

Effect of Colored Barley Flours on Quality Characteristics of Fermented Yogurt by *Lactobacillus spp.*

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ABSTRACT Quality characteristics of yogurt with added colored barely flour was investigated during fermentation by lactic acid bacteria. Chemical properties such as moisture, crude protein, starch, ash and β -glucan contents was measured. pH, acidity, brix, Hunter color value and growth of lactic acid bacteria in yogurt was investigated during fermentation by *L. acidophilus*, *L. bulgaricus*, and *S. thermophilus* mixed culture. Crude protein contents of Daeanchal and Boseokchal was 16.16 and 12.17%, respectively. Starch contents of daeanchal were shown lower score. The pH of yogurt by addition of barley flour (Daeanchal) addition 0 and 20% were 6.66 and 6.40, respectively. The pH of yogurt supplemented with barley flour tended to be lower than before control which was not added barely flours and oligosaccharide in yogurt. Titratable acidity of yogurt added barley flour was higher compared with that of control. Brix of yogurt was decreased during fermentation by lactic acid bacteria. Lightness of yogurt added barley flour (Daeanchal) addition 0 and 20% were 83.25 and 69.83, respectively. The original microbial population of the yogurt during 0, 5, 8, and 15 hr fermentation were 7.48, 7.79, 8.15, and 8.71 Log CFU/g, respectively. Moreover, the addition of colored barley flour was to promote the proliferation of lactic acid bacteria in yogurt. In our research, addition of colored barley flours added into the yogurt may also have contributed to growth of lactic acid bacteria.

Keywords : barley, colored barely, yogurt, lactic acid bacteria

Consumers are becoming more interested in incorporating healthy foods into their diet (Hekmat & Reid, 2006). Fermented beverages make up an important contribution to the human diet in many countries because fermentation is an inexpensive technology, which preserves the food,

improves its nutritional value and enhances its sensory properties (Gadaga *et al.*, 1999). Probiotic dairy products are considered to have functional properties because the probiotic bacteria added to the regular fermentation cultures provide therapeutic benefits such as modification of the immune system, reduction in cholesterol, alleviation from lactose intolerance, faster relief from diarrhea, and restoration of a healthy vaginal microbiota (Reid, 2001; Reid *et al.*, 2003). Probiotics are commonly incorporated into fermented dairy products worldwide among which yogurt is a popular delivery vehicle (Ng *et al.*, 2011). Yogurt is a well known fermented dairy food, which is usually manufactured from cow's milk with or without the addition of some natural derivatives of milk, and processes a gel structure that is the result of coagulation of the milk proteins by lactic acid produced by *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (Robinson, 2003). Recently, there has been an increasing interest in the use of natural food additives and incorporation of health-promoting substances into the diet (Varga, 2006).

However, maintain a high level of viable probiotic cell count in yogurts throughout the shelf life, is not a simple task. Many factors influence the viability of probiotics in yogurts such as strain variation, acid accumulation, interaction with starter cultures, level of dissolved oxygen and hydrogen peroxide, and storage condition (Donkor *et al.*, 2006; Kailasapathy *et al.*, 2007).

Cereals can be used as fermentable substrates for the growth of probiotic microorganisms. The main parameters that have to be considered are the composition and processing of the cereal grains, the substrate formulation, the growth capability and productivity of the starter culture, the stability

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of the probiotic strain during storage, the organoleptic properties and the nutritional value of the final product. Additionally, cereals can be used as sources of nondigestible carbohydrates that besides promoting several beneficial physiological effects can also selectively stimulate the growth of *Lactobacilli* and *Bifidobacteria* present in the colon and act as prebiotics (Charalampopoulos *et al.*, 2002). Of other cereals, barley (*Hordeum vulgare* L.) is an important miscellaneous grain in Korea and a widely consumed cereal because of its dietary health benefits, ready availability, reasonable cost, and processing properties of products such as beer, barley teas, and baked products. Based on grain composition, barley is classified as waxy, non waxy type, high lysine, high β -glucan, and proanthocyanidin-free (Lee *et al.*, 2010). Also, barley is mostly known for its high amount of dietary fiber such as β -glucan and also contains other important compounds such as arabinoxylan, tocol, and phenolic compounds (Holtekjølén *et al.*, 2008). The U.S. Food and Drug Administration (FDA) allowed the labels of foods containing the soluble fiber from barley products to claim that the consumption of these foods may reduce the risk of coronary heart disease (FDA, 2006). Increased fiber such as β -glucan consumption has been linked to a decrease in serum cholesterol and glycemic index (Behall *et al.*, 2004; Cavallero *et al.*, 2002). The objective of this study was to investigate the effect of addition of barely on quality characteristics of set type yogurt during fermentation by lactic acid bacteria.

MATERIALS AND METHODS

Sample preparation, Chemical properties and β -glucan compounds

Two hullless waxy barley cultivars, cvs. Daeanchal and Boseokchal, were obtained from Department of Rice and Winter cereal Crop, National Institute of Crop Science, Korea. The whole barley was grinding using a rotor mill (ZM 100; Retsch GmbH, Haan, Germany) equipped with a 0.5 mm screen and then barley flour were put into plastic bags and stored at -20°C until used.

Chemical properties (moisture, crude protein, and ash) of colored barely was determined using the standard method

described by the association of official analytical chemicals (AOAC 44-15A, 46-30, 08-01, 1990). Starch and β -glucan compounds was measured using Megazyme kit (Megazyme, Wicklow, Ireland) by McCleary and Glennie-Holmes (McCleary & Glennie-Holmes, 1985).

Yogurt preparation with barley flour

The yogurt base was prepared by dissolving whole milk powder (Seoulmilk Co, Seoul, Korea) in water and sterilized at 95°C for 20 min. It was supplemented with oligosaccharide (CJ cheiljedang Co, Seoul, Korea) that was added 20% concentration into the yogurt. Barley flour was mixed in water and it was gelatinization and sterilization at 120°C for 15 min. Addition concentration of the barley flour into the yogurt base was 10 and 20%, respectively. Control was not added barley and oligosaccharide. As strains for the manufacture of yogurt, *Lactobacillus bulgaricus* KCTC 3635, *Streptococcus thermophilus* KCTC 3778 and *Lactobacillus acidophilus* KCTC 3145 were used in this study and obtained from the Korean collection for type culture (KCTC, Daejeon, Korea). *L. bulgaricus*, *L. acidophilus*, and *S. thermophilus* were grown in a MRS broth (Difco, Laboratories, Sparks, MD, USA) at 37°C . The cell density of activated cultures was approximately $10^8\sim 10^9$ CFU/mL levels and it were used as yogurt starter. Yogurt mixture was blended and start culture was inoculated into mixed yogurt mixture with 5% concentration. And then it was fermented during 15 hr at 42°C . The yogurt fermented by *L. acidophilus*, *L. bulgaricus* and *S. thermophilus* mixed culture was used for investigation.

Properties of yogurt preparation with barley flours

The yogurt pH was measured using pH meter (MP220, Mettler Toledo, UK) with a pH metric probe and brix was measured with saccharometer (Atago, Tokyo, Japan). Titrate acidity expressed the value of titration with standard alkaline on total acid amount of the sample. The pH, brix and titrate acidity of the samples was measured three times for each samples and then averaged, respectively. Color of the sample was measured by a color difference meter (Color JS 55; Color Technology System Co., Tokyo, Japan). The color was measured 3 times for each samples and then averaged. The numerical value of the color was

expressed by Hunter L, a, and b values. Hunter L value indicates the lightness of the samples, a value indicates the +red/-green, and b value indicates the +yellow/-blue. Microbiological analysis was measured. A sample (10 g) was aseptically homogenized for 2 min in a sterile stomacher bag containing 90 mL of sterile 0.1% peptone water using a bag mixer[®] (Model 400, Interscience Co, France). Media for enumeration of the *Lactic acid bacteria* were prepared by MRS agar (Oxoid, Basingstoke, Hampshire, England). Plates were incubated at optimal temperature of bacteria for 72 hr and colony forming units (CFU) per gram were counted at a dilution of 30 to 300 CFU per plate. Experiments with each bacterial culture were conducted independently twice.

Statistical analysis

The experiment was designed as randomized block design with three replications. One-way analyses of variance were performed using SAS software (version 8.0, SAS Institute, Cary, NC) along with Duncan's post hoc tests to compare differences among mean values. Mean values and

standard errors of the mean (SEM) were reported, and the significance was defined at $p < 0.01$.

RESULTS AND DISCUSSION

Chemical composition and β -glucan compounds

The chemical properties and β -glucan compound of the samples was shown in Table 1. The moisture, crude protein, starch, ash and β -glucan of Daeanchal were 7.88, 6.16, 44.00, 2.16 and 2.35%, respectively. Crude protein, starch and β -glucan of Boseokchal were 12.17, 53.14, and 5.80% respectively. Starch contents of Daeanchal were shown tend to be lower. β -glucan content of Boseokchal was a higher score compared with Daeanchal. Cha *et al.*, (2012) reported that crude protein of naked, hulled and glutinous barley was 12.1, 11.9 and 12.6%, respectively and crude lipid, ash and total mineral contents were slightly decreased after germination. Lee *et al.*, (2012) reported that β -glucan of Saessalbori, Saechalssalbori and Hinchalssalbori were 4.79, 4.14 and 4.61% , respectively.

Table 1. Chemical properties and β -glucan compounds of colored barley.

Samples	Moisture (%)	Protein (%)	Starch (%)	Ash (%)	β -glucan (%)
Daeanchal	7.88 ^a	16.16 ^a	44.00 ^b	2.16 ^a	2.35 ^b
Boseokchal	8.71 ^a	12.17 ^b	53.14 ^a	1.35 ^b	5.80 ^a
SEM ^a	0.297	0.040	0.087	1.085	0.005

^aStandard error of the mean ($n=6$).

^{a-b}Different letters within the same column with the same sample differ significantly ($p < 0.01$).

Table 2. Change in pH of yogurt added colored barley flours during fermentation.

Samples	Con. (%)	pH			
		0 h	5 h	8 h	15 h
Control ^a		6.77±0.02 ^a	6.73±0.04 ^a	6.24±0.01 ^a	5.59±0.01 ^a
Daeanchal	0	6.66±0.01 ^b	5.18±0.01 ^b	4.99±0.01 ^b	4.76±0.01 ^b
	10	6.50±0.01 ^c	4.28±0.01 ^c	4.13±0.01 ^f	3.91±0.01 ^f
	20	6.40±0.01 ^c	4.37±0.01 ^d	4.10±0.01 ^g	3.97±0.01 ^g
Boseokchal	0	6.71±0.01 ^b	5.16±0.01 ^b	4.96±0.01 ^c	4.62±0.03 ^c
	10	6.53±0.06 ^c	4.16±0.01 ^c	4.34±0.01 ^e	4.05±0.01 ^e
	20	6.44±0.01 ^d	4.67±0.01 ^c	4.47±0.01 ^d	4.09±0.01 ^d
SEM ^b		0.015	0.010	0.003	0.006

^aControl was not add barley and oligosaccharide.

^bStandard error of the mean ($n=15$).

^{a-g}Different letters within the same column with the same sample differ significantly ($p < 0.01$).

Changes of chemical compositions of yogurt during fermentation

Effect of barley flour added on quality characteristics of yogurt during fermentation was investigated. The addition of barley flour has been shown to affect the fermentation efficacy of lactic acid bacteria in yogurt. In this study, not only barley but also oligosaccharide addition contributes to high pH of yogurt (Table 2). pH of yogurt added barley flour (Daeanchal) with 0 and 20% were 6.66 and 6.40, respectively. Gee *et al.* (2007) reported that addition of highly concentrated barley beta-glucan had no significant effect on the ability of the starter cultures to ferment. Low pH (4.6) and glucono delta lacton accumulation had no effect on the survival of *L. acidophilus* strains (Ng, 2011). As a similar result, Farnworth *et al.* (2007) reported that the falls of the pH values were faster in the soybeverage than in milk. Our research indicated that pH of yogurt supplemented with barley flour tended to be lower than

before addition.

Titrateable acidity of yogurt was increased by addition of barley flour. Also, titrateable acidity was similar result with pH of yogurt. Titrateable acidity of yogurt added barley flour was higher compared with those of control (Table 3). Donkor *et al.* (2006) concluded that the decrease in *L. acidophilus* cell numbers in yogurts was the result of lactic acid and acetic acids accumulation. Moreover, Ng *et al.* (2011) reported that the level of organic acids was found to have a more pronounced effect on the strain's variability than the pH value. Sahan *et al.* (2008) reported that use of β -glucan hydrocollidal composite in the manufacture of non-fat yogurt did not significantly influence pH, titrateable acidity, acetaldehyde, volatile fatty acid and tyrosine contents at any storage time.

Change in brix of yogurt added barley during fermentation are shown in Table 4. Brix of yogurt was decreased during fermentation by lactic acid bacteria. Also, addition of

Table 3. Change in titrateable acidity of yogurt added barley during fermentation.

Samples	Con. (%)	Titrateable acidity			
		0 h	5 h	8 h	15 h
Control		13.50±1.41 ^c	14.50±2.12 ^d	30.75±1.06 ^d	44.50±0.71 ^c
Daeanchal	0	21.50±2.12 ^b	54.25±0.35 ^c	62.25±2.47 ^c	66.50±1.41 ^b
	10	23.50±2.12 ^b	93.50±2.12 ^a	104.25±1.77 ^a	121.25±0.35 ^a
	20	31.75±1.77 ^a	96.25±1.77 ^a	108.50±2.83 ^a	128.25±8.13 ^a
Boseokchal	0	19.75±2.47 ^b	55.50±4.24 ^c	64.50±0.71 ^c	66.50±14.85 ^b
	10	20.50±0.71 ^b	70.75±1.06 ^b	74.00±5.66 ^b	112.25±2.47 ^a
	20	22.25±2.47 ^b	74.25±2.47 ^b	91.50±0.71 ^b	121.50±4.95 ^a
SEM ^a		1.386	1.637	1.916	4.780

^aStandard error of the mean ($n=15$).

^{a-d}Different letters within the same column with the same sample differ significantly ($p<0.01$).

Table 4. Change in brix of yogurt added barley flours during fermentation.

Samples	Con. (%)	Brix			
		0 h	5 h	8 h	15 h
Control		20.97±0.81 ^a	21.40±0.72 ^a	20.27±0.25 ^a	18.17±0.06 ^a
Daeanchal	0	20.17±0.15 ^{ab}	18.83±1.23 ^b	17.80±0.46 ^{bc}	17.93±0.58 ^{ab}
	10	21.00±0.70 ^a	18.47±0.32 ^b	17.73±0.12 ^c	17.77±0.21 ^{ab}
	20	21.10±0.36 ^a	18.67±0.15 ^b	18.33±0.25 ^b	18.37±0.21 ^a
Boseokchal	0	20.83±0.68 ^a	17.73±0.65 ^b	17.83±0.35 ^{bc}	17.37±0.35 ^b
	10	20.67±0.12 ^{ab}	18.48±0.59 ^b	17.60±0.35 ^c	17.40±0.10 ^b
	20	19.70±0.85 ^b	18.33±0.21 ^b	17.77±0.06 ^c	17.43±0.57 ^b
SEM ^a		0.346	0.377	0.169	0.205

^aStandard error of the mean ($n=15$).

^{a-c}Different letters within the same column with the same sample differ significantly ($p<0.01$).

oligosaccharide was decreased of brix of yogurt during fermentation. However, addition of barley flour was not influence on brix of yogurt during 5 hr fermentation. Hou *et al.* (2000) showed that concentration of sucrose, raffinose and stachyose decreased during fermentation, in spite of that concentration of the monosaccharides fructose, glucose and galactose increased. However, Farnworth *et al.* (2007) reported that fructose was the sugar most utilized and glucose, raffinose and stachyose were used much less by lactic acid bacteria.

Hunter color values and microbiological analysis of the yogurt added colored barley flour

Color contributes to measuring the quality of a food, which attracts the consumer and color from natural sources is determined by handling or processing (Hallagan *et al.*, 1995). Changes in the Hunter color value of yogurt added barley flour are shown in Table 5. Lightness of yogurt added barley flour (Daeanchal) addition 0 and 20% were 83.25 and 69.83, respectively. Yogurt during fermentation by lactic acid bacteria induced the color change of the samples. Lee *et al.* (2002) reported that Hunter L (Brightness) value of curd yogurt added with 3% maesil extracts was not significantly different from the control curd yogurt. However, in this research, addition of barley flour as cereal was to decrease lightness of yogurt.

Cereal fermentation processes are affected by characteristic

variables such as fermentable substrates, nutrients, growth factors, minerals, efficacy of growth inhibiting principle, water contents, and amylolytic activity (Hammes *et al.*, 2005). The original microbial population of the yogurt during 0, 5, 8, and 15 hr fermentation was 7.48, 7.79, 8.15, and 8.71 Log CFU/g, respectively. Lactic acid bacteria count of yogurt added oligosaccharides was higher than the control. Moreover, the addition of barley flour was to promote the proliferation of lactic acid bacteria in yogurt. Oligosaccharide, such as lactulose, fructo-oligosaccharides, and trans galacto-oligosaccharides have received interested attention, especially because they have been shown to be effective in stimulating the growth of *Bifidobacteria* and *Lactobacilli* in human large intestine (Charalampopoulos *et al.*, 2002). Moreover, cereals have higher content of some of the essential vitamins than milk, higher content of dietary fibre, and increased amount of minerals, especially phosphorus. Charalampopoulos *et al.* (2002) reported that the malt medium supported better cell growth than barley and wheat due to the increased amounts of maltose, sucrose, glucose, and fructose (approximately 15 g of total fermentable sugars) and free amino nitrogen (approximately 80 mg). Lactic acid bacteria can rarely convert starch into lactic acid. However, some strains of *Lactobacillus* and *Streptococcus* and do it (Prade *et al.*, 2008). Vasiljevic *et al.* (2007) reported that the barley beta-glucan addition suppressed proteolytic activity

Table 5. Hunter color value of yogurt added barley flours during fermentation.

Sample	Con (%)	Ferm. Time (hr)	L	a	b
Control		0	83.22±0.02 ^a	-3.15±0.01 ^b	3.71±0.03 ^a
		15	81.84±0.06 ^b	-2.65±0.03 ^a	2.43±0.01 ^b
Daeanchal	0	0	83.25±0.04 ^a	-2.57±0.01	6.00±0.01 ^b
		15	83.02±0.02 ^b	-2.55±0.02	7.43±0.09 ^a
	10	0	74.45±0.05	-0.68±0.01 ^b	10.57±0.07 ^b
		15	74.34±0.16	-0.59±0.02 ^a	11.44±0.11 ^a
	20	0	69.83±0.08	-0.17±0.01 ^b	12.53±0.08 ^b
		15	69.88±0.22	-0.12±0.02 ^a	13.20±0.20 ^a
Boseokchal	0	0	83.01±0.04 ^b	-2.70±0.02 ^b	5.60±0.07 ^b
		15	83.45±0.01 ^a	-2.63±0.01 ^a	7.54±0.03 ^a
	10	0	72.90±0.09	-0.13±0.01 ^b	8.23±0.07 ^b
		15	70.94±0.03	-0.06±0.02 ^a	9.86±0.02 ^a
	20	0	68.70±0.05 ^a	-0.26±0.01 ^b	8.93±0.05 ^b
		15	67.49±0.02 ^b	0.49±0.01 ^a	9.78±0.03 ^a

^{a-b}Different letters within the same column with the same sample differ significantly ($p < 0.01$).

Table 6. Effect of barley flours on the growth (Log CFU/g) of *Lactic acid bacteria* in yogurt during fermentation.

Samples	Con. (%)	Lactic acid bacteria (log CFU/g)			
		0 h	5 h	8 h	15 h
Control		7.48±0.03 ^c	7.79±0.13 ^e	8.15±0.17 ^c	8.71±0.04 ^c
Daeanchal	0	7.49±0.02 ^c	9.07±0.04 ^d	9.22±0.02 ^b	9.00±0.21 ^b
	10	7.87±0.04 ^{ab}	9.95±0.01 ^a	10.02±0.64 ^a	9.68±0.04 ^a
	20	7.90±0.01 ^a	10.10±0.01 ^a	9.42±0.01 ^{ab}	9.47±0.11 ^a
Boseokchal	0	7.48±0.03 ^c	9.27±0.08 ^{cd}	9.57±0.14 ^{ab}	9.47±0.12 ^a
	10	7.83±0.02 ^b	9.57±0.19 ^b	9.60±0.14 ^{ab}	9.15±0.01 ^b
	20	7.89±0.01 ^{ab}	9.39±0.01 ^{bc}	9.62±0.01 ^{ab}	9.61±0.01 ^a
SEM		0.019	0.065	0.187	0.073

^aStandard error of the mean ($n=15$).

^{a-d}Different letters within the same column with the same sample differ significantly ($p<0.01$).

more than that from oat. In our research, addition of barley flours added into the yogurt base may also have contributed to growth of lactic acid bacteria. Moreover, authors considered that results are good source to development of fermented food with the grain.

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