FOOD QUALITY and CULTURE
Food Quality Culture 1(1): 22~26 (2007)

Fermentation Properties of Yogurt with Added Nano-Filtered Sunmul Powder

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ABSTRACT This study was performed to determine the feasibility of developing a healthy yogurt using tofu whey concentrates separated by nanofiltration (NF). The curd yogurt was prepared from whole milk with added skim milk powder, in which the NF powder was substituted at 0, 6.25, 12.5, or 25% for the skim milk powder. The quality characteristics were evaluated for pH, titratable acidity, viscosity, color, and viable cell counts. There were no significant differences in pH or titratable acidity between the control (yogurt with added skim milk powder only) and the yogurts with added NF powder, after 24 hr of fermentation at 37°C. The apparent viscosities of the yogurts with added NF powder were higher (3,197~3,574 cps) than that of the control yogurt (3,196 cps). Lightness decreased, while yellowness increased, as the amount of NF powder increased. Sensory evaluations showed that the NF powder could be substituted for the skim milk powder at 6.25% without lowering the yogurt quality.

KEYWORDS: *sunmul, nanofiltered powder, yogurt*

INTRODUCTION

Yogurt is a semi-fluid product made by fermenting milk with lactic acid-producing bacteria such as *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. The milk is usually fortified with powdered milk, in order to increase the viscosity of the product as well as the total milk solids content to 12~14%. Yogurt consumption has increased rapidly in Korea, and the reason for this is its health promoting effects. Due to its positive nutritional attributes, much research has been conducted on developing new types of yogurt products. For example, garlic powder (1), mulberry extract (2), rice bran (3), chlorella (4), peanut (5), sweet persimmon powder (6), salted bamboo shoots (7), and maesil (8) were added to yogurt during manufacture, and the quality characteristics were investigated.

Sunmul is tofu whey that is produced after coagulating the protein component of soybeans during tofu production, and it is regarded as a waste product in the tofu industry. However, *sunmul* contains various low molecular weight substances such as isoflavones, oligosaccharides, phytic acids, and saponins, which possess diverse physiological activities (9-12). Isoflavones are reported to prevent some types of cancer, cardiovascular diseases, and osteoporosis (13,14). Oligosaccharides are known to stimulate the growth of *bifidobacteria* and *lactobacilli* in the intestine (15). Much effort has been made to separate these functional substances from *sunmul* (16-18). Kim *et al.* reported that a sequential membrane filtration process was effective for separating the isoflavones and oligosaccharides (16,17), and some food products were developed with these separated concentrates (19,20).

The aim of this study was to propose a use for tofu whey concentrates separated by nanofiltration (NF), within yogurt production. The curd yogurt was prepared from whole milk with added skim milk powder, in which the NF powder was substituted for the skim milk powder and the quality characteristics were investigated.

MATERIALS AND METHODS

Materials and yogurt cultures

The sunmul was supplied from Doosol Corporation (Yeisan-Gun, Korea), and the nanofiltered sunmul powder was prepared according to a previously described method (16,17). The yogurt cultures were purchased from Hanmi Co. (Korea), which contained a mixed strain culture of *Lactobacillus acidophilus* 3×10^8/g, *Lactobacillus bulgaricus* 5×10^8/g, *Lactobacillus yogurti* 2×10^8/g, and *Streptococcus thermophilus* 1×10^9/g as a powder form.

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Received Septeber 10, 2007; Accepted November 13, 2007

Preparation of yogurt

One hundred grams of whole milk (Seoulmilk Co.) was enriched with 4 g (W/V) of non-fat dried skim milk powder (Seoulmilk Co.), in which NF powder was substituted at 0, 6.25, 12.5, or 25% for the skim milk powder. The substitution level of the NF powder was determined from preliminary data based on a sensory test (data not shown). The mixtures were homogenized with a hand mixer (62680, Proctor-silex, USA) for 1 min, and heated at 60~65°C for 20 min. After cooling to room temperature, 1 g of the mixed strain culture was added and incubated at 37°C for 24 hours.

pH and titratable acidity determination

The pH of the yogurt was measured directly with a pH meter (level II, inoLab, Germany). The titratable acidity was determined by titrating with 0.1 N NaOH to pH 8.1, using a digital burette (Jencons, UK), and expressed in terms of lactic acid content (%).

Viscosity measurement

The apparent viscosity of each yogurt was determined at 11~14°C. The viscosity was measured using a viscometer with a No. 3 spindle (Viscostar L, Spain), which was set to 20 rpm. The data were recorded at 30 s intervals after 1 min of stirring up to 3 min, and average values were expressed as centipose (cp) units.

Color measurements

The color of the yogurt was determined by measuring tristimulus L (lightness), a (redness), and b (yellowness) values with a colorimeter (JX 777, Juki, Japan). An average of five values per one sample was taken, and three samples were conducted per each treatment.

Viable cell counts

Each yogurt sample of 0.1 g was weighed and blended with 9.9 mL of sterilized saline solution (0.85%), followed by serial dilution using the same sterile diluent. Each diluted sample (1.0 mL) was poured on BCP plate count agar (Eiken Chemical Co., Japan) and incubated for 24 hr at 37°C. The visible colonies were then counted, and the units were expressed as CFU (colony forming units)/mL.

Sensory evaluation

After fermenting for 24 hours, the yogurt samples were sweetened with sucrose (10%, w/w) and kept in the refrigerator (4 ± 1°C) for 2 hours before serving. The yogurt was placed in a plastic cup coded with a three-digit random number, and offered to 8 trained panelists in individual booths with lighting. The panelists were asked to rate the intensity of the color, smell, sour taste, mouthfeel, and aftertaste using a 9-point scale, where 1 = none, 5 = moderate, and 9 = intense. The overall acceptability was score based on a similar scale, where 1 = extremely bad, 5 = neither good nor bad, and 9 = extremely good.

Statistical analysis

All data were recorded as means ± standard deviations of at least triplicate measurements. The data were analyzed by SAS (Statistical Analysis System, version 8.12), and the Duncan's multiple range test was used to determine the differences among means at $\alpha = 0.05$.

RESULTS AND DISCUSSION

pH and titratable acidity

The changes in pH and titratable acidity of the yogurt with different levels of added NF powder, during 24 hr of fermentation, are presented in Table 1 and 2. The initial pH of the yogurt was highest in the control group (pH 6.53), and decreased with increasing amounts of NF powder (pH 6.23~6.40). This decrease may be explained by the presence of amino acids in the NF powder. At 6 hr of fermentation, the pH decreased remarkably to 4.24~4.61, and then slowly decreased thereafter, resulting in a final pH range of 3.67~3.69.

The initial titratable acidity was 0.20~0.22%, and it increased dramatically during the first 6 hr of fermentation as 0.95~1.18% (Table 2). Between 6~24 hr of fermentation, the titratable acidity increased steadily, and the final acidity

Table 1. Changes in pH of yogurts with different levels of added NF powder during fermentation at 37°C

Groups	Incubation time (hour)				
	0	6	12	18	24
NF-0[1)]	6.53 ± 0.03[a2)]	4.61 ± 0.29	3.89 ± 0.01	3.75 ± 0.00	3.68 ± 0.00
NF-6.25	6.40 ± 0.02[b]	4.27 ± 0.16	3.87 ± 0.02	3.75 ± 0.04	3.67 ± 0.04
NF-12.5	6.33 ± 0.01[b]	4.25 ± 0.17	3.88 ± 0.04	3.75 ± 0.03	3.69 ± 0.04
NF-25.0	6.23 ± 0.04[c]	4.24 ± 0.16	3.86 ± 0.00	3.73 ± 0.01	3.67 ± 0.01

[1)]NF-0: NF powder was substituted at 0% for skim milk powder.
NF-6.25: NF powder was substituted at 6.25% for skim milk powder.
NF-12.5: NF powder was substituted at 12.5% for skim milk powder.
NF-25.0: NF powder was substituted at 25.0% for skim milk powder.
[2)]Means with different letters within a column are significantly different from each other at $\alpha = 0.05$ as determined by Duncan's multiple range test.

Table 2. Changes in titratable acidity (%) of yogurts with different levels of added NF powder during fermentation at 37°C

Groups	Incubation time (hour)				
	0	6	12	18	24
NF-0	0.20 ± 0.01	0.95 ± 0.14	1.55 ± 0.02	1.84 ± 0.06	2.02 ± 0.05
NF-6.25	0.20 ± 0.00	1.16 ± 0.17	1.59 ± 0.02	1.84 ± 0.00	2.05 ± 0.04
NF-12.5	0.21 ± 0.00	1.18 ± 0.17	1.57 ± 0.09	1.81 ± 0.03	2.02 ± 0.02
NF-25.0	0.22 ± 0.01	1.14 ± 0.12	1.62 ± 0.01	1.86 ± 0.05	2.03 ± 0.04

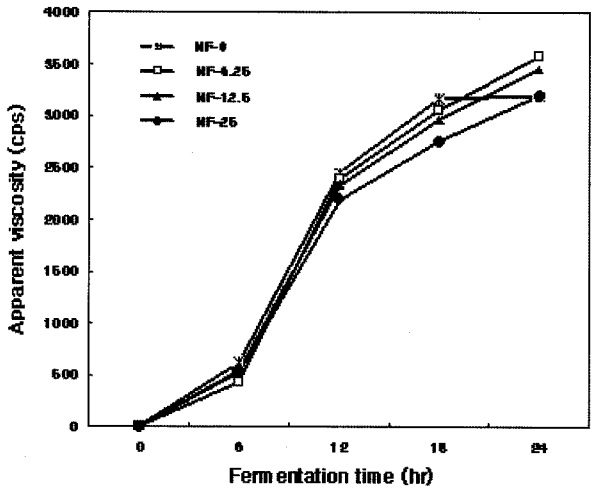

Fig. 1. Changes in viscosity of yogurts with different levels of added NF powder during fermentation at 37°C.

was 2.02~2.05%, showing no significant differences among the samples. Lee *et al.* (22) reported that yogurts with added *Saururus chinensis* (Lour) Bail exhibited a pH range of 3.60 ~3.97, and titratable acidities of 1.04~1.42%. Sung *et al.* (4) reported that yogurt formulated with chlorella showed pH values of 3.87~4.01, and titratable acidity values of 1.18~ 1.49%, after 18 hr of fermentation. It was previously reported that the pH values of commercial yogurts in Korea were 3.87~4.19, and the titratable acidity ranged from 0.97 to 1.43% (23). This is in agreement with our pH data, but disagrees with our titratable acidity data. The difference between our studies and the references might have resulted from certain factors such as the starter culture, ingredients, and manufacturing conditions.

Viscosity

The apparent viscosities of the yogurts with different levels of added NF powder are reported in Fig. 1. At 12 hr of fermentation, the viscosity increased drastically, and the results confirmed those of Kroger (24) who reported that yogurt milk became solid at an acidity of about 0.6% and a pH of about 5.3. Our results showed that for all the yogurts, the titratable acidity was above 0.6% and the pH was below 5.3 after 6 hr of fermentation. At the end of 24 hr of fermentation, the viscosity ranged from 3,197 to 3,574 cps,

Table 3. Hunter Lab color values of yogurts with different levels of added NF powder during fermentation at 37°C

Groups	Color value		
	L	a	b
NF-0	77.69 ± 0.08[a]	-0.12 ± 0.92	5.81 ± 0.39[a]
NF-6.25	77.70 ± 0.17[a]	-0.05 ± 1.05	6.58 ± 0.14[b]
NF-12.5	77.56 ± 0.01[ab]	-0.05 ± 1.12	6.88 ± 0.23[bc]
NF-25.0	77.30 ± 0.01[b]	-0.06 ± 1.16	7.48 ± 0.11[c]

[1)]Means with different letters within a column are significantly different from each other at $\alpha = 0.05$ as determined by Duncan's multiple range test.

and the NF-added yogurts had higher viscosity values than the control yogurt. Similar results have been observed in studies of defatted peanut powder-added yogurt (5), as well as Aloe vera-added yogurt (25), which showed viscosity values of 2,533~3,800 cps and 3,860~4,300 cps, respectively. Kim *et al.* (23) reported the viscosities of commercial yogurts to be 7,850~21,000 cps, which is in disagreement with our results. This may be explained by the differences in total solids content and the ingredients used. In order to increase the viscosity, viscosity modifiers such as starches, gums, gelatins, and pectins could be used.

Color

The results of the Hunter Lab color values of the yogurt are shown in Table 3. The L-value (lightness) of the control yogurt was 77.69 and those of the NF powder yogurts ranged from 77.30 to 77.70. The lightness of the NF powder yogurts decreased as the NF powder level increased, due to the loss of white color in the milk. Therefore, it could be expected that yogurt becomes darker with increasing amounts of added NF powder. Similar results were obtained in Jeung-Pyun made with UF powder (19). The Hunter a-value (redness) of the control yogurt was –0.12, and those of the NF powder groups were –0.06~–0.05. The Hunter b-value (yellowness) of the control yogurt was 5.81, and those of the NF powder group were 6.58~7.48, showing more yellowish color than the control. This was due to the yellow color of the NF powder. Lee *et al.* (8) reported that the incorporation of maesil into yogurt did not affect the L-value, but it increased the a- and b-values. Chun *et al.* (26) reported that the addition of purple sweet potato lowered the lightness and

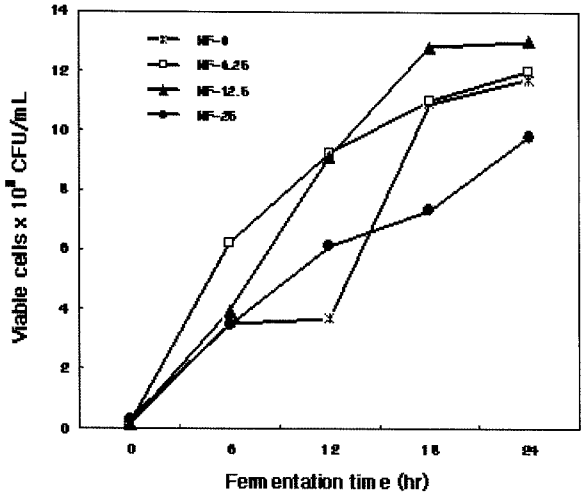

Fig. 2. Changes in viable cell counts of yogurts with different levels of added NF powder during fermentation at 37°C.

yellowness, but increased the redness of yogurt. From these results, it is suggested that the incorporation of different ingredients into yogurt products causes different color profiles.

Viable cell population

Changes in the viable cell counts during 24 hour of fermentation are presented in Fig. 2. The initial starter bacterial counts in the yogurts were 0.13×10^8~0.17×10^8 CFU/mL. At 6 hr of fermentation, the viable cells had increased markedly up to 3.48×10^8~6.20×10^8 CFU/mL, and steadily increased thereafter. At 24 hr, the cell numbers ranged from 9.16×10^8 CFU/mL to 13.00×10^8 CFU/mL, which meets the regulatory level of 10^8 CFU/mL. The yogurt with 12.5% NF powder added showed the highest cell numbers, and the 25% NF powder group showed the lowest. Jeong *et al.* (27) reported that the propagation of lactic acid bacteria was stimulated by adding sea tangle hot-water extract, and the number of viable cells were 1.4×10^8 CFU/mL. Similar results have been reported in cultured ginseng-added yogurt (28), and chlorella-added yogurt (4), which showed viable cell numbers of 61.4×10^8~82.4×10^8 cps CFU/mL and 1.95×10^8~8.60×10^8 cps CFU/mL, respectively.

Sensory evaluation

The results of the sensory evaluation for the yogurts are shown in Table 4. The color was evaluated as becoming darker as the NF powder level increased, which is due to the yellowish color of the NF powder. This is in agreement the results of the lightness data shown in Table 3. The scores for smell, mouthfeel, and aftertaste were higher in the yogurts with added NF powder as compared to the control, but no significant differences were observed among the samples. This could be attributable to the beany or tofu-like flavor present in the NF powder. The sour taste scores were higher in the yogurts formulated with NF powder (5.25~6.06) than in the control yogurt (5.06), probably due to the free amino acids present in the NF powder. Although tartness is an important attribute for the natural flavor of yogurt, a high acidity in products is not appealing to consumers (29). There were no significant differences in the scores of overall acceptability between the control and the 6.25% NF yogurt. However, significant differences ($p < 0.05$) were found with the higher percentages of NF powder, showing the lowest score in the yogurt with the 25% addition. Therefore, replacing up to 6.25% of the skim milk with NF powder would offer a yogurt without significant changes in its acceptability.

ACKNOWLEDGMENT This research was supported by the Agricultural R&D Promotion Center in Korea (ARPC, 102001-02-HD110). Authors deeply appreciated this financial supports.

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Table 4. Sensory scores of yogurts with different levels of added NF powder during fermentation at 37°C

Groups	Color	Smell	Sour taste	Mouthfeel	Aftertaste	Overall acceptability
NF-0	4.56[a]	4.75	5.06[a]	4.75	1.44	5.38[a]
NF-6.25	4.75[a]	4.70	5.25[a]	4.81	1.56	5.06[ab]
NF-12.5	4.81[a]	5.30	5.63[ab]	5.00	1.69	4.75[b]
NF-25	5.19[b]	5.29	6.06[b]	5.06	2.31	4.00[c]

[1)]Means with different letters within a column are significantly different from each other at $\alpha = 0.05$ as determined by Duncan's multiple range test.

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