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Fermentation of Green Coffee Bean (*Coffee Arabica*) with Lactic Acid Bacteria and Characterization of Its Biochemical Properties

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Abstract

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Coffee is one of the most popular beverages in the world. In this study, lactic acid bacteria, *Lactobacillus delbrueckii* YC KCCM11945P (YC), *Streptococcus thermophilus* Y2 KCCM11946P (Y2), and *Streptococcus thermophilus* R2 KCCM11947P (R2) were isolated from Korean traditional food; doenjang, and/or cheonggukjang. Using these strains, green coffee bean was fermented with various water or bacteria inoculation concentration based on sensory evaluation. The water optimization concentration (w/w) was 50% for YC, 50% for Y2, and 15% for R2, respectively. The optimization of bacteria concentration for coffee fermentation was 3.2 x 10^8 CFU/g coffee for YC and Y2, 6.4 x 10^8 CFU/g coffee for R2. The fermentation time was found though bacterial growth during fermentation, which was fixed for 12 h. Regular coffee and fermented coffee were analyzed by total phenol and flavonoid contents, antioxidant activity (DPPH, ABTS), angiotensin converting enzyme

inhibition activity, and the amount of trigonelline, caffeine, and chlorogenic acid determined by reverse-phase HPLC. Fermented coffee increases 1.2 times and 1.5 times in total phenol and flavonoid content. Fermented coffee improves 2 times of SC_{50} in DPPH, and 1.3 times in ABTS, and 1.7 times of IC_{50} in angiotensin converting enzyme. In an analysis of HPLC, content of chlorogenic acid (CGA) increases 1.3 times in fermented coffee than regular coffee, but trigonelline and caffeine were the same in regular coffee and fermented coffee. Additionally, to increase the flavor, cinnamon and pine needle extract added with green coffee bean during fermentation. Fermented coffee with cinnamon or pine needle also showed the similar results of fermented coffee, which improved more functionality than regular coffee.

Keywords : Lactic acid bacteria, *Coffee arabica*, antioxidant activity, fermentation, angiotensin converting enzyme

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Contents

List of Tables

List of Figures

Introduction

Coffee is one of the most popular beverages in the world and ranked as the second most traded global commodity after petroleum [1] and global coffee consumption has risen at an average annual rate of 1.9% over the past 50 years [2]. The coffee plant belongs to the genus *Coffee* (*Rubiaceae* family) that has over ninety different species, but only two of them, *Coffee arabica* (~60% of the world's production) and *C. canephora*, named also *robusta* (~40%) are widely cultivated and have economical value [3]. Frequent coffee consumption has been associated with a reduced risk of diabetes [4], Parkinson disease [5], cardiovascular disease [6], neurological disorders [7] and cancer [8]. These effects of coffee have been contributed to many different bioactive constituents of coffee such as caffeine, chlorogenic acids, diterpenes, and other phenolic compounds. In addition to, some of these compounds also played an important role in determining of coffee quality such as chlorogenic acid, trigonelline determined bitter-tasting of coffee [9-12], and is a precursor for the formation of different kinds of volatile compounds [10] during roasting. The sensory profiles in coffee are effected by growing, processing variables (fermentation, drying, and roasting), packaging, storage, and brewing conditions [13-15]. Kopi Luwak is made from coffee cherries that have been eaten by the Asian palm civet (*Paradoxurus hermophoditus*) though their gastrointestinal or intestinal fermentation. This exotic process could be made the world's most expensive coffee and add a unique flavor and taste to the beans [16]. For these profits, palm civets held captive and cages in the battery case with worse conditions [17]. In addition, there is no reliable, standardized method for determining the authenticity of Kopi Luwak and there is limited scientific information on Luwak coffee. Recently, a fraudulent Kopi Luwak was reported in the coffee market [18]. For these reasons, the

artificial fermentation process highlighted using several starters. One of them is lactic acid bacteria.

Lactic acid bacteria (LAB) play a critical role in food production and health maintenance. The health promoting ability of LAB in humans are inhibition of pathogenic microorganisms [19], increase in immune response [20], reduction of serum cholesterol levels [21]. In fermented food, LAB not only contribute to the flavor of the food, improve nutritional values but also is able to control pathogenic and spoilage microorganism though production of organic acids, bacteriocins, aroma compounds, exopolysaccharides [22-24]. The exopolysaccharide from lactic acid bacteria contributes to the texture, mouth-feel, taste perception and stability of the final product [25]. Previous studied reported that the fermented soya bean [26], kidney bean [27], chickpea [28], soy whey [29], small runner bean [26] by LAB increased total phenol contents as well as increase antioxidant activity. Diversity of LAB found in coffee cherries, showed antifungal activity against *Aspergillus flavus*, and produced bacteriocin [24]. Therefore, LAB was selected for coffee fermentation and to improve the flavor of fermented coffee. In addition, the cinnamon or pine needle were mixed in green coffee bean during coffee fermentation to modify flavor and improve functional component contents.

In this study, we investigated the sensory change of fermented coffee during optimization of fermentation and analyzed the amount of total phenol and flavonoid contents, chlorogenic acid, trigonelline, and caffeine. The biological functions of fermented coffee such as antioxidant effect, inhibitory activity against angiotensin converting enzyme (ACE) were also characterized.

Review of Literature

1. Coffee Bean Fermentation

The quality of a coffee is highly related to the chemical modification when roasting process but is also dependent on the postharvest processing. Microbial activity during fermentation solubilize the pulp material around the seeds, and produces a variety of metabolic products (e.g. alcohols and organic acids). Above the reason makes most important thing; even though characteristic flavor of coffee come from the composition of the bean, the microbe responsible for the fermentation may also make for the beverage sensory characteristics and other qualities because of the release of metabolites and their diffusion into the beans during the process.

Thee different methods are used in producing countries to process coffee fruit, referred to as dry, wet and semi-dry [30]. Wet processing used for Arabica coffee: the fruits are removed the pulp, submitted to underwater tank, and dried by a final water content of 10-12%. In dry processing, however, coffee fruits are dried in the sun on platforms without prior removal of the pulp. Semi-dry processing is a combination of wet and dry processing, in which coffee fruits are de-pulped, but the fermentation process do under the sun on a platform. In all these processing, a spontaneous fermentation carried out for removal of mucilage stuck to the beans and helps improve beverages flavor by microbial metabolites. This process produces a lot of metabolites, such as organic acids, higher alcohols and esters, which will plus complexity and depth to a coffee later [31]. Until now, coffees

made by wet process have better characteristics (quality, body, acidity, and aroma) than coffees made by other processes. Theses sensorial differences attributes by the fact that various metabolic process occur inside the coffee seeds which significantly alter the chemical composition of the green beans [32].

2. Lactic acid bacteria

Lactic acid bacteria are isolated in high populations during wet and semi-dry processing. It is possible that the anaerobic or low oxygen conditions present in wet process favor the development of lactic acid bacteria [33]. Lactic acid bacteria isolated from silage of coffee pulp have antifungal effect against Ochatoxigenic moulds like *Aspergillus carbonaruis* [24]. So far, little study has investigated the diversity of lactic acid bacteria in coffee fermentation. Thus, the function of lactic acid is not well known. Until now, lactic acid bacteria in coffee fruit have a function of antifungal activity. The potential of artificial inoculation of lactic acid bacteria to inhibit mold growth might affect during coffee fermentation. [24, 34].

Metabolically, homofermentative lactic acid bacteria (e.g., *Lactobacillus plantarum*) change the available energy source (sugar) to lactic acid via pyruvate to produce energy. Also, heterofermentative lactic acid bacteria (e.g., *Leuconostoc mesenteroides*) produces lactic acid and ethanol as well as several short-chain fatty acids, such as acetic acid and formic acid [35]. Theses chemical compounds

may contribute to the acidity of fermented coffee beans.

The use of starter cultures has emerged as a promising alternative to control the fermentation process and to promote quality development of coffee product. However, hardly information is available on the effects of controlled starter cultures in coffee fermentation performance and bean quality, making it impossible to use this technology in actual field conditions. A wide knowledge on the biochemistry, molecular biology and ecology could do easily the understanding and application of starter culture for coffee fermentation.

3. Major chemical compounds in coffee: Trigonelline, Chlorogenic acid and Caffeine

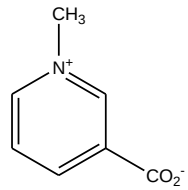
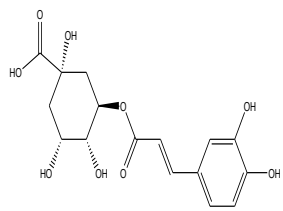
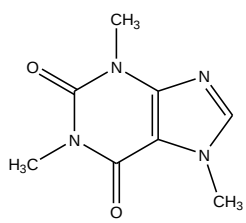
Trigonelline is an alkaloid and a zwitterion linked by the methylation of the nitrogen atom of niacin. It is a product of niacin metabolism that is excreted in urine. Coffee seeds have a lot of trigonelline. Trigonelline appears to have hypoglycemic, hypolipidemic, sedative, antimigraine, antibacterial, antiviral, anti-tumor effect and improve memory retention [36]. Trigonelline is a vitamin B6 derivative with a bitter taste. Its content in green coffee beans is 0.6 ~ 1%, which contributes to undue bitterness in coffee. When coffee is roasted, approximately 85% of trigonelline is broken down to nicotinic acid.

Chlorogenic acid is a natural chemical compound, which is the ester of caffeic acid and quinic acid. This compound known as an antioxidant, may also slow the release of glucose into the bloodstream after a meal [37]. Chlorogenic acid was mainly found in green and roasted coffee bean extract [38]. Chlorogenic acids are an important group of

biologically active dietary phenols; the best-known being 5-caffeoylquinic acid. The daily intake of chlorogenic acids by coffee drinkers is considered to be in the range 0.5 - 1 g and chlorogenic acids have been found to exhibit antioxidant activity in vitro [39].

Caffeine is a commercially important alkaloid, which belongs to group of purine alkaloids synthesized by plants. In nature, caffeine is distributed in the leaves and fruits of 13 different orders of plants, including coffee, tea, and cacao. Caffeine is an active psychostimulant which increase alertness and sustains concentration by overcoming fatigue [40]. This makes caffeine one of the most widely consumed dietary chemicals. Caffeine is widely used in pharmaceutical preparations as it enhances the effects of certain analgesics and antipyretic drugs [41]. Conventional decaffeination techniques like solvent extraction or use of supercritical carbon dioxide can be expensive, toxic to the environment and non-specific. Hence, there is a strong need for degradation of caffeine from products and waste streams by alternative routes other than conventional extraction techniques. The potential use of microorganisms obtained from microbial system for developing biological decaffeination techniques offer a much attractive alternative to the present existing techniques [42].

Table 1. Structure and functionality of major compounds in coffee

Compound	Structure	Functionality	Reference
Trigonelline	 Trigonelline	Antidiabetic activity, Antioxidant activity, Improve the insulin resistance.	Yoshinari et al.[43] Dutta et al. [44]
Chlorogenic acid	 Chlorogenic acid (5-caffeoylquinic acid)	Antioxidant, antimicrobial, Inhibitory effects on carcinogenesis in the large intestine, liver and tongue.	Pimia et al. [45] Park et al. [46] Azuma et al. [47]
Caffeine	 Caffeine	Alleviating muscular pain, Increase in serum free fatty acids	Kalmar et al. [48] Van Soeren et al.[49]

Materials and methods

2.1. Lactic acid bacteria preparation

The LAB were isolated from Korean soy bean paste on *Lactobacilli* deMan, Rogosa and Sharpe medium (MRS, Difco, Seoul, Korea) and determined as *Lactobacillus delbrueckii* YC KCCM11945P (YC), *Streptococcus thermophilus* Y2 KCCM11946P (Y2), and *Streptococcus thermophilus* R2 KCCM11947P (R2) by using Biolog (Biolog Inc., CA, USA) and 16s rRNA sequence (Table S1). LAB were grown on MRS broth at 37℃ for 24 h until OD_{600} reached at 2.0 (1.6×10^{11} CFU/mL).

2.1.1. Isolation of lactic acid bacteria

Korea traditional fermented food, cheonggukjang or doenjang 0.1g were separately blended with 900 μL 0.85% NaCl solution. Each of these carried out serial dilution. Diluted samples spread in MRS Agar plate and grown at 37℃ for 24 h.

2.1.2. Identification of lactic acid bacteria

Cultivated bacterial was pure cultured on MRS Agar plate. A single colony of bacteria was incubated in MRS broth at 37℃ for 24 h. Genomic DNA from 5 mL of culture media was extracted using Accuprep Genomic DNA Extraction kit (Bioneer, Daejeon, Korea). The method of extraction was followed supplier's the manual. The 5 mL sample was centrifuged at 12,000 x g for 5 min. The cell pellet was resuspended in 50 μL of tissue lysis buffer (TL buffer), added with 20 μL of lysozyme and incubated at 37 ℃ for 1 h. Then 20 μL of proteinase K, 200 μL of TL buffer and 200 μL of binding buffer (GC buffer) was added and then vortexed immediately. The sample was incubated at 60 ℃ for 10 min. 100 μL of

isopropanol was added and then centrifuged at 8,000 x g for 1 min. The supernatant was transferred carefully into binding column tube. Binding column tube was centrifuged at 8,000 x g for 1 min. The column was washed with 500 μL of washing buffer 1 and 2. The column was centrifuged at 12,000 x g for 1 min and then transferred to a new 1.5 mL tube and 30 μL of elution buffer was added to binding column tube. After at least 1 min waiting, the column was centrifuged at 8,000 x g for 1 min. The isolated DNA was commissioned to Biosesang (co., Ltd., Korea). Universal primer set (27F; 5'-AGAGTTTGATCCTGGCTCAG-3' and 1492R; 5'-GGTTACCTTGTTACGACTT-3') was used to analysis of 16S rDNA. 16S rDNA sequence was edited using Chomaspro (version 1.49, Technelysium Pty Ltd., Helensville, Queensland, Australia). Sequence-similarity searches were conducted using GenBank data (NCBI offers the GenBank blast searches at http://www.ncbi.nlm.nih.gov/genbank (Table. S1)

2.1.3. Characterization of lactic acid bacteria

A single colony was selected and emulsified into IFA (inoculating fluid A) for subsequent inoculation on to the Microplate test plate. According to the manufacturer's instructions, inocula prepared to a specified transmittance using a turbidimeter until an optical density 98% was reached. For each isolate, 100μL of the cell suspension was inoculated into each well of the MicroPlate, using a multichannel pipette and incubated at 37℃ for 24 h. Microplates were read in the MicroStation reader and results interpreted by the identification system's software (GEN III database, version 5.2.1) (Table. S2)

Table. S1 Identification of YC, Y2, and R2 16S rRNA sequence and description

	16S rRNA sequence
YC	GTTATCCCACCGACTTTGGGCATTGCAGACTTCCATGGTGTGACGGGCGGTGTGTA CAAGGCCCGGGAACGTATTCACCGCGGCGTGCTGATCCGCGATTACTAGCGATTC CAGCTTCGTGCAGGCGAGTTGCAGCCTGCAGTCCGAACTGAGAACAGCTTTAAG AGATCCGCTTACCCTCGCGGGTTCGCTTCTCGTTGTACTGCCCATTGTAGCACGTG TGTAGCCCAGGTCATAAGGGGCATGATGACTTGACGTCATCCCCACCTTCCTCCGG TTTGTCACCGGCAGTCTCTTTAGAGTGCCCAACTTAATGATGGCAACTAAAGACA AGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGA CAGCCATGCACCACCTGTCTCTGCGTCCCCGAAGGGAACCACCTATCTCTAGGTG TAGCACAGGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCA CATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAACCTTGCGG TCGTACTCCCCAGGCGGAGCGCTTAATGCGTTTGCTGCGGCACTGAGGACCGGAA AGTCCCCAACACCTAGCGCTCATCGTTTACGGCATGGACTACCAGGGTATCTAATC CTGTTCGCTACCCATGCTTTCGAGCCTCAGCGTCAGTTGCAGACCAGAGAGCCGC CTTCGCCACTGGTGTTCTTCCATATATCTACGCATTCCACCGCTACACATGGAATTC CACTCTCCTCTTCTGCACTCAAGAATGACAGTTTCCGATGCAGTTCCACGGTTGA GCCGTGGGCTTTCACATCAGACTTATCATTCCGCCTGCGCTCGCTTTACGCCCAAT AAATCCGGACAACGCTTGCcACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAG CCGTGACTTTCTGGTTGATTACCGTCAAATAAAGACCAGTTACTGCCTCTATCCTT CTTCACCAACAACAGAGCTTTACGATCCGAAAACCTTCTTCACTCACGCGGCGTT GCTCCATCAGACTTGCGTCCATTGTGGAAGATTCCCTACTGCTGCCTCCCGTAGGA GTTTGGGCCGTGTCTCAGTCCCAATGTGGCCGATCAGTCTCTCAACTCGGCTACG CATCATTGCCTTGGTAGGCCTTTACCCCACCAACTAGCTAATGCGCCGCGGGCTCA TCCTAAAGTGACAGCTTACGCCGCCTTTCAAACTTGAATCATGCGATTCATGTTGT TATCCGGTATTAGCACCTGTTTCCAAGTGGTATCCCAGTCTTTAGGGCAGATTGCC CACGTGTTACTCACCCATCCGCCGCTAGCGTCCAACAAATCATCCCGAAGGAATCT TTGAATTCAGCTCGCTCGACTGCATGATAG

Table. S1 Identification of YC, Y2, and R2 16S rRNA sequence and description

Y2	TATAATGCAGTAGAACGCTGAAGAGAGGAGCTTGCTCTTCTTGGATGAGTTGCGA
	ACGGGTGAGTAACGCGTAGGTAACCTGCCTTGTAGCGGGGGATAACTATTGGAAA
	CGATAGCTAATACCGCATAACAATGGATGACACATGTCATTTATTTGAAAGGGGCA
	ATTGCTCCACTACAAGATGGACCTGCGTTGTATTAGCTAGTAGGTGAGGTAATGGC
	TCACCTAGGCGACGATACATAGCCGACCTGAGAGGGTGATCGGCCACACTGGGAC
	TGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCGGCAATG
	GGGGCAACCCTGACCGAGCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGTA
	AAGCTCTGTTGTAAGTCAAGAACGGGTGTGAGAGTGGAAAGTTCACACTGTGAC
	GGTAGCTTACCAGAAAGGGACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGT
	AGGTCCCGAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTGA
	TAAGTCTGAAGTTAAAGGCTGTGGCTCAACCATAGTTCGCTTTGGAAACTGTCAA
	ACTTGAGTGCAGAAGGGGAGAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAG
	ATATATGGAGGAACACCGGTGGCGAAAGCGGCTCTCTGGTCTGTAACTGACGCTG
	AGGCTCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCG
	TAAACGATGAGTGCTAGGTGTTGGATCCTTTCCGGGATTCAGTGCCGCAGCTAAC
	GCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAAT
	TGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGA
	AGAACCTTACCAGGTCTTGACATCCCGATGCTATTTCTAGAGATAGAAAGTTACTT
	CGGTACATCGGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGT
	TGGGTTAAGTCCCGCAACGAGCGCAACCCCTATTGTTAGTTGCCATCATTCAGTTG
	GGCACTCTAGCGAGACTGCCGGTAATAAACCGGAGGAAGGTGGGGATGACGTCA
	AATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGTTGGTACAAC
	GAGTTGCGAGTCGGTGACGGCGAGCTAATCTCTTAAAGCCAATCTCAGTTCGGAT
	TGTAGGCTGCAACTCGCCTACATGAAGTCGGAATCGCTAGTAATCGCGGATCAGC
	ACGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGAG
	AGTTTGTAACACCCGAAGTCGGTGAGGTAACCTTTGGAG

Table. S1 Identification of YC, Y2, and R2 16S rRNA sequence and description

R2	TGCAGTAGAACGCTGAAGAGAGGAGCTTGCTCTTCTTGGATGAGTTGCGAACGG
	GTGAGTAACGCGTAGGTAACCTGCCTTGTAGCGGGGGATAACTATTGGAAACGAT
	AGCTAATACCGCATAACAATGGATGACACATGTCATTTATTTGAAAGGGGCAATTG
	CTCCACTACAAGATGGACCTGCGTTGTATTAGCTAGTAGGTGAGGTAATGGCTCAC
	CTAGGCGACGATACATAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGA
	GACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCGGCAATGGGG
	GCAACCCTGACCGAGCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAG
	CTCTGTTGTAAGTCAAGAACGGGTGTGAGAGTGGAAAGTTCACACTGTGACGGT
	AGCTTACCAGAAAGGGACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGG
	TCCCGAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTGATAA
	GTCTGAAGTTAAAGGCTGTGGCTCAACCATAGTTCGCTTTGGAAACTGTCAAACT
	TGAGTGCAGAAGGGGAGAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGATAT
	ATGGAGGAACACCGGTGGCGAAAGCGGCTCTCTGGTCTGTAACTGACGCTGAGG
	CTCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAA
	CGATGAGTGCTAGGTGTTGGATCCTTTCCGGGATTCAGTGCCGAAGCTAACGCATT
	AAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACG
	GGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAAC
	CTTACCAGGTCTTGACATCCCGATGCTATTTCTAGAGATAGAAAGTTACTTCGGTA
	CATCGGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGT
	TAAGTCCCGCAACGAGCGCAACCCCTATTGTTAGTTGCCATCATTCAGTTGGGCAC
	TCTAGCGAGACTGCCGGTAATAAACCGGAGGAAGGTGGGGATGACGTCAAATCAT
	CATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGTTGGTACAACGAGTTG
	CGAGTCGGTGACGGCGAGCTAATCTCTTAAAGCCAATCTCAGTTCGGATTGTAGG
	CTGCAACTCGCCTACATGAAGTCGGAATCGCTAGTAATCGCGGATCAGCACGCCG
	CGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTG
	TAACACCCGAAGTCGGTGAGGTAACCTTTGGAGCCA

NAME	Description	Max score	Total score	Query cover	E value	Max ident	Accession
YC	Lactobacillus delbrueckii strain SP1.1 16S ribosomal RNA gene, partial sequence	2608	2608	100%	0	99%	KJ939317.1
Y2	Streptococcus thermophilus strain M4 16S ribosomal RNA gene, partial sequence	2601	2601	100%	0	99%	GU195648.1
R2	Streptococcus thermophilus strain KDLLJ4-1 16S ribosomal RNA gene, partial sequence	2597	2597	100%	0	99%	KJ890358.1

Table. S2 Chemical characterization of YC, Y2, and R2 used Biolog™

Chemical	YC	Y2	R2	**Chemical**	YC	Y2	R2
Negative control				L-Glutamic acid			+
Dextrin	+	+		L-Histidine	+	+	+
Maltose				L-Pyroglutamic acid		+	+
D-Trehalose				L-Serine			
D-Cellobiose				Lincomycin	+	+	+
Gentiobiose	+			Guanidine hydrochloride	+		+
Sucrose		+	+	Niaproof 4	+	+	+
D-Turanose	+	+	+	Pectin		+	+
Stachyose				D-Galacturonic acid			
Positive control	+	+	+	L-Galactonic acid- γ -lactone			
pH 6.0	+		+	D-Gluconic acid			
pH 5.0				D-Glucuronic acid	+	+	+
D-Raffinose				Glucuronamide	+	+	+
α-D-Lactose	+	+	+	Mucic acid			
D-Melibiose				Quinic acid			
3-methyl-D-glucoside				D-saccharic acid			
D-Salicin				Vancomycin	+		
N-Acetyl-D-glucosamine				Tetrazolium violet	+	+	+
N-Acetyl-D-Mannosamine				Tetrazolium blue	+		+
N-Acetyl-D-galactosamine				4-Hydroxyphenyl acetic acid			
N-Acetyl-neuraminic acid		+	+	Pyruric acid methyl ester			
1% Nacl	+		+	D-Lactic acid methyl ester			
4% Nacl				L-Lactic acid			

8% Nacl				Citric acid			
α-D-glucose		+	+	α-Ketoglutaric acid	+	+	+
D-Manose				D-Malic acid			
D-Fructose	+	+	+	L-Malic acid			+
D-Galactose				Bromosuccinic acid			
3-Methy glucose				Nalidixic acid	+	+	+
D-Fucose	+			Lithium chloride			
L-Fucose				Potassium iellurite	+	+	+
L-Ramnose	+		+	Tween 40			
Inosine				γ –Amino-N-butiric acid			
1% Sodium Lactate			+	α-Hydroxybutiric acid			
Fusidic acid	+			β-Hydroxybutiric acid			
D-Serine				α-Ketobutyric acid			
D-Sorbitol				Acetoacetic acid	+	+	+
D-Mannitol				Propionic acid			
L-Arabitol				Acetic acid			
Myo-inositol				Formic acid			
Glycerol				Aztreonam	+		
D-glucose-6-phosphate				Sodium butyrate	+	+	+
D-Fructose-6-phdsphate	+		+	Sodium Bromate	+		+
D-Aspartic acid				Glycyl-L-proline			
D-Serine	+		+	L-Alanine			
Troleandomycin				L-Arginine			
Rifamycin SV	+		+	L-Aspartic acid			
Minocycline	+		+	Gelatin			

(+) : positive reaction, Nothing : Negative reaction

2.2. Optimization of green coffee bean fermentation

Green coffee bean (Ethiopia Yigacheffe, Arabicas) was supplied by CoffeeCupper™ (Gangneung, Korea). The effects of water amount from 5% (w/w-water amount added/coffee weight used) to 50% (w/w) and of bacterial concentration from 8.0 x 10^9 to 8.0 x 10^{10} CFU/g coffee into coffee were investigated at 37℃ for 12 h. After fermentation, coffee bean was dried at 80℃ until moisture content was reached at 10~12% by using moisture analyzer machine (MB35 Moisture analyzer, NJ, USA). Depending on the water concentration, the drying time of fermented bean at 80°C was varied such as follow: 15 min for 5% (w/w) or 10% (w/w) water concentration, 30 min for 15% (w/w) or 20% (w/w) water concentration, 45 min for 25% (w/w) or 30% (w/w) water concentration, 60 min for 35% (w/w) or 40% (w/w) water concentration, and 75 min for 45% (w/w) or 50% (w/w) water concentration. Fermented coffee bean were roasted with a roaster (Gene Café, Gyeonggi, Korea) at 250℃ for 12 min (medium – dark) and ground with 14 mm particle size using a grinder (FEIMA, Seoul, Korea).

For the sensory evaluation, 50 g of roasted and ground regular and fermented coffee beans were put on coffee filter machine and then 500 mL hot water was added. Sensory tests of coffees were performed by 20 panelists (9 men, 11 women) aged of 26.9 ± 1.6 years. Indexes from low (value 1) to high (value 7) were awarded to each sensorial attribute depending on their intensity in the samples [50]. Evaluation categories were aroma, flavor, sweet, bitter, sour, and overall preference. The protocol was approved by the Seoul National University Institutional Review Board and the study was registered with IRB No. 1701/001-001. (Table S3)

The cinnamon or pine needle extract was added from 0.2 to 1% (w/v) during coffee fermentation at the above optimized water concentration and bacterial concentration. The sensory tests of cinnamon or pine needle fermented coffee were also evaluated as described above (Table 4).

Table. S3 Sensory evaluation of fermented coffee in water concentration and inoculated by different strain concentration

Water vol.	YC						Y2						R2					
	Aroma	Flavor	Sweet	Bitter	Sour	Overall	Aroma	Flavor	Sweet	Bitter	Sour	Overall	Aroma	Flavor	Sweet	Bitter	Sour	Overall
Ctrl	3.2 ± 1.3^{cd}	4.1 ± 1.5^{ab}	4.1 ± 1.3^{ab}	4.1 ± 1.6^{a}	3.1 ± 1.5^{b}	$3.9\pm1.4^{a}_{bc}$	$3.2\pm1.3^{ab}_{c}$	4.1 ± 1.5^{ab}	$4.1\pm1.3^{ab}_{c}$	4.1 ± 1.6^{bc}	3.1 ± 1.5^{c}	3.9 ± 1.4^{b}	3.2 ± 1.3^{a}	4.1 ± 1.5^{ab}	3.7 ± 1.3^{a}	4.1 ± 1.6^{a}	3.1 ± 1.5^{e}	$3.9\pm1.4^{ab}_{cd}$
10%	3.0 ± 1.7^{d}	3.5 ± 1.6^{b}	4.2 ± 1.7^{ab}	2.2 ± 1.4^{b}	4.1 ± 2.1^{ab}	$3.3\pm2.0^{b}_{c}$	2.8 ± 1.3^{bc}	$2.9\pm1.9^{bc}_{d}$	$3.2\pm1.5^{ab}_{cd}$	2.6 ± 1.0^{d}	3.2 ± 1.9^{c}	2.6 ± 1.4^{c}	3.9 ± 1.1^{a}	4.2 ± 1.0^{ab}	3.9 ± 0.9^{a}	3.7 ± 1.5^{a}	$3.6\pm1.4^{bc}_{de}$	4.5 ± 1.1^{ab}
20%	$4.2\pm1.3^{ab}_{cd}$	4.3 ± 1.6^{ab}	4.3 ± 0.8^{ab}	2.9 ± 1.2^{ab}	4.5 ± 1.6^{ab}	$4.2\pm1.5^{a}_{bc}$	3.5 ± 1.6^{ab}	3.3 ± 1.5^{bc}	4.2 ± 1.4^{ab}	3.0 ± 1.5^{cd}	$4.4\pm1.9^{ab}_{c}$	2.7 ± 1.2^{c}	3.7 ± 1.3^{a}	4.4 ± 1.1^{ab}	3.9 ± 0.8^{a}	4.1 ± 1.0^{a}	$3.5\pm1.5^{cd}_{e}$	$4.4\pm1.4^{ab}_{c}$
30%	$4.1\pm1.5^{ab}_{cd}$	4.2 ± 1.3^{ab}	4.3 ± 0.9^{ab}	2.9 ± 1.2^{ab}	4.1 ± 1.4^{ab}	$4.1\pm1.3^{a}_{bc}$	4.3 ± 0.9^{a}	4.9 ± 0.8^{a}	4.3 ± 1.1^{ab}	$3.1\pm0.7^{bc}_{d}$	3.5 ± 0.9^{bc}	5.1 ± 1.3^{a}	3.7 ± 1.2^{a}	3.4 ± 1.4^{bc}	3.6 ± 1.2^{a}	3.5 ± 1.6^{a}	4.7 ± 1.7^{ab}	$3.4\pm1.5^{cd}_{e}$
40%	$4.1\pm1.5^{ab}_{cd}$	3.8 ± 1.3^{ab}	3.9 ± 0.7^{ab}	3.4 ± 1.4^{ab}	3.6 ± 1.9^{ab}	$4.0\pm1.4^{a}_{bc}$	$3.1\pm1.2^{ab}_{c}$	3.6 ± 1.4^{bc}	$4.1\pm1.2^{ab}_{c}$	$3.1\pm1.6^{bc}_{d}$	3.1 ± 1.9^{c}	3.5 ± 1.4^{bc}	3.9 ± 1.1^{a}	3.5 ± 1.1^{bc}	3.5 ± 1.4^{a}	3.9 ± 1.2^{a}	$4.3\pm1.5^{bc}_{d}$	3.2 ± 1.3^{de}
50%	3.0 ± 1.0^{d}	3.5 ± 1.2^{b}	3.3 ± 1.1^{b}	3.5 ± 1.8^{a}	3.2 ± 1.2^{b}	3.0 ± 1.0^{c}	3.8 ± 1.4^{ab}	4.1 ± 1.6^{ab}	4.5 ± 1.3^{a}	$3.2\pm1.2^{bc}_{d}$	$3.9\pm1.6^{ab}_{c}$	3.9 ± 1.8^{b}	3.8 ± 1.4^{a}	4.3 ± 1.3^{ab}	3.9 ± 1.4^{a}	3.7 ± 1.3^{a}	$3.8\pm1.8^{bc}_{de}$	$4.1\pm1.4^{ab}_{cd}$
60%	4.6 ± 1.2^{ab}	4.5 ± 1.3^{ab}	4.1 ± 1.4^{ab}	3.2 ± 1.2^{ab}	4.0 ± 1.4^{ab}	$4.3\pm1.2^{a}_{b}$	2.1 ± 0.8^{c}	2.0 ± 0.8^{d}	$3.0\pm2.2^{bc}_{d}$	2.7 ± 1.9^{d}	4.7 ± 1.9^{ab}	2.3 ± 1.0^{c}	4.2 ± 1.0^{a}	4.3 ± 1.6^{ab}	3.9 ± 1.1^{a}	3.6 ± 1.4^{a}	$3.5\pm1.3^{bc}_{de}$	$4.3\pm1.3^{ab}_{c}$
70%	4.6 ± 1.3^{ab}	4.5 ± 1.7^{ab}	3.8 ± 1.3^{ab}	3.1 ± 1.6^{ab}	4.1 ± 1.7^{ab}	$4.3\pm1.4^{a}_{b}$	3.7 ± 1.4^{ab}	4.2 ± 1.2^{ab}	$3.3\pm1.1^{ab}_{cd}$	4.5 ± 1.7^{b}	$4.6\pm0.9^{ab}_{c}$	3.4 ± 0.9^{bc}	3.9 ± 1.3^{a}	4.2 ± 1.2^{ab}	4.2 ± 0.9^{a}	3.7 ± 0.9^{a}	$4.3\pm1.4^{bc}_{d}$	$4.4\pm1.2^{ab}_{c}$
80%	4.8 ± 1.5^{a}	4.8 ± 1.4^{a}	4.3 ± 1.3^{ab}	3.8 ± 1.6^{a}	5.0 ± 1.5^{a}	$4.1\pm1.6^{a}_{bc}$	2.8 ± 0.9^{bc}	$2.9\pm1.1^{bc}_{d}$	2.8 ± 1.3^{cd}	$3.5\pm2.3^{bc}_{d}$	$3.8\pm1.6^{ab}_{c}$	2.8 ± 1.0^{bc}	3.2 ± 1.2^{a}	3.7 ± 1.3^{bc}	3.4 ± 1.5^{a}	3.4 ± 1.3^{a}	$4.6\pm1.8^{ab}_{c}$	$3.6\pm1.4^{bc}_{de}$
90%	$4.3\pm1.2^{ab}_{c}$	4.7 ± 1.2^{ab}	4.2 ± 1.3^{ab}	3.0 ± 1.2^{ab}	3.4 ± 1.9^{b}	$4.3\pm1.4^{a}_{b}$	3.6 ± 1.9^{ab}	2.7 ± 1.1^{cd}	2.4 ± 0.9^{d}	5.7 ± 1.1^{a}	$3.9\pm1.0^{ab}_{c}$	2.8 ± 1.0^{bc}	3.9 ± 1.4^{a}	3.1 ± 1.6^{c}	3.4 ± 1.4^{a}	4.2 ± 1.8^{a}	5.5 ± 1.6^{a}	2.9 ± 1.6^{e}
100%	$3.5\pm1.2^{bc}_{d}$	4.4 ± 0.9^{ab}	4.8 ± 1.1^{a}	2.9 ± 1.2^{ab}	4.2 ± 1.3^{ab}	5.0 ± 1.0^{a}	3.7 ± 1.4^{ab}	3.6 ± 1.3^{bc}	$3.0\pm1.3^{bc}_{d}$	$3.6\pm0.9^{bc}_{d}$	5.1 ± 1.4^{a}	2.6 ± 0.5^{c}	3.7 ± 1.5^{a}	4.8 ± 1.2^{a}	3.8 ± 1.2^{a}	3.4 ± 1.4^{a}	3.2 ± 1.3^{de}	4.9 ± 1.3^{a}

% : water concentration into green coffee bean (w/v)

Bact Conc. (CFU/g coffee)	YC						Y2						R2					
	Aroma	Flavor	Sweet	Bitter	Sour	Overall	Aroma	Flavor	Sweet	Bitter	Sour	Overall	Aroma	Flavor	Sweet	Bitter	Sour	Overall
Ctrl	3.9±0.9^{a}	4.2±1.1^{a}	4.0±1.3^{ab}	4.3±1.5^{a}	3.6±1.2^{b}	4.2±1.1^{a} $_{bc}$	3.9±0.9^{a}	4.2±1.1^{a}	4.0±1.3^{a}	4.3±1.5^{a}	3.6±1.2^{bc}	4.2±1.1^{b}	3.9±0.9^{a}	4.2±1.1^{ab}	4.0±1.3^{a}	4.3±1.5^{a}	3.6±1.2^{b}	4.2±1.1^{ab}
1.6 x 10^{8}	4.3±1.3^{a}	4.6±1.3^{a}	4.5±1.1^{ab}	3.3±1.3^{ab}	4.4±1.3^{b}	4.4±1.3^{a} $_{b}$	3.7±1.2^{a}	4.4±1.4^{a}	4.5±0.8^{a}	3.3±1.2^{a}	4.8±1.3^{a}	4.1±1.2^{b}	4.2±0.8^{a}	3.6±1.2^{ab}	3.6±1.2^{a}	3.6±1.3^{ab}	5.1±1.4^{a}	3.4±1.2^{b}
3.2 x 10^{8}	4.0±1.1^{a}	4.5±1.1^{a}	4.3±0.9^{ab}	3.5±1.4^{ab}	4.3±1.6^{ab}	4.5±1.1^{a}	4.3±1.0^{a}	4.7±1.0^{a}	4.3±1.2^{a}	4.0±1.4^{a}	3.17±1.03^{c}	5.2±1.2^{a}	3.8±1.4^{a}	3.5±1.4^{b}	3.3±1.1^{a}	3.3±1.8^{ab}	4.3±1.4^{ab}	3.8±0.9^{ab}
4.8 x 10^{8}	4.3±1.4^{a}	3.9±1.4^{a}	3.7±1.2^{b}	3.3±1.4^{ab}	5.5±1.3^{a}	3.2±1.3^{c}	4.6±0.8^{a}	3.8±1.0^{a}	4.1±1.0^{a}	3.3±0.9^{a}	4.9±1.6^{a}	4.1±1.0^{b}	3.7±0.9^{a}	4.1±0.7^{ab}	4.1±1.0^{a}	3.3±1.0^{ab}	4.2±1.4^{ab}	4.0±0.7^{ab}
6.4 x 10^{8}	4.0±1.4^{a}	4.0±1.4^{a}	4.3±1.4^{ab}	2.8±0.8^{b}	4.8±1.4^{a}	4.3±1.2^{a} $_{bc}$	4.2±1.3^{a}	3.8±1.2^{a}	4.3±0.9^{a}	3.9±1.4^{a}	3.7±1.4^{bc}	3.9±1.4^{b}	3.8±1.1^{a}	4.6±1.4^{a}	4.0±1.2^{a}	3.7±1.6^{ab}	3.8±1.5^{b}	4.8±1.3^{a}
8 x 10^{8}	4.0±1.9^{a}	4.2±1.3^{a}	4.8±0.8^{a}	2.8±0.9^{b}	5.3±1.8^{a}	4.3±1.6^{a} $_{bc}$	4.0±1.0^{a}	3.9±1.2^{a}	4.1±1.0^{a}	3.5±1.2^{a}	4.5±1.3^{ab}	3.7±1.0^{b}	4.2±0.7^{a}	4.0±0.9^{ab}	3.4±1.1^{a}	4.0±1.2^{ab}	4.2±1.5^{ab}	3.6±0.9^{b}
9.6 x 10^{8}	3.8±1.5^{a}	4.4±1.4^{a}	4.3±1.3^{ab}	2.8±1.6^{b}	4.7±1.6^{ab}	4.3±1.7^{a} $_{bc}$	3.7±1.3^{a}	3.7±1.2^{a}	3.8±1.0^{a}	3.8±1.6^{a}	3.3±1.3^{c}	3.6±1.4^{b}	3.5±1.5^{a}	3.4±1.3^{b}	4.1±1.2^{a}	2.9±0.9^{b}	4.6±1.9^{ab}	3.3±1.5^{b}
1.1 x 10^{9}	4.1±1.2^{a}	3.8±1.6^{a}	3.9±0.9^{ab}	3.1±1.1^{b}	5.0±1.5^{ab}	3.3±1.8^{b} $_{c}$	4.1±1.2^{a}	3.8±1.5^{a}	3.8±1.1^{a}	4.0±1.6^{a}	5.3±1.0^{a}	3.8±1.1^{b}	4.3±1.6^{a}	4.3±1.3^{ab}	3.4±1.2^{a}	4.1±1.4^{ab}	4.2±1.5^{ab}	3.9±1.1^{ab}
1.3 x 10^{9}	3.7±1.2^{a}	4.3±1.4^{a}	4.4±1.0^{ab}	2.8±1.3^{b}	4.3±1.5^{a}	3.8±1.5^{a} $_{bc}$	3.7±1.0^{a}	3.7±1.0^{a}	3.7±1.2^{a}	3.5±1.2^{a}	4.8±1.5^{a}	3.6±1.4^{b}	3.7±1.2^{a}	3.5±1.2^{b}	3.8±1.1^{a}	3.5±1.6^{ab}	4.5±1.8^{ab}	3.4±1.4^{b}
1.4 x 10^{9}	4.0±1.7^{a}	3.3±1.5^{a}	4.8±1.1^{a}	2.9±1.2^{b}	5.4±1.2^{a}	3.5±1.1^{a} $_{bc}$	3.7±1.2^{a}	4.0±0.7^{a}	3.8±1.4^{a}	3.7±1.6^{a}	3.3±1.2^{c}	4.1±1.4^{b}	3.6±0.9^{a}	3.6±1.1^{ab}	3.6±0.8^{a}	3.6±0.5^{ab}	4.8±1.2^{ab}	3.6±0.9^{b}
1.6 x 10^{9}	4.2±1.6^{a}	4.1±1.6^{a}	4.4±0.8^{ab}	3.0±1.2^{b}	5.1±1.4^{a}	4.3±1.3^{a} $_{bc}$	3.9±1.1^{a}	4.3±1.2^{a}	4.1±1.4^{a}	3.2±0.8^{a}	5.1±1.1^{a}	4.0±1.3^{b}	4.0±0.9^{a}	4.3±0.9^{ab}	3.8±0.7^{a}	3.6±0.9^{ab}	3.8±0.9^{b}	4.3±0.9^{ab}

Values are mean ± standard deviation. Different letters indicate statistically significant differences by Duncan's multiple range test at $p< 0.05$.

2.3. Biological characterization of fermented coffee

2.3.1. Preparation of coffee samples

For water extraction, 2 g of roasted coffee powder was mixed with 20 mL of boiled water and kept at 80℃ for 1 h, and then centrifuged at 8000 x g for 10 min. The supernatant was transferred to 50 ml falcon conical tube for further studies. For ethanol extraction, 2 g of roasted coffee powder was mixed with 20 mL of 70% (v/v) ethanol and vortex for 30 min, and then centrifuged at 8000 x g for 10 min. The supernatant was transferred to 50 ml falcon conical tube for further studies. To optimize fermentation time, the samples were fermented at 3 h intervals. Green coffee bean prepared 15g of YC, Y2, and R2 were prepared centrifuged at 8000 rpm for 15min and inoculated to each samples without media mixed with distilled water. The fermentation time was 3, 6, 9, 12, 15, 18, 21, 24 h. The samples were fermented at 37℃ incubator.

2.3.2. pH, titratable acidity, and total carbohydrate

The pH of coffee samples was measured with a pH meter (SP-2100, SUNTEX, Taipei, Taiwan). The coffee samples were titrated using a 0.1 N NaOH solution and the acidity was calculated as acetic acid based on the volume of NaOH used to pH 8.2 for titration. The total carbohydrate was measured by the phenol sulphuric acid method with glucose as standard [51]. (Table. 2)

2.3.3. Determination of total phenols or flavonoids content

The total polyphenols content in water extracted coffees were determined by using Folin-Ciocalteu reagent with gallic acid (GAE, Sigma) as standard [52]. 120 μL of samples and gallic acid (1 ~ 50 μg/mL) were added into each well of 96 well plate and 15 μL Follin-Ciocalteu reagent (Sigma) was mixed for 3 min. Then 15 μL of 10 % (w/v) Na_2CO_3 was added and reacted with the samples for 30 min at dark condition.

The flavonoids content in each 70% ethanol extracted coffee was determined by using aluminum chloride reagent with catechin (CE, Sigma) as standard [53]. 150 μL distilled water was added to each of the 96 wells, followed by 10 μL of 5% sodium nitrite and 25 μL of samples and catechin (1 μg ~ 100 μg/mL) solution as was added into 96 well plate and mixed for 5 min. Then 15 μL of 10% aluminum chloride (Sigma) was added to the mixture. After 6 min, 50 μL of 1M NaOH were added and then reacted for 30 min at dark condition. The phenol and flavonoid content was determined using SpectraMax M3 (Molecular Devices, Sunnyvale, CA, USA) at 760 nm and 510 nm, respectively. (Table. 3)

2.3.4. Analysis of trigonelline, caffeine, and chlorogenic acid in fermented coffee

The HPLC-UV analyses were achieved as described by Farah et al. [54] with slight modifications. An analytical HPLC unit (Waters, USA) consists of 2545 binary gradient module pump, 2998 PDA detector, and 2767 sample manager (Injector). A reverse-phase Sunfire™ C_{18} column (4.6 mm × 100 mm, 5 μm, Milford, MA, USA) was used. The solvent system used was a gradient of phosphoric acid (50 mM) and acetonitrile performed at a constant flow rate of 1.0 mL min^{-1} at room temperature. A gradient elution of solvent A (50 mM phosphoric acid) and solvent B (100% acetonitrile) was used starting and kept with 100% solvent A until pressure stabilization then to used 90% solvent A and 10% solvent B for 20 min. Then the system was returned to initial conditions after 10 min. Detection was accomplished with a UV detector, and chomatograms were recorded at 210 ~ 400 nm. The components were identified by their retention times, chomatographic comparisons with standards, and their UV spectra. Quantification was based on the external standard method. Under the assay conditions described, a linear relationship between the concentration and the UV absorbance was obtained at 272 nm for trigonelline and caffeine, and 325 nm for chlorogenic acid. (Table. 3)

2.3.5. Antioxidant activity

2.3.5.1. DPPH radical scavenging activity assay

The antioxidant activities of regular coffee and fermented coffees were evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method [55]. Samples were dissolved in water and mixed with 100 μM (DPPH) in methanol solution to give a final concentration of 0.1 to 2 mg/mL. After 30 min of incubation at 25°C in total darkness, the absorbance of each mixture was measured using SpectraMax M3 at 517 nm. A mixture containing all reagents except the test sample was used as a negative control, α-tocopherol and Trolox (6-hydroxy-2,5,7,8,-tetramethylchomane-2-carboxylic acid) were used as a positive control. DPPH radical-scavenging activity was calculated as our previous reports [55].

2.3.5.2. ABTS radical cation decolorization assay

ABTS radical cation decolorization assay was carried out according to Gomez et al [56] with some modification. The $ABTS^{\bullet+}$ chomophore was produced by the oxidation of 7 mM ABTS with 2.5mM potassium persulfate in water. Then the mixture was place in the dark at 4 ℃ for overnight to yield a dark-green solution and then was diluted with phosphate-buffered saline (PBS) was mixed with 5 μL of $ABTS^{\bullet+}$ reagent for 10 min. The absorbance of each mixture was measured using SpectraMax M3 at 510 nm. Trolox was used as a positive control. (Table. 3)

2.3.6. Angiotensin converting enzyme inhibitory activity

Rabbit lung ACE (Sigma) assay was performed using the method described by Ghanbari & Zarei [57] with some modifications. The reaction mixture contained 0.5 mU ACE, 1.5 mM HHL (N-hippuryl-L-histidyl-L-leucine, Sigma), 1 mg/mL OPA (o-Phthaldialdehyde, Sigma), 400 mM NaCl and fermented coffee (0.1 ~ 2 mg/mL) in 100 mM sodium borate (pH 8.3). After addition of 1.5 mM HHL, assay mixture was added with 2 M NaOH at zero time. The reaction was terminated after incubation at 37℃ for 60 min by the addition of 2 M NaOH. After reaction stop, 1 mg/mL OPA was added and transferred to the 96-well plate. The reaction was measured in a SpectraMax M3 (λ_{ex} = 365, λ_{em} = 460) at 25°C for 1 h with triplicate. (Table. 3)

2.4. Statistical analysis

Statistical analyses were conducted by one-way analysis of variance followed by Duncan's multiple range test using an SPSS Statistics Program 23 (SPSS Statistics 23.0, IBM, U.S) [58].

Results and Discussion

3.1. Fermentation of coffee using lactic acid bacteria

3.1.1. Optimum of water concentration during coffee fermentation

Sensory evaluation of the fermented coffee was performed by quantitative descriptive analyses. The chosen parameters for coffee sensory evaluation were aroma, flavor, sweet, bitter, sour, and overall acceptability. The effects of water concentration to fermented coffee sensory compared with regular coffee are shown in Table S3 and Figure 1A. The aroma, flavor, sweet, bitter, sour, and overall acceptable score of regular coffee are 3.2, 4.1, 4.1, 4.1, 3.1, and 3.9, respectively. When the water concentration was varied from 5 to 50% (w/w), the overall acceptable scores of fermented coffee was also varied from 3.0 to 5.0 for YC, 2.3 to 5.1 for Y2, and 2.9 to 4.9 for R2 (Table S3). The aroma, flavor, sweet, bitter, sour scores at highest overall acceptable scores of YC strain at 50% (w/w) water concentration were 3.5, 4.4, 4.8, 2.9, and 4.2, respectively (Figure 1A). The aroma, flavor, sweet, bitter, sour scores for highest overall acceptable scores of Y2 strain at 15% (w/w) water concentration were 4.3, 4.9, 4.3, 3.1, and 3.5, respectively (Figure 1A). The aroma, flavor, sweet, bitter, sour scores of fermented coffee at highest overall acceptable scores of R2 strain at 50% (w/w) water concentration were 3.7, 4.8, 3.8, 3.4, and 3.2, respectively (Figure 1A). The fermented coffees with YC at 50% (w/w) water concentration, Y2 at 15% (w/w) water concentration, and R2 at 50% (w/w) water concentration showed significant higher intensities in aroma score (1.1, 1.3, and 1.2 times), flavor score (1.1, 1.2, and 1.2 times), sourness scores (1.4, 1.1, and 1.0 times), and overall acceptable scores (1.3, 1.3, and 1.2 times) than those of regular coffee. The sweetness scores of YC at 50% (w/w) water concentration and Y2 at 15% (w/w) water concentration was 1.2 and 1.0 higher than that of regular coffee. However, the sweetness scores of R2 at 50% (w/w) water concentration was 1.1 times lower than that of regular coffee. The fermented coffees with these strains showed decreased bitterness scores compared to regular coffee.

3.1.2. Optimum of inoculation amount added to coffee during fermentation

The LAB inoculation amount concentration effected to sensory of coffee during fermentation. When LAB inoculation concentration was varied from 1.6 x 10^8 to 1.6 x 10^9 CFU/g coffee, the overall acceptable scores of fermented coffee were also varied from 3.2 to 4.5 for YC, 3.6 to 5.2 for Y2, and 3.3 to 4.8 for R2 (Table S3). The aroma, flavor, sweet, bitter, sour scores for the highest overall acceptable scores at 3.2 x 10^8 CFU YC/g coffee were 4.0, 4.5, 4.3, 3.5, and 4.3, respectively (Figure 1B). The aroma, flavor, sweet, bitter, sour scores for the highest overall acceptable scores at 3.2 x 10^8 CFU Y2/g coffee were 4.3, 4.7, 4.3, 4.0, and 3.2, respectively (Figure 1B). The aroma, flavor, sweet, bitter, sour scores for the highest overall acceptable scores at 6.4 x 10^8 CFU R2/g coffee were 3.8, 4.6, 4.0, 3.7, and 3.8, respectively (Figure 1B). The fermented coffees with 3.2 x 10^8CFU YC/g coffee, 3.2 x 10^8CFU Y2/g coffee, and 6.4 x 10^8CFU R2/g coffee showed higher aroma scores (1.2, 1.3, and 1.2 times), flavor scores (1.1, 1.1, and 1.11 times), sour scores (1.4, 1.3, and 1.2 times), and overall acceptable scores (1.1, 1.3, and 1.2 times) than those of regular coffee. The sweet scores coffee fermentation at 3.2 x 10^8CFU YC/g coffee and 3.2 x 10^8CFU Y2/g coffee was 1.0 and 1.1 time higher than that of regular coffee. However, the sweet scores of coffee fermentation at 6.4 x 10^8CFU R2/g coffee was 1.0 times lower than that of regular coffee. The fermented coffees with these strains showed decrease bitter scores compared to regular coffee. From the sensory evaluation, the coffee fermented with 50% (w/w) water concentration and 3.2 x 10^8 CFU/g coffee for YC, 15% (w/w) water concentration and 3.2 x 10^8 CFU/g coffee for Y2, and 50% (w/w) concentration for R2 and 6.4 x 10^8 CFU/g coffee for R2 were selected for further study.

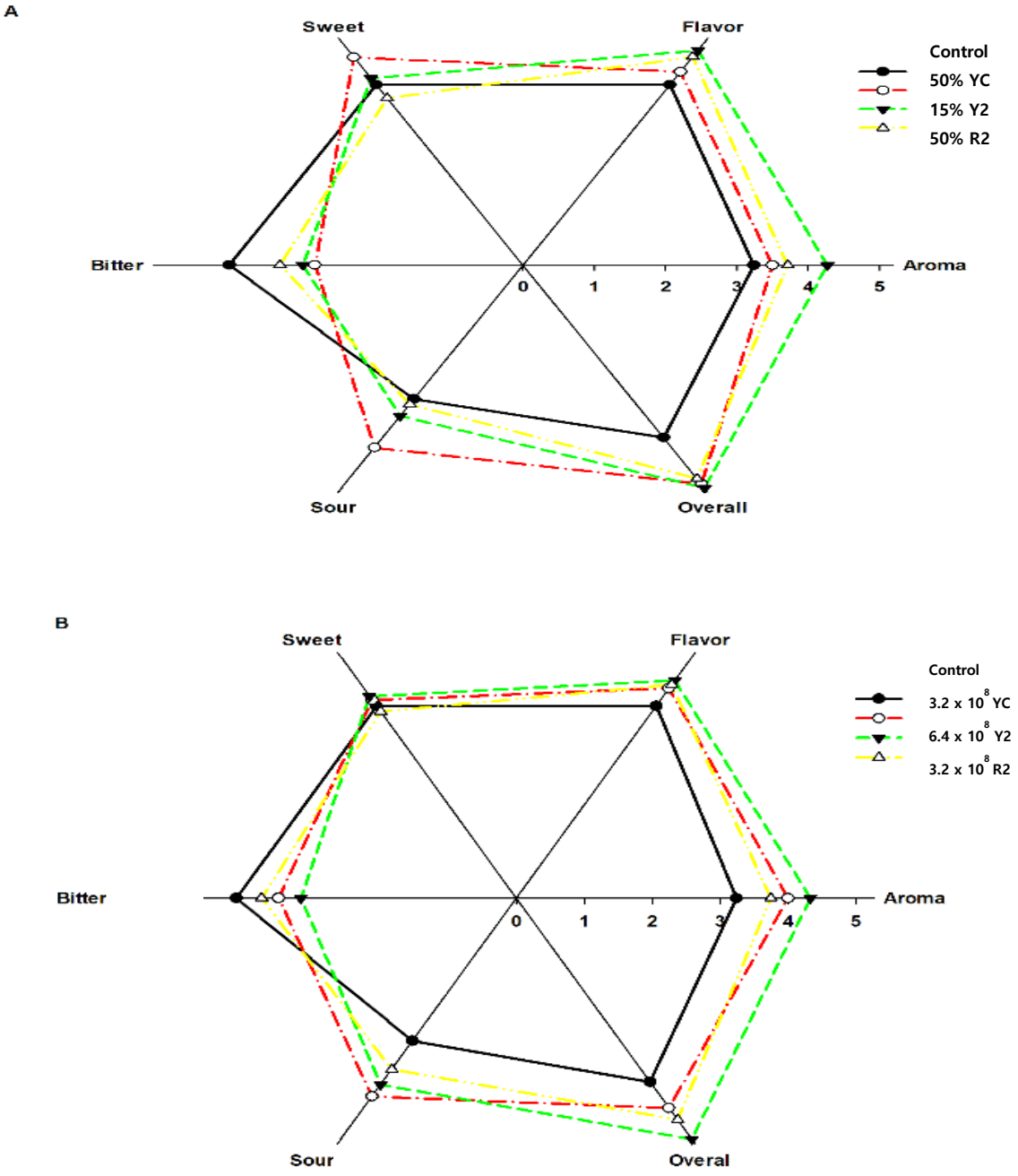


Figure. 1 Sensory evaluation of regular coffee and fermented coffee by lactic acid bacteria (YC, Y2, R2)

(A) High score sample of regular coffee and fermented coffee by YC, Y2, and R2 in water concentration optimization (w/w) (B) High score sample of regular coffee and fermented coffee by YC, Y2, and R2 in bacterial inoculation optimization (CFU/g coffee)

3.2. Optimization of fermentation time to coffee during fermentation

To optimize the fermentation time of green coffee bean, pH and growth curve of each strain were used to check. YC, Y2 and R2 cell growth patterns were traced for 24 h to know the proper time for inoculation (Fig. 2). Y2 and R2 strain go to stationary phase before 10 h and pH level goes down steadily until pH level 4 ~ 4.5. In YC, it goes to stationary phase started at around 18 h after and pH goes down for 24 h. It might be that *Streptococcus thermophiles* usully grow 4.2 ~ 4.5 in acidic pH. *Lactobacillus delbrueckii* could grow lower pH around 3.5 ~ 4.0 [22].

The activity of cell is the most optimal at latest time of exponential phase. Therefore, the most proper time of inoculation is different. The graphs below show that it is suitable to inoculate when pH level is between 4 and 4.5, OD_{600} is around 10 h (Y2, R2) and 15 h (YC). Thus, to give an equality of 3 strains, the fermentation time fixed for 12 h though cell number and pH change.

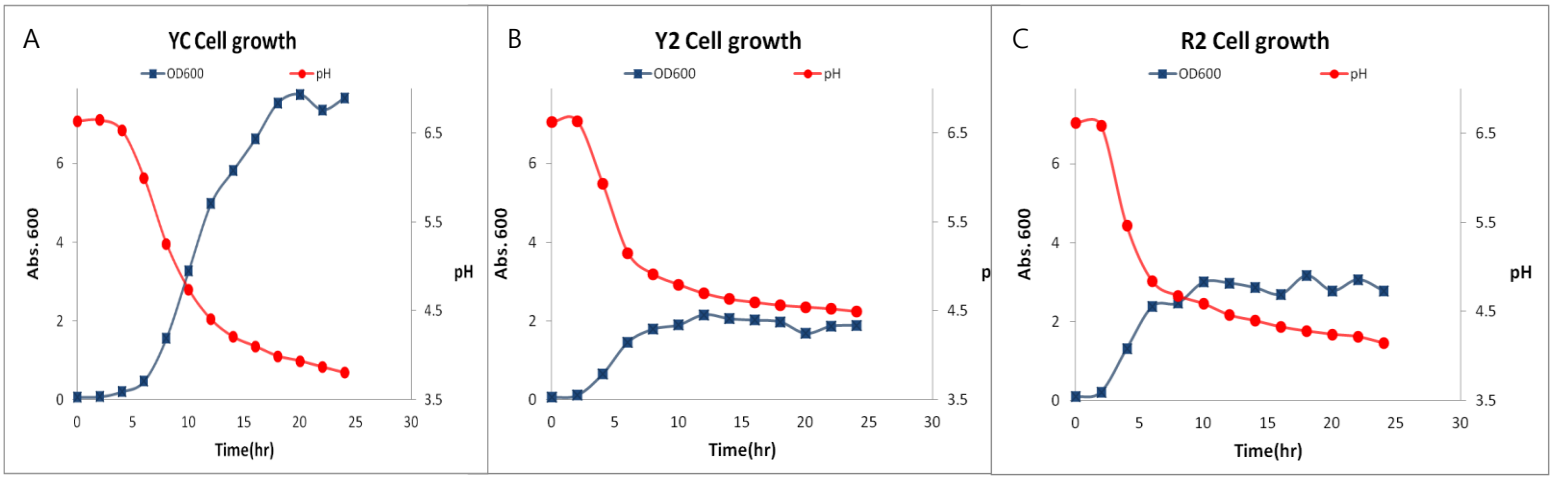


Figure 2. Optimization of fermentation time through pH and growth curve in fermented green coffee bean for 24 hr. (A), (B), and (C) shows the ph and growth curve in OD600 in fermented green coffee bean for each YC, Y2, and R2.

3.2. pH, titratable acidity, and total carbohydrate concentration of fermented coffee

The fermented coffee prepared with YC, Y2, and R2 showed decrease of pH compared to regular coffee (Table 2) from 6.2 ± 0.1 to 5.6 ± 0.2 for YC fermented coffee, to 5.8 ± 0.1 for Y2 fermented coffee, and to 5.5 ± 0.1 for R2 fermented coffee. The titratable acidities was shown in Table 2. The titratable acidities of regular coffee, YC fermented coffee, Y2 fermented coffee, and R2 fermented coffee were 1.7 ± 0.1, 2.9 ± 0.1, 2.9 ± 0.2, and 2.5 ± 0.1 mL 0.1M NaOH/g coffee, respectively. The titratable acidities of fermented coffee were higher from 1.5 to 1.7 times than that of regular coffee. In this study, thee LAB strains YC, Y2, R2 were isolated and used for fermentation of green coffee beans. Because of acid production during growing up of LAB, there decrease of pH and increase of titratable acidity of fermented coffee were found as previously reported [1]. The total carbohydrate amount of fermented coffee prepared with YC, Y2, and R2 showed increase of 1.3, 1.3, and 1.4 times compared to regular coffee respectively.

In relation with sensory evaluation result, the sourness in bacterial concentration increases compared to regular coffee. This is because when lactic acid bacteria grows green coffee bean, they produced organic acid especially lactic acid, which gives a change to decrease pH. Because of this, panelists gave a high score of sourness category in fermented coffee. In addition, panelists give a high score of sweetness in fermentation coffee. Sweetness in sensory evaluation could explain though lactic acid bacteria metabolism. Lactic acid bacteria produce exopolysaccharides, which have some content of sweetness. When lactic acid bacteria grow in green coffee bean, they could use sugar and nitrogen contents in green coffee bean. By using these sources, they synthesized the metabolites in several kinds of exopolysaccharides, which are secondary metabolite. Total carbohydrate was increased in fermented coffee though fermentation. This result could be proved through exopolysaccharide.

Table. 2 pH, titratable acidity and total carbohydrate of fermented coffee.

	pH	Titratable acidity (mL 0.1M NaOH/g coffee)	Total carbohydrate (mg Glc/10mg coffee)
Regular coffee	6.2 ± 0.1^{a}	1.7 ± 0.1^{a}	0.6 ± 0.1^{a}
YC	5.6 ± 0.2^{c}	2.9 ± 0.1^{c}	0.9 ± 0.2^{b}
Y2	5.8 ± 0.1^{b}	2.9 ± 0.2^{c}	0.9 ± 0.1^{b}
R2	5.5 ± 0.1^{d}	2.5 ± 0.1^{b}	0.8 ± 0.1^{b}

Different letters indicate statistically significant differences by Duncan's multiple range test at $p < 0.05$. Glc: Glucose

3.3 Total amount of phenol and flavonoid contents in fermented coffees

The total phenol amount of regular coffee and fermented coffee are shown in Table 3. The total phenol amount of regular coffee was 0.7 ± 0.1 mg/g coffee extraction. They were 0.8 ± 0.2 mg/g YC fermented coffee, 0.9 ± 0.1 mg/g Y2 fermented coffee, 0.8 ± 0.1 mg/g R2 fermented coffee. The total flavonoid amount of regular coffee displayed the lowest amount of flavonoid content of 15.6 ± 1.0 mg/g followed by Y2 (22.5 ± 1.5 mg/g), R2 (23.1 ± 1.7 mg/g), and YC (23.6 ± 1.8 mg/g). The fermented coffee prepared with LAB showed higher total phenol and flavonoid amount than that of regular coffee and the total phenol contents were increased from 1.1 to 1.2 times and the total flavonoid amount were increased from 1.4 to 1.5 times compare to regular coffee. After fermentation, the total phenol and flavonoid contents were increased than that of regular coffee. The increasing of total phenol contents in fermented coffee was resulted because LAB can produce several enzymes such as tannase, phenolic acid decarboxylase, benzyl alcohol dehydrogenase, and β-glucosidase able to degrade some phenolic compounds [59]. Those are involved in the formation of useful volatile phenols derivatives which contribute naturally to aroma in fermented foods and beverages [23]. Flavonoids are present as glycosylated forms in plants and foods. When flavonoid glycosides are absorbed in the body, they changes a form of aglycone, like de-glycoside form. In this step, several enzymes of microbe in human small intestine worked to hydrolyze glycoside in flavonoid compounds. β-glucosidase act as a major enzyme, which activity may be a factor determining variation in flavonoid bioavailability. Flavonoid deglycoside has the same capacity of antioxidant activity, as flavonoid glycoside has [60]. During fermentation, β-glucosidase in lactic acid bacteria might work to hydrolyze flavonoind glycosides in green coffee beans to flavonoind aglycone form.

3.4. Trigonelline, caffeine, and chlorogenic acid contents

Chlorogenic acids, trigonelline, and caffeine in regular coffee and fermented coffee were analyzed by

using HPLC-UV (Table. 3). The amounts of chlorogenic acid, trigonelline, and caffeine in regular coffee were 1.4 ± 0.1 mg/g, 0.8 ± 0.1 mg/g, and 0.8 ± 0.2 mg/g coffee extract, respectively. The amounts of trigonelline in fermented coffee with YC, Y2, and R2 were ranged from 0.8 ± 0.1 mg/g coffee extract to 0.9 ± 0.2 mg/g coffee extract. The amounts of caffeine in fermented coffee with YC, Y2, and R2 were ranged from 0.8 ± 0.1 mg/g coffee extract to 0.8 ± 0.1 mg/g coffee extract. The amounts of chlorogenic acid were 1.9 ± 0.2 mg/g for YC fermented coffee, 1.8 ± 0.1 mg/g for Y2 fermented coffee, 1.7 ± 0.1 mg/g for R2 fermented coffee. There are non-significant difference in the amounts of trigonelline and caffeine between coffee and fermented coffees with LAB. However, the amounts of chlorogenic acid in fermented coffee were increased from 1.2 to 1.3 times comparison to regular coffee.

Comparison of regular coffee, fermented coffee increase chlorogenic acid contents, even though trigonelline and caffeine showed the same amount in both coffee. There is little research about increase of chlorogenic acid used lactic acid bacteria. In addition, it does not have any researches why trigonelline and caffeine did not show the difference when lactic acid bacteria fermentation. Thus, it could not exactly explain why chlorogenic acid in fermented coffee increase and trigonelline and caffeine did not increase or decrease. However, there is a few clue to explain of these results. Lactic acid bacteria, which are used for this study, have several methyl transferase and esterase. However, they do not have any demethylases. A linkage with caffeic acid and quinic acid synthesizes chlorogenic acids. When chlorogenic acid synthesize, both compounds linked by esterification. Above the reason, chlorogenic acid might be had more contents in fermented coffee than regular coffee. Trigonelline and caffeine are synthesized by methylase, which attaches a methyl group in hydroxyl group they have. Thus, when they need to increase or decrease the amount of them, they are released methyl group by de-methylase. However, lactic acid bacteria do not have de-methylase, so trigonelline and caffeine might be show the similar content of regular coffee.

3.5. Antioxidant effect

The antioxidant activities of coffee and fermented coffees were compared using a DPPH and ABTS radical-scavenging activity assay (Table. 3). The antioxidant activity was represented by SC_{50} and compared to regular coffee. In DPPH, the antioxidant activity (SC_{50}) was 70.3 ± 3.7 µg/g for regular coffee, 31.4 ± 0.5 µg/g for YC fermented coffee, 38.6 ± 4.7 µg/g for Y2 fermented coffee, 37.3 ± 4.8 µg/g for R2 fermented coffee (Table. 3). The fermented coffee with YC, Y2 and R2 showed from 1.8 to 2.2 times higher antioxidant activity than that of regular coffee. Trolox was used as positive control having SC_{50} values of 6.3 ± 1.0 µg/g.

In ABTS assay, the antioxidant acitivities (SC_{50}) were 30.6 ± 1.6 µg/g regular coffee, 22.9 ± 0.8 µg/g YC fermented coffee, 25.1 ± 1.2 µg/g Y2 fermented coffee, and 24.2 ± 2.2 µg/g R2 fermented coffee. The fermented coffee with YC, Y2, and R2 showed from 1.2 to 1.3 fold higher ABTS antioxidant activity than that of regular coffee. Trolox was used as positive control in ABTS had SC_{50} value of 8.1 ± 1.2 µg/g. Velioglu et al. reported the total phenol contents, and the antioxidant activity increase [61]. The increase of antioxidant activity of fermented coffee compared to regular coffee was due to the increase of total phenol contents in LAB fermentation coffee.

3.6. ACE inhibitory activity

Angiotensin converting enzyme (ACE EC 3.4.15.1, dipeptidyl carboyxypeptidase) is a glycoprotein peptidyldipeptide hydrolase that cleaves histidyl-leucine dipeptide from angiotensin 1, forming the potent vasoconstrictor angiotensin 2, which is an important target for inhibition because octapeptide angiotensin 2 is involved in the release of the sodium-retaining steroid aldosterone from the adrenals. Thus, ACE raises blood pressure by increasing both vascular resistance and fluid volume [62]. Angiotensin-converting enzyme inhibitors were developed as therapeutic agents targeted for the treatment of hypertension. In this study, the ACE inhibitory activity (IC_{50}) were 10.8 ± 2.4 mg/g for

regular coffee, 6.0 ± 1.4 mg/g for YC fermented coffee, 7.3 ± 0.2 mg/g for Y2 fermented coffee, 7.1 ± 1.0 mg/g for R2 fermented coffee (Table. 3). The fermented coffees by YC, Y2, and R2 showed 1.5 – 1.8 times higher ACE inhibitory activity than that of regular coffee. Trigonelline, chlorogenic acid and caffeine were used as positive control. Among them, trigonelline did not inhibit ACE, while caffeine and chlorogenic acid showed against 8.7% and 67.4% ACE activity at 1 mg/g.

Kozuma et al. [63] and Suzuki et al. [64] reported that green coffee extracts with chlorogenic acid as main component showed lower blood pressure in rats than non-treated groups. Among 3 compounds, chlorogenic acid (CGA) was reported as main inhibitor against ACE enzyme [63]. The increase of chlorogenic acid during coffee fermentation effected to ACE inhibitory activity of coffee.

Table. 3 Total phenol and flavonoid contents, DPPH and ABTS radical scavenging activity, HPLC-UV analysis and ACE inhibition assay of fermented coffee compared to regular coffee

	Total phenol contents (mg GAE/g coffee extract)	Total flavonoid contents (mg CE/ g coffee extract)	DPPH radical scavenging activity (μg/g coffee extract, SC_{50})	ABTS radical scavenging activity (μg/g coffee extract, SC_{50})	HPLC-UV Analysis (mg/g coffee extract)			ACE inhibition (mg/g coffee extract, IC_{50})
					Trigonelline	Caffeine	Chlorogenic acid	
Regular Coffee	0.7 ± 0.1	15.6 ± 1.0	70.3 ± 3.7	30.6 ± 1.6	0.8 ± 0.1	0.8 ± 0.2	1.4 ± 0.1	10.8 ± 2.4
YC	0.8 ± 0.2	23.6 ± 1.8	31.4 ± 0.5	22.9 ± 0.8	0.9 ± 0.1	0.9 ± 0.1	1.9 ± 0.2	6.0 ± 1.4
Y2	0.9 ± 0.1	22.5 ± 1.5	38.6 ± 4.7	25.1 ± 1.2	0.9 ± 0.1	0.8 ± 0.1	1.8 ± 0.1	7.3 ± 0.2
R2	0.8 ± 0.1	23.1 ± 1.7	37.3 ± 4.8	24.2 ± 2.2	0.9 ± 0.2	0.8 ± 0.1	1.7 ± 0.1	7.1 ± 1.0

Values are mean ± standard deviation in triplicates. GAE : Gallic Acid Equivalent. CE : Catechin Equivalent

3.7 Sensory evaluation of co-fermented coffee with cinnamon and/or pine needle extract

To improve the flavor and increase the functionality of lactic acid bacteria fermented coffee, cinnamon and/or pine needle extract were added into fermented green coffee bean during fermentation. Among thee LAB for coffee fermentation, YC showed the highest in total flavonoid content, antioxidant activity, chlorogenic acid amount, and ACE inhibitory activity. Thus, YC was selected for coffee co-fermentation with cinnamon and/or pine needle extract. The sensory evaluation of cinnamon and/or pine needle extract co-fermented coffee was shown in Table 4. In cinnamon fermented coffee, aroma, flavor, sweet, and sour category were higher than that of fermented coffee without adding cinnamon (Table 4). With 0.7% (w/v) of cinnamon extract addition, the cinnamon co-fermented coffee showed the highest flavor, sweet, bitter, sour scores. Among the tested cinnamon concentration, 1% (w/v) cinnamon co-fermented coffee showed the strongest overall preference (Table 4). The overall preference of 1% (w/v) cinnamon extract co-fermented coffee was increased 48% compared to fermented coffee without addition. The more increase of cinnamon percentage, the higher score of overall preference score.

In pine needle extract added co-fermented coffee, aroma, flavor, sweet, sour, and overall preference scores were higher than that of fermented coffee without adding pine needle extract (Table 4). The highest score of aroma, flavor, bitter, sour, and overall preference scores were obtained when 0.5% (w/v) of pine needle extract was co-fermented using YC.

Many traditional plants possessed medicinal properties such as controling blood glucose levels. Cinnamon has known to be effective in improving glycaemia [65]. The essential oil of pine needles, which is obtained by steam distillation, is used extensively as fragrance and flavor components in medicine, detergents, beverages, candy, baked goods, and perfumes [66]. When cinnamon was added to coffee during fermentation, the bitterness of coffee was increased. The bitterness in fermented coffee with cinnamon was increased because cinnamon has several polyphenolic compounds, such as hydroxyl cinnamic and benzoic acids [67] that contributed to bitter taste of coffee [68]. The increase

sweetness in fermented coffee with cinnamon due to the reducing sugars and volatile oil constituents [69] in cinnamon contributed to sweetness taste. And pine needle have several volatile flavor compounds and phenolic compounds such as dimeric and trimeric proanthocyanidins [70], which give a unique flavor into coffee [71]. Lea et al [72] reported the effect of DP of procyanidins to bitterness and astringency of beverage.

Table. 4 Sensory evaluation of fermented coffee blend with Cinnamon and/or Pine needle

	Aroma	Flavor	Sweet	Bitter	Sour	Overall
YC fermented coffee	3.5±0.8 ^{a}	3.6±0.9^{a}	3.0±0.9^{a}	2.3±0.7^{a}	3.8±0.9^{a}	3.0±1.1^{a}
C 0.2%	4.0± 1.3^{a}	4.1±1.3^{a}	3.5±1.0^{a}	4.9±1.5^{b}	4.1±1.4^{a}	3.6±1.2^{ab}
C 0.5%	4.1±1.2^{a}	4.4±1.5^{a}	3.6±1.2^{a}	4.9±1.5^{b}	4.1±1.3^{a}	3.7±1.1^{ab}
C 0.7%	3.9±1.2^{a}	4.6±1.4^{a}	3.6±1.3^{a}	5.1±1.0^{b}	4.3±1.5^{a}	3.8±1.2^{ab}
C 1 %	3.6±1.1^{a}	4.3±1.1^{a}	3.4±1.0^{a}	5.0±1.2^{b}	4.1±1.2^{a}	4.4±0.9^{b}
P 0.2%	3.4±0.8^{a}	3.8±1.0^{ab}	4.6±0.5^{c}	3.1±1.2^{b}	3.2±0.9^{a}	4.0±0.7^{b}
P 0.5%	4.2±1.1^{a}	4.7±0.8^{b}	3.6±0.7^{ab}	4.1±0.9^{c}	4.3±1.0^{b}	4.8±0.8^{c}
P 0.7%	3.8±0.4^{a}	3.8±1.0^{ab}	3.5±0.7^{ab}	3.4±0.7^{bc}	3.8±1.1^{ab}	4.2±0.4^{bc}
P 1%	4.0±0.7^{a}	3.8±1.0^{ab}	4.3±1.2^{bc}	3.5±1.0^{bc}	3.4±1.1^{ab}	4.2±0.7^{bc}

Values are mean ± standard deviation. Different letters indicate statistically significant differences by Duncan's multiple range test at $p< 0.05$. (C: YC fermented coffee blended with cinnamon P: YC fermented coffee blended with pine needle)

% : Cinnamon and pine needle concentrates concentration (w/v) into green coffee bean

3.8 Functionality of co-fermented coffee with cinnamon and/or pine needle extract

The total phenol and flavonoid concentration, antioxidant activity and the amount of trigonelline, caffeine, and chlorogenic acid in cinnamon or pine needle extract co-fermented coffee is shown in Table 4. The total phenol and flavonoid concentration were 1.1 ± 0.2 mg/g, 24.3 ± 1.9 mg/g for YC co-fermented coffee with 1% cinnamon extract, and 1.0 ± 0.1 mg/g, 25.2 ± 3.2 mg/g for co-fermented coffee with 0.5% pine needle extract. The total phenol content was increased 10% and 36% for YC co-fermented coffee with 1.0% cinnamon extract or 0.5% pine needle extract, respectively, compared to YC fermented coffee. The DPPH and ABTS radical scavenging activity of YC co-fermented coffee with cinnamon and/or pine needle extract (SC_{50}) is shown in Table 4. The DPPH and ABTS radical scavenging activities (SC_{50}) were 42.9 ± 0.8 μg/g, 22.2 ± 2.0 μg/g for YC co-fermented coffee with 1% cinnamon extract and 40.4 ± 1.1 μg/g, 21.8 ± 0.3 μg/g for YC co-fermented coffee with 0.5% pine needle extract. Compared to YC fermented coffee, the YC co-fermented coffee with cinnamon and/or pine needle extract showed slightly higher than that of YC fermented coffee. The amount of trigonelline, caffeine, and chlorogenic acid in YC co-fermented coffee with 1% cinnamon extract were 0.9 ± 0.1 mg/g, 0.8 ± 0.1 mg/g, and 2.2 ± 0.1 mg/g, respectively. The amount of trigonelline, caffeine, and chlorogenic acid in YC co-fermented coffee with 0.5% pine needle extract was 0.9 ± 0.1 mg/g, 0.9 ± 0.1 mg/g, or 2.7 ± 0.3 mg/g, respectively. The amount of chlorogenic acid in YC co-fermented coffee with 1% cinnamon extract showed similar amount compared to YC fermented coffee, but the amount of chlorogenic acid in YC co-fermented coffee with 0.5% pine needle extract showed 1.4 times higher than that of YC fermented coffee.

Table 5. Total phenol and flavonoid contents, DPPH and ABTS radical scavenging activity, HPLC-UV analysis of fermented coffee blend with Cinnamon and/or Pine needle

	Total phenol contents (mg GAE/g coffee extract)	Total flavonoid contents (mg CE/g coffee extract)	DPPH radical scavenging activity (μg/g coffee extract, SC_{50})	ABTS radical scavenging activity (μg/g coffee extract, SC_{50})	HPLC-UV analysis (mg/g coffee extract, IC_{50})		
					Trigonelline	Caffeine	Chlorogenic acid
YC fermented coffee	0.81 ± 0.1	23.6 ± 1.8	31.4 ± 0.5	22.9 ± 0.8	0.9 ± 0.1	0.9 ± 0.1	1.9 ± 0.1
1% Cinnamon coffee	1.1 ± 0.2	24.3 ± 1.9	42.9 ± 0.8	22.2 ± 2.0	0.9 ± 0.1	0.8 ± 0.1	2.2 ± 0.1
0.5% Pine coffee	1.0 ± 0.1	25.2 ± 3.2	40.4 ± 1.1	21.8 ± 0.3	0.9 ± 0.1	0.9 ± 0.1	2.7 ± 0.3

Values are mean ± standard deviation in triplicate. GAE : Gallic Acid Equivalent. CE : Catechin Equivalent

Conclusion

Until now, most fermented coffee studies only focused on the enhancement of the functional properties of green coffee bean such as antioxidant activity, inhibition about glucosidase, lipase [73], tyrosinase [74] and starch hydrolase [75], natural compounds' variation [76], decaffeination [77], or coffee aroma [78] without sensory evaluation. Previous studies reported that the volatile aroma, sensory and quality of coffee were improved by using LAB fermentation together with yeast for coffee cherries during on-farm wet processing [79]. This study shows the difference among previous studied researches. Usually, researches on fermentation related to food demonstrated the difference between fermented something and non-fermented one. Some of researches are finished included sensory evaluation or not. In food industry to sell the product, taste or flavor is the most important thing to appeal to consumers. For this reason, this study selected conditions to optimize several factors, which affects the taste or flavor, through sensory evaluation. Due to this research process, not only fermented coffee guaranteed taste or flavor, but also they had functionalities through fermentation.

According to our best knowledge, the characteristics of fermented green coffee bean by using lactic acid bacteria were reported in the first time. The fermented coffee with LAB were higher overall index and increased total phenol and flavonoid contents, the amounts of chlorogenic acid, antioxidant activity as well as ACE inhibitory activity compared to regular coffee. To give a unique characteristic, cinnamon or pine needle were added into fermented green coffee bean. In sensory evaluation test of cinnamon or pine needle fermented coffee, they showed the characteristic of each ingredient. In cinnamon fermented coffee, sweetness and bitterness got high score than YC fermented coffee. Cinnamon has a sweetness compound like glucose and fructose and bitterness compound like alkaloid group like tannin. Cinnamon or pine needle fermented coffee showed higher overall index and total phenol and flavonoid contents, antioxidant activity and amount of chlorogenic acid compared to fermented coffee. The coffee fermented coffee with LAB shows great potential for industrial productions.

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국문초록

커피는 전세계에서 가장 인기있는 음료 중 하나이다. 본 연구에서는 우리나라 전통식품에서 유래한 젖산균을 이용하여 커피 생두를 발효하였다. 커피 발효에서의 최적의 수분과 균 접종 농도를 관능평가를 통해 선정하였다. 커피 발효에서의 수분 농도 최적 조건의 경우, 세 종류의 젖산균 *Lactobacillus delbrueckii* KCCM11945P YC 50% (w/w-넣어준 수분의 양/사용된 커피 생두 무게), *Streptococcus thermophilus* KCCM11946P Y2 15% (w/w), and *Streptococcus thermophilus* KCCM11947P R2 50% (w/w)로 확인되었다. 접종 균 최적 조건의 경우, YC, Y2는 3.2 x 10^8 CFU/g coffee, R2는 6.4 x 10^8 CFU/g coffee으로 확인되었다.

일반커피와 발효커피간의 비교를 위해, 총 페놀, 총 플라보노이드 함량, 항산화효과 (DPPH, ABTS), 안지오텐신전환효소 저해능, HPLC를 통한 트리고넬린, 카페인, 클로로겐산을 분석하였다. 일반커피 대비 발효커피 경우, 총 페놀함량 1.2배, 총 플라보노이드 1.5배 증가하였으며, 항산화능의 경우 (SC_{50}), DPPH 2배, ABTS 1.3배 향상되었다. 또한, 안지오텐신전환효소 저해능은 (IC_{50}) 1.7배 향상되었다. 성분분석 결과, 트리고넬린과 카페인은 일반커피와 발효커피간의 유의미한 차이를 보이지 않았으며 클로로겐산의 경우, 발효커피에서 1.3배 증가되었다.

발효커피의 맛을 증가시키기 위해, 계피, 솔잎 추출물을 커피 생두 발효에 추가하여 혼합 발효를 진행한 결과, 일반커피보다 더 높은 기능성을 지닌것으로 나타났다.

주요어 : 젖산균, 커피 아라비카, 항산화능, 발효, 안지오텐신전환효소

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