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Production of Fermented Kale Juices with *Lactobacillus* Strains and Nutritional Composition

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ABSTRACT: Fermented kale juices using four types of lactobacilli were produced in the present study. After 48 h of fermentation time, viable cell counts of all ferments reached an above 10^9 CFU/mL. The viability of the ferments after cold storage in the refrigerator for 4 weeks showed 10^8 CFU/mL in all ferments. Among four types of fermented kale juices, the ferment of *Lactobacillus acidophilus* IFO 3025 indicated a good nutritional composition, including neutral sugar (1,909.76 μg/mL), reducing sugar (564.00 μg/mL, $P<0.05$), and protein contents (160.06 μg/mL, $P<0.05$). The results of mineral composition analysis had the highest potassium value in all ferments (854.16~895.07 μg/mL), particularly in the ferment of *Lactobacillus brevis* FSB-1 ($P<0.001$), which is necessary to sustain osmotic pressure, prevention of high blood pressure, and protein synthesis. Moreover, calcium, phosphorus, and magnesium contents related to bone health were generally sufficient in all ferments. Consequently, in this study, fermented kale juices may be suggested as a healthy fermented beverage with essential nutrients. However, the acceptability of the fermented kale juice to the Korean taste should be further investigated with a trained taste panel to determine whether inoculated fermentation could be an option for the consumers.

Keywords: fermented kale juice, lactobacilli, nutritional composition, potassium, viable cell count

INTRODUCTION

Nowadays, consumers' claim for healthy foods is motivating the food industry to develop products based on ingredients with high content of bioactive compounds. Fruit and vegetable juices, perceived as healthy and refreshing products, have been suggested as an ideal vehicle of functional health ingredients, such as minerals, vitamins, dietary fiber, and bioactive compounds, such as phenolics, choline, carotenoids, phytoestrogen, and glucosilolates (1,2).

Among many vegetables, green leafy vegetables including kale, have been reported to have hypocholesterolemic effects, reduction of cholesterol absorption, and enhancement of cholesterol catabolism to bile acids (3,4). Further biological activities of kale extracts have been demonstrated in *in vitro* systems, such as antioxidant activity, inhibitory effect of abnormal cell growth, and inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase activity (5). Kale juice also showed a strong antimutagenic activity against aflatoxin B_1 (6) as well as improvements in serum lipid profiles, especially with respect to high-density lipoprotein (HDL)- and low-density lipoprotein (LDL)-cholesterol levels, the ratio of HDL- to LDL-cholesterol, and in the antioxidant status of hypercholesterolemic men (4).

Fermentation can be defined as a desirable process of biochemical modification of primary food products brought about by microorganisms and their enzymes (7). Lactic acid fermentation of vegetable juices is a preservation method in the food industry, which has gained attention because of the nutritional value, shelf-life, texture, taste, and health effects (8).

Probiotics are live microorganisms, which confer beneficial effects to the host when administered in adequate amounts (9). The most studied probiotics belong to the genera lactobacilli and bifidobacteria. These genera have considerable safety records within the fermented foods industry where they have been used for many years in probiotic foods (10). In particular, lactobacilli may aid the digestion of lactose in lactose-intolerant individuals, reduce constipation and infantile diarrhea, help resist infections such as salmonellae, and help to relieve irritable bowel syndrome (9,11). Moreover, bifidobacteria is one of the strict anaerobic bacterial species, whereas lactobacilli, a microaerophilic bacteria, can grow more easily than

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bifidobacteria in the natural environment. Therefore, lactobacilli species as probiotics can simply be applied in the food fermentation. The desirable properties of fermented vegetable juices can be achieved by choosing *Lactobacillus* strains suitable for lactic acid fermentation of individual raw materials. Commercially offered strains in fermented vegetable juices are *Lactobacillus plantarum*, *Lactobacillus bifidus*, *Lactobacillus brevis*, and *Lactobacillus xylosus* (12). Therefore, the objective of present study was to produce a fermented kale juice using kale and four types of representative probiotic lactobacilli and to investigate the nutritional composition of the ferments.

MATERIALS AND METHODS

Lactobacillus strains and growth condition

Lactobacillus acidophilus IFO 3025 and *L. brevis* FSB-1 were kindly provided by the laboratory of Prof. Lee of Kyonggi University (Suwon, Korea), and *Lactobacillus casei* KCTC 12452 and *Lactobacillus plantarum* KCTC 3104 were obtained from the Korean Collection for Type Cultures (Daejon, Korea). The strains were maintained at −70°C in Man Rogasa Sharpe (MRS) broth (dextrose 20.0 g/L, meat peptone 10.0 g/L, beef extract 10.0 g/L, yeast extract 5.0 g/L, sodium acetate 5.0 g/L, disodium phosphate 2.0 g/L, ammonium citrate 2.0 g/L, tween 80 1.0 g/L, magnesium sulfate 0.1 g/L, and manganese sulfate 0.05 g/L) with 20% (v/v) glycerol before use and were twice activated grown in MRS broth at 37°C for 24 h.

Production of fermented kale juice

Kale (*Brassica oleraceae* L. var. *acephala* DC.) was purchased from an organic local market (Suwon, Korea) in July, 2012. It was washed with tap water twice and then air dried for 3 h at room temperature. Kale was then ground with an electronic grinder (Hanil Electronics Corp., Wonju, Korea) after adding three times the amount of distilled water based on the sample weight and then filtrated with a four-fold gauze. The sample was autoclaved at 121°C for 15 min, which was then inoculated with a 24 h culture (>10^7 CFU/mL) and incubated at 37°C for 48 h.

Total acidity and microbiological analysis

Total acidity, expressed as a percent of lactic acid, was determined by titrating with 0.01 N NaOH to pH 8.2. Microbiological analysis was determined by the plate count agar method with lactobacilli MRS medium after 48 h of incubation at 37°C.

Nutritional composition analysis

General component (neutral, acidic, reducing sugars, and protein content) and mineral composition characteristics (macro- and micro-mineral) for nutritional composition analysis of the fermented samples were determined.

Neutral and acidic sugar contents were analyzed by the phenol-sulfuric acid (13) and *m*-hydroxybiphenyl (14) methods, and then glucose and galacturonic acid were used as the respective standards. Reducing sugar content was measured by the 3,5-dinitrosalicylic acid (DNS) method (15) using glucose as the standard. Protein content was determined by the Bradford's method (16) using bovine serum albumin as the standard.

Mineral composition analysis was determined as follows: 2 g sample was treated with a mixture of 10 mL of 60% HNO_3 solution (v/v) and 3 mL of 60% $HClO_4$ solution (v/v) for 2 h. It was heated gradually from 100°C to 150°C for 2 h and then decomposed at 180°C for 2 h. After cooling the sample, it was filtrated with filter paper (No. 4, Whatman International Ltd., Kent, UK) and adjusted to volume in a 25 mL volumetric flask. Ten species of minerals (Ca, P, K, Na, Mg, Fe, Zn, Cu, Mn, and Se) and five species of heavy metals (As, Cd, Pb, Cr, and Ni) were analyzed using inductively coupled plasma optical emission spectrometry (ICP-OES, Varian 730-ES, Mulgrave, Victoria, Australia). The measurement wavelength is as follows: Ca, 370.602 nm; P, 213.618 nm; K, 766.491 nm; Na, 568.821 nm; Mg, 277.983 nm; Fe, 259.940 nm; Zn, 213.857 nm; Cu, 324.754 nm; Mn, 257.610 nm; Se, 196.026 nm; Ge, 209.426 nm; Cd, 226.502 nm; Pb, 220.353 nm; Cr, 267.716 nm; Ni, 231.604 nm.

Effect of cold storage on cell viability

After 48 h of fermentation at 37°C, the fermented samples were stored in the refrigerator at 4°C for 4 weeks. The samples were taken at weekly intervals, and the viability of *Lactobacillus* strains in the fermented samples was determined by the plate count agar method with lactobacilli MRS medium after 48 h of incubation at 37°C.

Statistical analysis

All statistical analyses were conducted using the Statistical Package for Social Sciences, version 12.0 (SPSS Inc., Chicago, IL, USA). The differences among the samples were evaluated statistically by a one-way analysis of variance and Duncan's multiple tests. All data were estimated using the significant differences among the means at $P<0.05$, $P<0.01$, and $P<0.001$.

RESULTS AND DISCUSSION

Total acidity and microbiological analysis

Kale juice was fermented by four types of *Lactobacillus* strains, including *L. acidophilus* IFO 3025, *L. brevis* FSB-1, *L. casei* KCTC 12452, and *L. plantarum* KCTC 3104. The results of total acidity and viable cell count of the four types of fermented kale juices are presented in Table 1.

Table 1. Total acidity and viable cell count of four types of fermented kale juices

Variables	Total acidity (%, lactic acid)	Viable cell count (CFU/mL)
Lactobacillus acidophilus IFO 3025	0.88±0.02[b]	1.1×10^{10}
Lactobacillus brevis FSB-1	1.01±0.05[a]	7.0×10^{9}
Lactobacillus casei KCTC 12452	0.87±0.03[b]	7.5×10^{9}
Lactobacillus plantarum KCTC 3104	1.01±0.05[a]	2.5×10^{10}

Data are expressed as mean±standard deviation.
Different letters (a,b) mean significant differences among samples (P<0.01).

After 48 h of fermentation time, the results of viable cell counts of *L. acidophilus* IFO 3025 and *L. plantarum* KCTC 3104 reached 10^{10} CFU/mL, whereas *L. brevis* FSB-1 and *L. casei* KCTC 12452 showed 10^{9} CFU/mL. The total acidity was higher in the ferments of *L. brevis* FSB-1 (1.01) and *L. plantarum* KCTC 3104 (1.01) than that of *L. acidophilus* IFO 3025 (0.88) and *L. casei* KCTC 12452 (0.87, P<0.01). Among the ferments, *L. plantarum* KCTC 3104 showed the highest value in total acidity (1.01) and viable cell count (2.5×10^{10} CFU/mL).

Nutritional composition

The nutritional value of a particular food depends on its digestibility and its content of essential nutrients, which may be improved by lactic acid fermentation. During fermentation, the enzymatic activity of a microbial culture may predigest the macronutrients (17). For this reason, general components and mineral composition characteristics among the fermented kale juices were determined and are presented in Table 2 and 3, respectively.

The range of neutral sugar contents of fermented kale juices were 1,434.52~1,909.76 μg/mL and did not show significant differences among the samples (Table 2). Acidic sugar contents showed a higher value in the fermented kale juice of *L. plantarum* KCTC 3104 (162.53 μg/mL) when compared to the other fermented kale juices (P<0.05). The reducing sugar content was lower in the fermented kale juice of *L. brevis* FSB-1 (510.67 μg/mL) than the other fermented kale juices (P<0.05). Protein content showed a higher value in the fermented kale juice of *L. acidophilus* IFO 3025 (160.06 μg/mL) and *L. plantarum* KCTC 3104 (152.86 μg/mL) than that of *L. casei* KCTC 12452 (97.18 μg/mL, P<0.01).

In the mineral composition characteristics of fermented kale juices, K content was observed at the highest value in all samples regardless of lactobacilli types, occurring in the following order: Ca> Na> Mg or P (Table 3). In general, the fermented kale juice of *L. brevis* FSB-1 showed high values in the contents of macro-mineral with the exception of Na content, K (895.07 μg/mL, P<0.001) and Mg (82.57 μg/mL, P<0.05). The fermented kale juice of *L. acidophilus* IFO 3025 was high in the contents of Na (186.47 μg/mL, P<0.001) and Zn (1.72 μg/mL, P<0.001). P content was higher in the fermented kale juice of *L. brevis* FSB-1 (82.65 μg/mL) and *L. plantarum* KCTC 3104 (82.37 μg/mL) than the other fermented kale juices (P< 0.001).

Generally, lactic acid fermentation leads to a decrease in the level of carbohydrates as well as some non-digestible polysaccharides and oligosaccharides (7), and it enhances protein solubility and the availability of limiting amino acids in some cases by as much as 50% (18). The micronutrient availability is enhanced because of significant reduction in phytates and breakdown of complex substances (18,19). It has been shown that metal chelates originating from vegetables, such as phytate, tannins, and oxalate may be degraded during fermentation as a result of microbial and/or plant enzymatic activities (20). This has been suggested as a major cause for improved mineral availability by lactic acid fermentation (21,22).

Several studies reported that prebiotic-rich, micronutrient-rich, and low-calorie diets play important roles in supporting intestinal health with potential to promote gut health. Enriched-prebiotic products are becoming more popular in the belief that they may also have probiotic effects (23,24). Kale has recently received attention from the health and nutrition sectors due to its nutrient profile with 8% carbohydrates, 3.5% dietary fiber, vitamins, and minerals, including K, Ca, Mg, and Zn (25,26). Thavarajah et al. (27) reported that a serving of fresh

Table 2. General components of four types of fermented kale juices

Variables	Neutral sugar (μg/mL)	Acidic sugar* (μg/mL)	Reducing sugar* (μg/mL)	Protein content** (μg/mL)
Lactobacillus acidophilus IFO 3025	1,909.76±212.17[NS]	150.91±8.02[ab]	564.00±0.00[a]	160.06±24.19[a]
Lactobacillus brevis FSB-1	1,597.86±189.39	157.98±9.74[ab]	510.67±23.09[b]	134.21±7.67[ab]
Lactobacillus casei KCTC 12452	1,434.52±374.93	143.33±2.62[b]	554.00±17.32[a]	97.18±17.76[b]
Lactobacillus plantarum KCTC 3104	1,724.05±290.17	162.53±8.35[a]	560.67±15.28[a]	152.86±4.30[a]

Data are expressed as mean±standard deviation.
Significantly different at *P<0.05 and **P<0.01.
Different letters (a,b) mean significant differences among samples.
NS: No significant differences.

Table 3. Mineral composition characteristics of four types of fermented kale juices

Variables	Macro-mineral (μg/mL)				
	Ca***	K***	Na***	Mg*	P***
Lactobacillus acidophilus IFO 3025	484.55±2.17[a]	866.56±2.31[b]	186.47±2.85[a]	77.92±2.36[b]	75.87±0.67[b]
Lactobacillus brevis FSB-1	495.51±3.58[a]	895.07±2.82[a]	156.82±1.73[b]	82.57±1.69[a]	82.65±2.29[a]
Lactobacillus casei KCTC 12452	472.16±3.07[b]	854.16±2.92[c]	146.07±2.07[c]	80.10±1.88[ab]	72.51±0.91[b]
Lactobacillus plantarum KCTC 3104	487.07±1.26[a]	858.34±2.77[bc]	140.44±3.20[c]	79.88±2.52[ab]	82.37±1.15[a]
	Micro-mineral (μg/mL)				
	Zn***	Mn***	Fe	Cu	Se
Lactobacillus acidophilus IFO 3025	1.72±0.02[a]	0.53±0.02[a]	ND	ND	ND
Lactobacillus brevis FSB-1	0.60±0.01[b]	0.34±0.03[b]	ND	ND	ND
Lactobacillus casei KCTC 12452	0.60±0.02[b]	0.28±0.02[b]	ND	ND	ND
Lactobacillus plantarum KCTC 3104	ND	0.35±0.01[b]	ND	ND	ND

Data are expressed as mean±standard deviation.
Significantly different at $^{*}P<0.05$ and $^{***}P<0.001$.
Different letters (a-c) mean significant differences among samples.
ND: Not detected.

kale (100 g) from 25 genotypes can also provide approximately 0.4～6.7 g of prebiotic carbohydrates, including sugar alcohols (45.4～59.8 mg), simple sugars (0.4～3,348 mg), and hemicellulose (245～703 mg). In the carbohydrate analysis of fresh kale, the highest value was for glucose at 993 mg/100 g followed by fructose (545 mg/100 g)> mannose (241 mg/100 g)> arabinose (73.5 mg/100 g)> xylose (59.9 mg/100 g)> sucrose (39.3 mg/100 g). The mean protein content was 4.4 mg/100 g and the range was 1.6～5.9 mg/100 g.

Some studies also reported that fresh kale has the potential to provide significant quantities of several essential micronutrients, including minerals (27-29). Kawashima and Soares (28) compared K, Ca, and Mg levels in Brazilian-grown kale to other green vegetables, including Chinese cabbage, cabbage, butter head lettuce, and spinach. They found fresh kale to have the highest concentration of K (712 mg/100 g), Ca (283 mg/100 g), and Mg (51 mg/100 g). Thavarajah et al. (27) reported that a 100 g single serving of fresh kale can provide 188～873 mg of K, 35～300 mg of Ca, and 20～100 mg of Mg. In the present study, K content was the highest mineral in all fermented kale juices, which was particularly high in the fermented kale juice of *L. brevis* FSB-1 ($P<0.001$). K is an essential nutrient and has an important role in the synthesis of amino acids and proteins (30) as well as in the reduction of hypertension risk in adults (31). Ca content was the second largest amount in all ferments, and Mg ($P<0.05$) and P contents ($P<0.001$) were high in the ferments of *L. brevis* FSB-1 and *L. plantarum* KCTC 3104. Therefore, we confirmed that fermented kale juice may be an excellent nutrient source of K, Ca, Mg, and P. Among micro-minerals, Zn is essential for bone formation, and recent research suggests that bone loss and cardiovascular disease are functionally interwoven (32). Thus, the supply of sufficient micro-minerals, including Zn, may be essential for the prevention of chronic disease and osteoporosis. In this study, Zn and Mn contents of *L. acidophilus* IFO 3025 showed the highest values (Zn: 1.72 μg/mL; Mn: 0.53 μg/mL) after the fermentation time of 48 h among the fermented kale juices.

Effect of cold storage on cell viability in fermented kale juice

The effect of cold storage on the cell viability of four types of lactobacilli in fermented kale juice was determined and are presented in Table 4. The results of the viable cell count showed to be 10^8 CFU/mL in all fermented kale juices, which occurred in the following order: *L. plantarum* KCTC 3104 (6.7×10^8 CFU/mL)> *L. casei* KCTC 12452 (2.6×10^8 CFU/mL)> *L. acidophilus* IFO 3025 (1.9×10^8 CFU/mL)> *L. brevis* FSB-1 (1.5×10^8 CFU/mL). Shah (33) reported that the minimum number of probiotic organisms in a food product should be 10^6 CFU/mL for maximum health benefits. The viability of probiotic bacteria is affected by inhibitory substances, such as lactic acid produced during cold storage of the ferment (34), which is the most important factor for the viability of lactic acid bacteria. However, the present results of viable cell counts of kale ferments by the four types of lac-

Table 4. Effect of cold storage on the cell viability of four types of fermented kale juices (unit: CFU/mL)

Time (week)	*Lactobacillus acidophilus* IFO 3025	*Lactobacillus brevis* FSB-1	*Lactobacillus casei* KCTC 12452	*Lactobacillus plantarum* KCTC 3104
1	6.8×10^9	2.9×10^8	5.0×10^8	1.8×10^8
2	2.3×10^8	3.5×10^8	2.9×10^8	5.2×10^8
3	1.5×10^8	1.8×10^8	2.4×10^8	3.3×10^8
4	1.9×10^8	1.5×10^8	2.6×10^8	6.7×10^8

tobacilli maintained at about 10^8 CFU/mL after 4 weeks in a cold refrigerator. This result suggested that kale was suitable as a raw material for the production of probiotic kale juice ferment using lactic acid bacteria.

Consumption of diets rich in fruits and vegetables can reduce the prevalence of chronic diseases, including diabetes, cancer, heart disease, and stroke (35). Kale is strongly associated with reducing the risk of heart disease and other non-communicable disease (25,26,36). In the present study, probiotic kale juices using four types of lactobacilli were finely produced after 48 h of fermentation time at 37°C with the viable cell counts of above 10^9 CFU/mL. The nutritional composition analyses showed a good result including neutral sugar, reducing sugar, and protein contents particularly in the ferment of *L. aciophilus* IFO 3025. Whereas the ferment of *L. brevis* FSB-1 showed a great mineral composition related to bone health such as Ca, P, and Mg contents, and all the probiotic kale juices produced in this study had the highest K value which is necessary to help prevent hypertention. In conclusion, probiotic kale juices produced in the present study may be useful for the prevention of chronic diseases and are suggested as healthy probiotic fermented beverages with high essential nutrients.

AUTHOR DISCLOSURE STATEMENT

The author declares no conflict of interest.

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