Linum usitatissimum* L. (flaxseed) fraction could potentially affect insulin secretion and its mechanism of action in maintaining plasma glucose homeostasis. Lignans had antioxidant activity and thus could contribute to the anticancer activity of *Linum usitatissimum* L. (flaxseed) (Kangas *et al.*, 2002; Ganorkar and Jain, 2014).

Among various fermented milk products, Koumiss was a fermented mare milk beverage and also was popular in regions of Central Asia (Mongolia, Kazakhstan, Turkey, etc) and Eastern Europe (Danova *et al.* 2005). The production of the ancient beverage koumiss could be traced to the ancient Greeks and Romans in Europe (Wszolek *et al.*, 2006). Also Koumiss had a long history as a popular drink among traditional nomadic people in China, and also had been considered as a complete food, rich in nutrients with medicinal properties. In general, Koumiss was a common fermented mare's milk with beneficial therapeutic effects on cardiovascular disease, tuberculosis, dyspepsia, abdominal tumors, and diarrhea as it could nourish vessels, relieve ill moods, and improve digestion (Thompson and Leamington, 1879; Wu *et al.* 2009; Liu *et al.* 2011). Recently, Kazakhstan had introduced koumiss into military rations for preventing from tuberculosis (Ishii and Samejima, 2001). And Koumiss was rich in angiotensin I-converting enzyme inhibitory peptides, which had antihypertensive properties (Chen *et al.*, 2010), and angiotensin I-converting enzyme inhibitory activity in yeast strains isolated from Colombian koumiss (Chaves-López *et al.*, 2012). Yeasts were the main microorganisms in Koumiss which played an significant role in Koumiss fermentation and endowing them with its therapeutic effects (Khrisanfova 1969; Quan *et al.* 2006). Hence, the purpose of this study was to make the health-promoting Koumiss added with *Linum usitatissimum* L. (flaxseed) for improving sensory profiles as new concept taking several health benefits of Koumiss. Then, we analyzed sensory profiles including TA and pH of the health-promoting Koumiss added with *Linum Usitatissimum* L. (flaxseed) produced in this experiment.

Materials and Methods

1. Crude ingredients extracted from *Linum usitatissimum* L. (flaxseed)

Linum Usitatissimum L. (flaxseed) was provided from Center for One Health, College of Veterinary Medicine, Konkuk University in Seoul, Korea. The dried *Linum Usitatissimum* L. (flaxseed) was macerated in 100% sterilized distilled water for about 2 days with occasionally stirring at ambient temperature. Hence, the soluble ingredients were filtrated through 0.45 μm Millipore and stored at -20°C before use.

2. Producing the health-prompting Koumiss added with *Linum usitatissimum* L. (flaxseed)

Crude ingredients extracted from *Linum usitatissimum* L. (flaxseed) was added to premix of Koumiss at concentrations of 0% (control), 1, 2, 3% and then homogenized. *Lactobacillus acidophilus* ATCC43587 and *Lactobacillus kefir* DH5 as lactic acid bacteria and also *Kluyveromyces marxianus* DH6 as yeast were inoculated and fermented, and then the health-prompting Koumiss was stored at 4°C for 24 h. The health-prompting Koumiss was made by modification of Lee *et al.* (2011) and Jeong *et al.* (2017) (Fig. 1).

3. The pH and TA of the health-prompting Koumiss added with *Linum usitatissimum* L. (flaxseed)

The pH of the homogenized yoghurt was determined using a digital pH meter (Orion Star A211, USA), and the titratable acid (TA) was determined by titration with 0.1 N NaOH and according to method of Jeong *et al.* (2017).

4. Sensory profiles of the health-prompting Koumiss added with *Linum usitatissimum* L. (flaxseed)

The sensory profiles was carried out by 10 trained evaluators between 20 and 50 years of age. The samples were coded with three digit numbers and randomly served at about 7 to 10°C in plastic cups (approximately 10 mL). All evaluators completed a test assessment form to compare the five sensory attributes (taste, flavor, color, and texture) by using a five-point hedonic scale (1, extremely poor; 2, poor; 3, fair; 4, good; 5, excellent). More details please

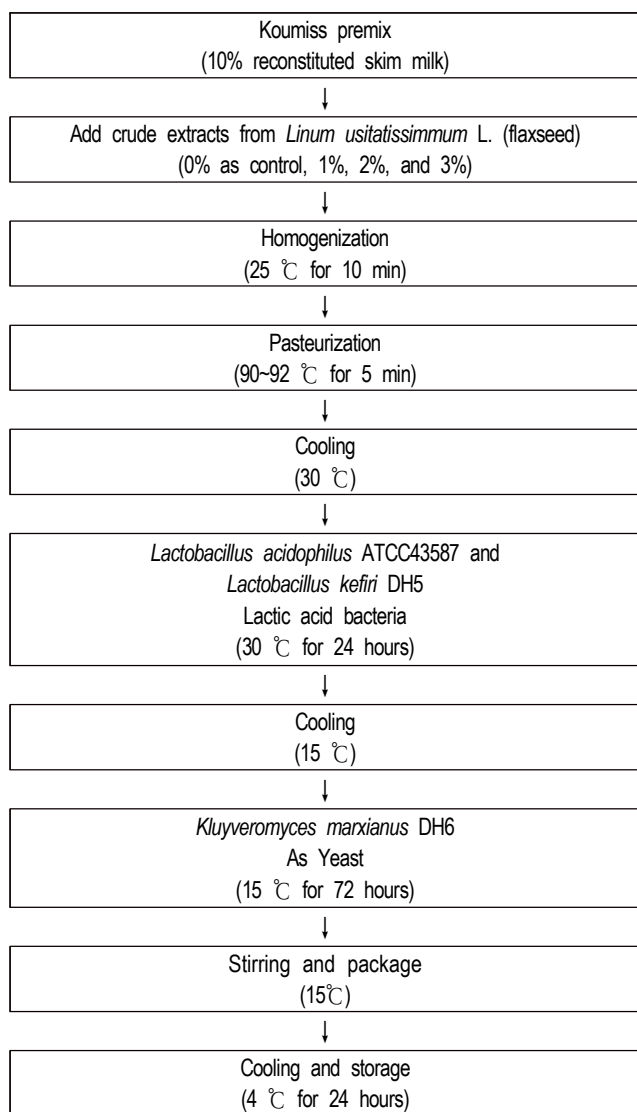


Fig. 1. Procedure for the manufacture of functional Koumiss added with *Linum usitatissimum* L. (flaxseed).

refer to Table 2.

Assays were performed in duplicate in at least three independent experiments and data are expressed as mean (\pm SD).

5. Statistical analysis

Assays were performed in duplicate in at least three independent experiments and data are expressed as mean (\pm SD). Statistical analysis was performed using one-way analysis of variance (ANOVA; SPSS 19.0, USA) followed by Duncan's *post hoc* test for mean comparison. Statistical significance was established as $p < 0.05$.

Results and Discussion

1. The pH and TA of the health-prompting Koumiss added with 0% to 3% *Linum usitatissimum* L. (flaxseed)

Generally, the pH was high depending on the increase of the TA. And Koumiss could be divided by 3, (1) light type of Koumiss showed 4.5 to 5.0 of pH, 0.54 to 0.72 of titratable acidity, and 0.7 to 1.0 of alcohol contents, (2) moderate type of Koumiss showed 3.9 to 4.5 of pH, 0.73 to 0.90 of titratable acidity, and 1.1 to 1.8 of alcohol contents, and (3) strong type of Koumiss showed 3.3 to 3.6 of pH, 0.91 to 1.08 of titratable acidity, and 1.8 to 2.5 of alcohol contents types (Robinson *et al.*, 2002; Danova *et al.*, 2005). In this study, the pH was decreased to around 4.42 to 4.43, but the TA was increased to around 0.78 to 0.82% after the fermentation of Koumiss premix (Data not shown). Hence, the pH and TA contents of the health-prompting Koumiss added with *Linum usitatissimum* L. (flaxseed) (1% to 3%) showed the moderate type.

When compared to similar study, in the functional Koumiss added with *Cichorium intybus* L. (chicory), the pH was decreased to about 4.34 to 4.35, the TA was increased to about 0.85 to 0.88%, and showed the moderate type in the functional Koumiss added with *Cichorium intybus* L. (chicory) (Jeong *et al.*, 2017).

There was no significant difference among these 3 groups including control group in pH and TA in this study. Furthermore, the results of this study showed similar trend with the those of various previous studies (Robinson *et al.*, 2002; Danova *et al.*, 2005; Lee *et al.*, 2011; Carrick, 2012; Zhang and Zhang, 2012; Jeong *et al.*, 2017).

2. Sensory profiles of the health-prompting Koumiss added with *Linum usitatissimum* L. (flaxseed)

The sensory profiles of the health-prompting Koumiss were evaluated by 10 trained evaluators of ages 20 to 50 years, and the results are summarized in Table 1.

The health-prompting Koumiss were prepared with *Linum usitatissimum* L. (flaxseed) at concentrations of 0%, 1%, 2%, and 3%, respectively. The taste scores for the health-prompting Koumiss with *Linum usitatissimum* L. (flaxseed)

(1% to 3%) ranged from 2.9 point to 2.8 point, which were higher than those for conventional Koumiss without addition *Linum usitatissimum* L. (flaxseed) (0% as control) (2.7 points). The flavor score of the health-prompting Koumiss with *Linum usitatissimum* L. (flaxseed) (1% to 3%) ranged from 2.7 points to 2.5 point, whereas that of the conventional Koumiss without addition of *Linum usitatissimum* L. (flaxseed) (0% as control) showed 2.7 points. The color value of the health-prompting Koumiss with *Linum Usitatissimum* L. (flaxseed) (1% to 3%) ranged from 4.5 points to 4.3 point, which was comparable to that of conventional Koumiss without addition of *Linum usitatissimum* L. (flaxseed) (0% as control) showed 4.7 points. The texture of the health-prompting Koumiss with *Linum usitatissimum* L. (flaxseed) (1% to 3%) and the conventional Koumiss without addition of *Linum usitatissimum* L. (flaxseed) (0% as control) showed about 4.3 points. And the overall acceptability of the health-prompting Koumiss with *Linum Usitatissimum* L. (flaxseed) (1% to 3%) ranged from 3.6 points to 3.5 points, which was comparable to that of conventional Koumiss without addition of *Linum usitatissimum* L. (flaxseed) (0% as control) showed 3.6 points. Namely, the sensory profiles did not have sharply changed with increasing amounts of added *Linum Usitatissimum* L. (flaxseed). Among the experimental group, high scores were received by *Linum usitatissimum* L. (flaxseed)-containing Koumiss with 1% and 2% compared with the control group. Summarizing the results of this study, flavor, color, texture, and overall acceptability except the taste, decreased in proportion to the added amount of *Linum usitatissimum* L. (flaxseed) (Table 1).

When compared to similar study, according to Jeong *et al.* (2017), the functional Koumiss was manufactured added

with *Cichorium intybus* L. (chicory) at concentrations of 0%, 1%, 2%, and 3%, respectively. The sensory evaluation decreased with increasing amounts of added *Cichorium intybus* L. (chicory). The high scores were received by *Cichorium intybus* L. (chicory)-containing Koumiss with 1% compared with the control group. And the taste, flavor, color, texture, and overall acceptability generally decreased in proportion to the added amount of *Cichorium intybus* L. (chicory). Also, according to Lee *et al.* (2011), Korean-type Koumiss was made using whey powder (0% as control, 2%, 4%, 6%, and 8%). When whey powder content increased in sensory evaluations, the scores increased. In the appearance, there were no great differences among the samples (Lee *et al.*, 2011).

Recently, Interest in koumiss production has dramatically grown (IDF, 2009). Milk from eight species of domesticated mammals (cow, buffalo, sheep, goat, camel, horse, reindeer and yak) were used for making traditional fermented milk products, and there were three types of fermented milks- (1) lactic fermentations, (2) yeast-lactic fermentations, and (3) mould (*Geotrichum candidum*)-lactic fermentations. Among them, koumiss and kefir belonged to the yeast-lactic fermentation group where alcoholic fermentation by yeasts was used in combination with a lactic acid fermentation (Tamine and Marshall, 1984). In general, koumiss contains about 90% moisture, 2~2.5% protein (1.2 % casein and 0.9% whey proteins), 4.5~5.5% lactose, 1~1.3% fat and 0.4~0.7% ash. The end-products of microbial fermentation in Koumiss were lactic acid (1.8%), ethanol (0.6~3.0%) and CO₂ (0.5~0.9%) and provided 37 to 40 kcal per 100 mL. Furthermore, Koumiss was thought to be more effective in disease treatment due to the additional peptides and bactericidal substances produced during microbial metabolism (Doreau and

Table 1. The sensory evaluation of the health-prompting Koumiss added with *Linum usitatissimum* L. (flaxseed)

Attributes	Addition of <i>Linum usitatissimum</i> L. (flaxseed) (%)				A five-point hedonic scale 1, extremely poor; 2, poor; 3, fair; 4, good; 5, excellent
	0(Control)	1	2	3	
Taste	2.7±0.4	2.9±0.3	2.9±0.5	2.8±0.4	The higher the intensity of the bitterness, the lower the score
Flavor	2.7±0.4	2.7±0.4	2.7±0.4	2.5±0.5	The lower the intensity of Koumiss's aroma, the lower the score
Color	4.7±0.4	4.5±0.5	4.4±0.6	4.3±0.6	The lower the intensity of the white color, the lower the score
Texture	4.3±0.7	4.3±0.6	4.3±0.6	4.3±0.6	The lower the intensity of Koumiss's texture, the lower the score
Overall acceptability	3.6±1.1	3.6±0.9	3.6±0.9	3.5±1.0	The higher the total score, the higher overall acceptability.

All points are expressed as mean (±SD) of duplicate determinations.

Within a row, means with different superscripts are significantly different ($p < 0.05$).

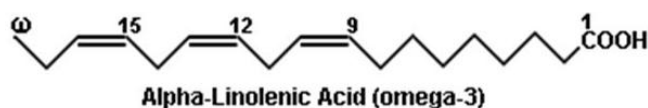


Fig. 2. Structure of alpha-linolenic acid (omega-3)
Resource: XXX, year.

Marti-Rosset, 2002). According to the results of previous studies, Koumiss was found to have a beneficial effect on the chronic diseases such as tuberculosis, bronchitis, catarrh, anemia, and so on.

Alpha-linolenic acid (ALA) was a kind of omega-3 fatty acid found in plants, and seed oils were the richest sources of α -linolenic acid, notably those of chia, perilla, flaxseed (linseed oil), rapeseed (canola), walnut oils, and soybeans (Fig. 2). In general, Alpha-linolenic acid was recognized as the omega-3 fatty acids called Eicosapentaenoic acid (EPA; 20:5, $n-3$) and docosahexaenoic acid (DHA; 22:6, $n-3$) as fish oil. Human's body could change alpha-linolenic acid into EPA and DHA. Until now, Omega-3 fatty acids such as EPA and DHA had been shown to reduce inflammation and could help prevent chronic diseases as arthritis and heart disease. Furthermore, it could be very significant factors for brain's health and development. For preventing and treating heart disease, the best ways was to eat a diet with low level of saturated and trans fats but with rich level of monounsaturated and polyunsaturated fats, particularly omega-3 fatty acids. For example, the daily servings of Mediterranean Diet were contained fruit, fish and poultry, olive and canola oils, and alpha-linolenic acid (found in flaxseed oil). Also, when people follow a Mediterranean-style diet tend, they would have higher levels of High Density Lipoprotein (HDL, good cholesterol). Since a Mediterranean-style diet tend generally showed rich levels of alpha-linolenic acid. Especially, among various plants having alpha-linolenic acid, flaxseed oil contained the high in alpha-linolenic acid. Flaxseed oil effectively showed to help lower cholesterol owing to alpha-linolenic acid. The diets in rich level of omega-3 fatty acids could effectively reduce the blood pressure in people with hypertension. According to previous study, when people with hypertension ate the diet in rich contents of alpha-linolenic acid, it could lower the risk of high blood pressure by approximately 30%. Until now, alpha-linolenic acid as omega-3 fatty acid could decrease

inflammation and improve lung function in some people with asthma and improve dry eye that are comparable to those seen with corticosteroids. Especially the ALA was linked with improvements in dry eye and also linked with lower inflammatory markers among patients with chronic obstructive pulmonary disease (reference).

In summary, the pH was decreased and the TA was increased in the health-prompting Koumiss added with 0%, 1%, 2%, and 3% of *Linum usitatissimum* L. (flaxseed), respectively, and in the sensory profiles, the health-prompting Koumiss containing 1% and 2% concentration of *Linum usitatissimum* L. (flaxseed) received higher scores for taste, flavor, texture, and overall acceptability. Furthermore, it urgently needs further studies to make multi-purpose health-prompting Koumiss through upgraded availability of *Linum usitatissimum* L. (flaxseed) for human.

Disclaimer

The views expressed herein do not necessarily reflect those of the US Food and Drug Administration or the US Department of Health and Human Services

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