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Fermentation of Green Coffee Bean with *Rhizopus oryzae* and Its Biochemical Properties

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Fermentation of Green Coffee Bean with *Rhizopus oryzae* and Its Biochemical Properties

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Abstract

Coffee is one of the most popular beverages in the world with its health benefits. In this study, *Rhizopus oryzae* was used for the fermentation of green coffee beans. The L-carnitine content in regular and fermented coffee was determined using LC-MS method. Compared to regular coffee, the fermented coffee extract produced L-carnitine. Based on the L-carnitine content, the optimum period for fermentation by *Rhizopus oryzae* was determined. The total phenol content in fermented coffee was increased 1.2 times than regular coffee. In an analysis using HPLC, the content of chlorogenic acid, caffeine and trigonelline were almost same in both regular and fermented coffee. The fermented coffee has the potential to be utilized as a functional nutrients fortified beverage.

Keywords: Antioxidant activity; Coffee; Fermentation; L-carnitine; *Rhizopus oryzae*

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Introduction

1. Coffee

Coffee has been the most consumed beverage in the world. Likewise, many people enjoy drinking coffee regardless of their nationality [1]. The reason for this continuous increase in coffee consumption includes improved coffee quality through selection of varieties and breeding, better agriculture practices [1]. Coffee contains functional materials such as caffeine, chlorogenic acid, HHQ(hydroxyl hydro quinine). Especially, chlorogenic acids act as antioxidants and showed hepato-protective (preventing damage to the liver) and antiviral activities. Thus, drinking coffee in appropriate amount has impact on the cardiovascular system and on the metabolism of carbohydrates and lipids [2]. Coffee reduces the incidence of cancer, diabetes and liver disease. Coffee protects against Parkinson's disease and even reduces mortality risk [2].

2. Solid-state fermentation using *Rhizopus oryzae*

There are different fermentation technologies such as solid-state and submerged fermentation. Solid-state fermentation is considered as effective process for production of enzymes and other components such as phenolics, vitamin, flavor compounds [3]. Fungi, the main microorganism used in solid-state fermentation, produce several enzymes to degrade plant cell walls. Some enzymes could also enhance chemical composition and bioactivities of the substrates. The genus *Rhizopus* includes several species used industrially for enzyme production (glucoamylase, cellulase, tannase), organic acids (lactic acids, fumaric acid), as well as traditional food production such as tempeh [4]. *Rhizopus oryzae* is general recognized as safe (GRAS) and consumes a great range of carbon sources [5].

3. L-carnitine

L-Carnitine is non-essential amino acid and natural compound occurring most in red meat such as ground beef, lamb chop, pork ham [6]. L-carnitine transfers fatty acid into mitochondria as a carrier. It related to the lipid metabolism. In addition, L-carnitine modulates the ratio of Coenzyme A and sustains its homeostasis by forming acyl-carnitine under abnormal condition [7]. It has two isomers but only L-isoform has biological functionality in organism. L-carnitine is considered as weight-loss product because it has function related to fat oxidation. Clinical researches presented that regular intake of L-carnitine makes people lose weight [8]. L-carnitine is effective antioxidants [9]. In mammals, L-carnitine is biosynthesized from two essential amino acids, lysine and methionine [10].

4. Major chemical compounds in a coffee: Chlorogenic acid, Caffeine, Trigonelline

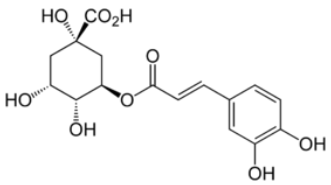
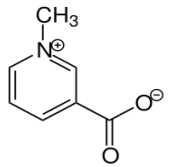
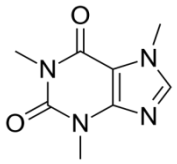
Chlorogenic acid is a main compound in green and roasted coffee bean [11]. It is a natural chemical compound, which is the ester of caffeic acid and quinic acid. This compound known as an antioxidant and makes the release of glucose slow in the bloodstream after a meal [12]. Chlorogenic acid is important to activate biological dietary phenols; the best known as being 5-caffeoylquinic acid. The daily intake of chlorogenic acid by coffee drinkers is considered to be in the range 0.5 g - 1 g and exhibits antioxidant activity in vitro [13].

Caffeine is a commercially important alkaloid, which belongs to group of purine alkaloids synthesized by plants. In nature, caffeine is distributed to the leaves and fruits of 13different orders of plants, including coffee, tea and cacao. Caffeine is an active psycho-stimulant, which increases alertness and sustains concentration by overcoming fatigue [14].

Trigonelline is an alkaloid biologically derived from enzymatic methylation of nicotinic acid [15]. It is a product of niacin metabolism. It contributes to the bitterness of the coffee. It has hypoglycemic,

hypolipidemic, sedative, antimigraine, antibacterial, antiviral, anti-tumor effect and improves memory retention [16].

Table 1. Structure and functionality of major compounds in coffee

Compound	Structure	Functionality	Reference
Chlorogenic acid		Antioxidant, Antimicrobial, Inhibitory effects on carcinogenesis in the large intestine, liver and tongue	Pimia et al. [17] Park et al. [18] Azuma et al. [19]
Trigonelline		Antidiabetic activity, Antioxidant activity, Improves the insulin resistance	Yoshinari et al. [20] Dutta et al. [21]
Caffeine		Alleviating muscular pain, Increase in serum free fatty acids	Kalmar et al. [22] Van Soeren et al.[23]

5. Research trends of fermented coffee

There were several studies about fermented coffee with *Rhizopus*. Lee, L.W., et al. reported modulation of aroma compounds via the fermentation of green coffee bean with *Rhizopus oligosporous*. This study aims to evaluate how to change the volatile and non-volatile profiles of coffee by the fungi fermentation [24]. Furthermore, depending on the different roasting levels, the aroma compounds were analyzed [25]. However, there is no research about functionality of fermented coffee and L-carnitine fortified coffee with *Rhizopus oryzae*.

Materials and Methods

1. Fermentation of green coffee bean

Green coffee bean was purchased from GSC(Global Soft Commodities). Arabica, yirgacheffe green coffee bean was used as the sample. Before fermentation, samples were autoclaved at 121℃ for 15 min. 50 g coffee bean, 100% (w/v) diluted water and 0.005% (w/v) *R.oryzae* was prepared for solid-state fermentation. Each fungus was inoculated 2×10^5 spores into 50 g autoclaved coffee bean samples. Fermentation time of *R. oryzae* was optimized at 3 days. Each sample was solid- state fermented at 30℃. All of the fermentation were performed in triplicate.

2. Preparation of roasted coffee samples

The green coffee bean samples were roasted by roaster (Gene café, Korea) at 240℃ for 14 min (medium roasting) and grinded. 4 g roasted coffee powder was mixed with 20 ml distilled water. Then, the samples were centrifuged at 8000 rpm for 15 min, after that the 10 ml supernatant was took out to 50 ml conical tube.

3. Determination of pH and moisture content

The pH values of the samples were measured with a pH meter (SP-2100, SUNTEX, Taipei, Taiwan). 1 g of fermented green coffee bean before roasting was mixed with 3 ml of distilled water. Each sample was extracted for an hour at room temperature. Determination of the pH of the samples was performed in triplicate.

The moisture content of the samples was determined with a moisture analyzer. 20 g of green coffee bean was prepared for the measurement. The water content was measured at 100℃.

4. Analysis of L-carnitine

1 g of the fermented coffee was mixed with 5ml distilled water. The sample was extracted at 80℃ for 30 min. Then, the extract was centrifuged at 10,000 rpm for 10 min, after that 1 ml supernatant was took out. The sample was diluted with acetonitrile and filtered using a 0.2 μm membrane syringe filter, then 1 μL of the sample was injected into the LC/MS system. Solvent A was 15 mmol/L ammoinm formate with 1 mL/L formic acid and solvent B was

acetonitrile with 1 mL/L formic acid. The sample and column temperatures were 10 and 40℃, respectively. The following elution gradient was applied: 0-3 min, 10% A; 3.1-5 min, 10-30% A; 5.1-6 min, 30-60% A; then a 4 min equilibrium step [26].

5. Determination of total phenols and flavonoids content

After preparing extracted coffee, 0, 1, 3, 5, 7.5, 10, 12, 15, 17, 20, 25, 30, 50 ug/ml gallic acid were prepared with distilled water as a standard. 120 ul samples and gallic acid were added into 96 well plate and 15 ul follin ciocalteu reagent was mixed for 3 min. Then 15 ul 10% (w/v) sodium carbonate added and reacted with the samples for 30 min at dark condition. The detection absorbance is 760 nm at spectrometer [27].

The flavonoid content in each 100% ethanol extracted coffee was determined with quercetin as standard [28]. An addition of 90 µl ethanol, 6 µl aluminum chloride at 10% (w/v), 6 µl sodium nitrite at 5%(w/v) and 170 µl distilled water to each extract sample was performed. Samples were maintained during 30min in the dark at room temperature. The absorbance of

the mixture was measured at 415 nm. A calibration curve was prepared with a standard solution of quercetin. The content of total flavonoid was expressed as milligram quercetin equivalent per dry weight material (mg QE/g coffee).

6. Analysis of chlorogenic acid, caffeine and trigonelline

The samples were freeze dried for 2 days. Freeze dried samples were analyzed using quantitative method. Quantitative analysis of chlorogenic acid, caffeine and trigonelline was performed. The HPLC-UV analyses were achieved as described by Farah et al. [29] with slight modifications. HPLC-UV was carried out on an HPLC system consisting of WATERS 2545 Binary Gradient Module (pump), 2767 sample manager (injector), 2998 PDA detector HPLC system equipped with a SunFire™ C18(4.6 mm× 100 mm, 5 µm) column and a detector. Mobile phase A was acetonitrile. Mobile phase B was 50 mM phosphoric acid. The flow rate was 1 ml/min. UV detection was set at wavelength of 325 nm for CGA and 272 nm for caffeine, trigonelline; injection volume was 20 µl. Concentration of CGA was calculated using the regression equation of their concentration and peak area.

7. Antioxidant activities : DPPH and ABTS radical scavenging activities

Antioxidant activities of coffee samples were measured by using DPPH radical scavenging activity. The DPPH assay measures the reduction of the stable radical 2,2-diphenyl-1-picrylhydrazyl by monitoring the decrease in its absorbance at 517 nm [30]. Freeze drying samples were prepared to obtain 0.01g/ml. Then, samples were diluted to obtain 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45, 0.5, 0.55, 0.6, 0.7, 0.8, 1.0, 1.25, 1.5, 2 mg/ml.

60 µl of distilled water and 20 µl of sample were mixed. Then, 100 µl of ethanol was added. The sample's absorbance at 517 nm was detected for a blank using a spectrophotometer. The blank was prepared from the reaction mixture without DPPH. 20 µl of 1mM DPPH solution were mixed. After 30 min, absorbance was measured at 517 nm.

Radical scavenging activity(%)

$$= \frac{\text{Control optical density} - \text{Sample optical density}}{\text{Control optical density}} \times 100$$

ABTS radical cation decolorization assay was carried out according to Gomez et al. [31] with some modification. The ABTS chromophore was produced by the oxidation of 7mM ABTS with potassium persulfate(2.45 mM) in water(final concentration). The mixture was reacted in the dark condition at room temperature for 11-12 hours. Before experiments, this reaction was performed. The radical was diluted with phosphate-buffered saline(PBS) at pH 7.4 to give an absorbance of 0.7±(0.02) at 734 nm. Each sample and a standard material were dissolved in 80% (w/v) ethanol. Freeze drying samples were prepared to obtain 0.01 g/ml. Each sample was diluted to obtain 1.0, 1.25, 1.5, 2, 2.5, 3, 5, 7, 10 mg/ml. Each analyzed solution was composed with 0.02, 0.25, 0.03, 0.035, 0.05, 0.06, 0.14, 0.2 mg/ml. Then, 5 μl of the sample solution was mixed with 245 μl of ABTS reagent, and the absorbance was monitored at 30℃ for 10 min using UV spectrophotometer. The decrease of absorbance at 734 nm was detected at 10 min. Trolox, the water soluble analogue of vitamin E, was used as a standard. A standard curve was prepared by measuring the percent inhibition values at different concentration of Trolox. The Trolox equivalent antioxidant capacity (TEAC) of each sample represents the concentration of Trolox. The activity of the

samples was measured in triplicate.

8. Sensory evaluation

The group of 17 students and staff served as the sensory panel. Every panelist was given seven pairs of random coded samples representing different extracts. Panelists were asked to estimate the samples compared to control. Each panelist evaluated organoleptic properties which represent aroma, flavor, sweetness, sourness, and bitterness of the samples.

9. Analysis of co-fermentation with herbal extracts

Different kinds of herbal extracts such as angelica gigas (dangquai), astragalus (huangqi) procured from Healing-nature (Korea). Before fermentation, samples were autoclaved at 121℃ for 15 min. 50g coffee bean, 100% (w/v) diluted water and 0.005% (w/v) *R. oryzae* was prepared for solid-state fermentation. Each fungus was inoculated 2×10^5 spores into 50 g autoclaved coffee bean samples. 0.1% (w/v) dang qui, huangqi extracts were added to the samples. Fermentation time of *R. oryzae* was optimized at 3 days. Each sample was fermented at 30℃. All of the fermentation were

performed in triplicate.

10. Statistical analysis

The means and standard deviation values reported were calculated from data derived from triplicate batches of fermentation and extraction. Statistical differences at $p < 0.05$ between the mean values were evaluated with t-test. Statistical analysis was performed on SPSS statistic 23 (IBM, USA) [32].

Results and Discussion

1. pH and moisture content of fermented coffee

Depending on the fermentation time, the pH of fermented coffee was getting decreased. It was known that *R. oryzae* produces L-(+) lactic acid in medium with minimal condition by employing different carbon sources. The pH value is reduced because production of lactic acid during fermentation. This reduction cause an inhibition effect over microorganisms and decreasing productivity. Similar to lactic acid production, during fumaric acid production a decrease in pH affects production performance.

The moisture content of unfermented green coffee was 7.8%. The fermented green coffee bean has 9.6% moisture content. Before roasting, the moisture content of fermented coffee was controlled by using 3 hours drying.

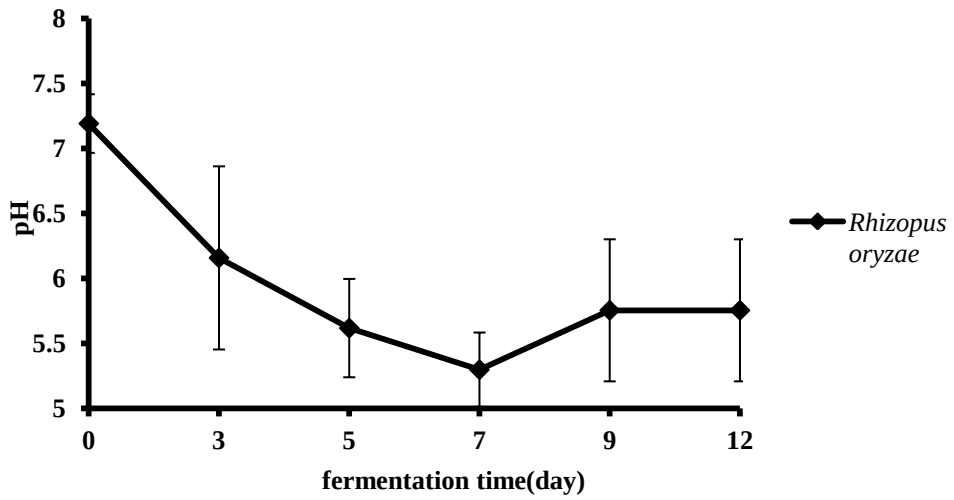


Figure 1. pH of fermented green coffee bean with *Rhizopus oryzae*

Table 2. Moisture content of fermented green coffee bean

	Unfermented GB	Fermented GB (D)
moisture content (%)	7.79	9.55

GB: Green Bean, GB (D): 3 hours Dried Green Bean

2. Determination of L-carnitine in fermented coffee with different factors

The amount of L-carnitine was checked with different factors such as fermentation time, extraction temperature and roasting condition.

Depending on the fermentation time, regular tendency could not found. The L-carnitine content at 5 days fermentation was the highest level. However, when fermentation time is getting increased, mycelium of the fungi is getting disturbed roasting. Therefore, fermentation time was optimized at 3 days.

Table 3 shows the amount of L-carnitine in fermented coffee. In unfermented green coffee bean, L-carnitine could not detected. After fermentation, it was produced by *Rhizopus oryzae*. Fermented green coffee bean has 1.03 ± 0.05 mg /100 g coffee extracted at 80℃. Even after roasting, L-carnitine was remained 30% over. There was no significant difference between extraction temperature 80 ℃ and 20 ℃. It means that L-carnitine withstand the heat up to 80 ℃. When the roasted coffee was brewed at 80 ℃ hot water, L-carntine was still maintained.

However, the roasting condition affects the L-carnitine content in the roasted coffee. When the roasting time is getting longer and the temperature is getting higher, fewer L-carnitine was determined. Therefore, it is important to select an appropriate roasting time and temperature.

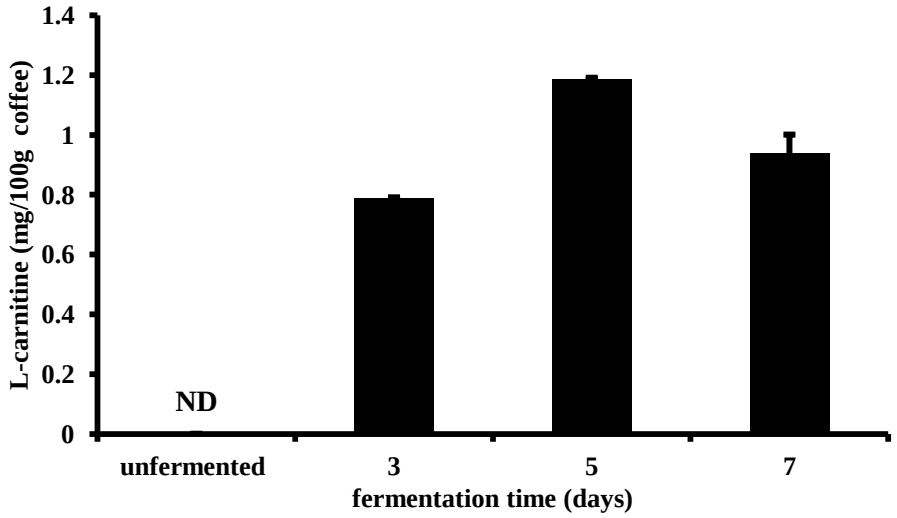


Figure 2. L-carnitine content of fermented coffee depending on fermentation

Table 3. L-carnitine content of fermented coffee depending on fermentation time

	L-carnitine (mg/100g coffee)
Unfermented	ND
Fermented at 3 days	0.79 ± 0.004
Fermented at 5 days	1.19 ± 0.004
Fermented at 7 days	0.94 ± 0.061

Table 4. L-carntine content of fermented coffee with different extraction temperature

	L-carnitine (mg/100g coffee)	
	Unfermented	Fermented
GB_80	ND	1.03 ± 0.05^{a}
RB_80	ND	0.32 ± 0.01^{b}
GB_20	ND	1.14 ± 0.04^{a}
RB_20	ND	0.35 ± 0.02^{b}

Different letters indicate significant differences by t-test at $p < 0.05$.

GB: Green coffee bean, RB: Roasted coffee bean, 80, 20: Extraction temperature (℃), ND: Not detected

Table 5. L-carnitine content of fermented coffee depending on roasting condition

Roasting condition (temp, time)	L-carnitine (mg/100g coffee)
230 ℃, 10 min (light)	0.16±0.05
235 ℃, 11 min (cinnamon)	0.11±0.01
240 ℃, 12 min (medium)	0.01±0.01
240 ℃, 14 min	ND
245 ℃, 14 min (high)	ND

3. Total amount of phenol and flavonoid contents in fermented coffee

Table 5 shows the total phenol and flavonoid contents in unfermented and fermented coffee. The total phenol amount of unfermented coffee was 7.3 ± 0.8 mg (GAE)/ g. Fermented coffee contains 9.0 ± 1.0 mg (GAE)/ g. Total phenol content of fermented coffee was 1.2 times higher than unfermented coffee. Total flavonoid content was almost same in both unfermented and fermented coffee. There were no significant differences in the unfermented and fermented coffee.

In other research, increase in phenolic compounds is attributed to the enzymatic activity of α-amylases, β-glucosidase and xylanase, which hydrolyzed bound phenols during fermentation [16]. When solid-state fermentation used, the antioxidant activity was better than before fermentation. Most phenolic compounds were released in glycoside form [33]. During fermentation, the β-glucosidase activity increase, therefore resulting in release of phenolic attached to wall polysaccharides and in increasing these compounds [24]. It could improve the bioavailability of phenolic compounds.

Table 6. Total phenol and flavonoid content and antioxidant activities of unfermented and fermented coffee

Antioxidant assay type	Roasted coffee beans	
	Unfermented	Fermented
Total phenol content (mg GAE/g)	7.3±0.8[a]	9.0±1.0 [a]
Total flavonoid content (mg QE/g coffee)	7.2±0.6 [a]	7.8±0.5 [a]
DPPH radical scavenging activity (µg/mL, SC50)	72.2±10.5 [a]	69.9±16.0 [a]
ABTS radical scavenging activity (µg/mL, SC50)	15.3±2.3 [a]	16.2±1.8 [a]

Mean and standard deviation values in triplicate. GAE: Gallic Acid Equivalent QE: Quercetin Equivalent

Table 7. Major component of unfermented and fermented coffee

	HPLC-UV analysis (g/100g coffee)		
	CGA	Caffeine	Trigonelline
Unfermented coffee	0.91±0.16[a]	0.84±0.20 [a]	0.80±0.20 [a]
Fermented coffee with *R. oryzae*	1.04±0.05 [a]	0.78±0.01 [a]	0.77±0.02 [a]

4. Trigonelline, caffeine and chlorogenic acid contents in fermented coffee

Trigonelline, caffeine and chlorogenic acid in unfermented and fermented coffee were analyzed by using HPLC-UV. The amount of chlorogenic acid, caffeine and trigonelline in unfermented coffee were 0.91 ± 0.16 mg, 0.84 ± 0.20 mg and 0.80 ± 0.20 mg / 100 g coffee extract, respectively. The amount of chlorogenic acid, caffeine and trigonelline in fermented coffee with *Rhizopus oryzae* were 1.04 ± 0.05 mg, 0.78 ± 0.01 mg, 0.77 ± 0.02 mg / 100 g coffee extract. There is no significant difference between unfermented and fermented coffee with *Rhizopus oryzae*.

5. Antioxidant activities of fermented coffee: DPPH and ABTS radical scavenging activities

Two figures and previous table present the DPPH and ABTS radical scavenging activities of fermented coffee. The antioxidant activity was expressed as SC_{50} (the concentration required to scavenge 50% radicals). Chlorogenic acids mainly contribute the antioxidant capacities of coffee. Like the amount of chlorogenic acid, the antioxidant activities were almost same even after fermentation. There were no significant differences in DPPH and ABTS radical scavenging activities between unfermented and fermented coffee.

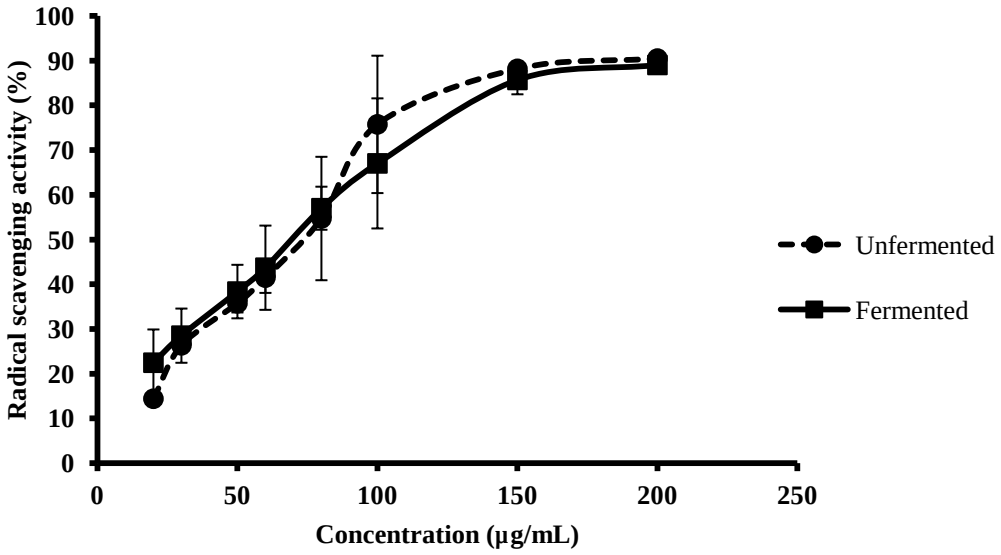


Figure 3. DPPH radical scavenging activity of fermented coffee compared to unfermented coffee

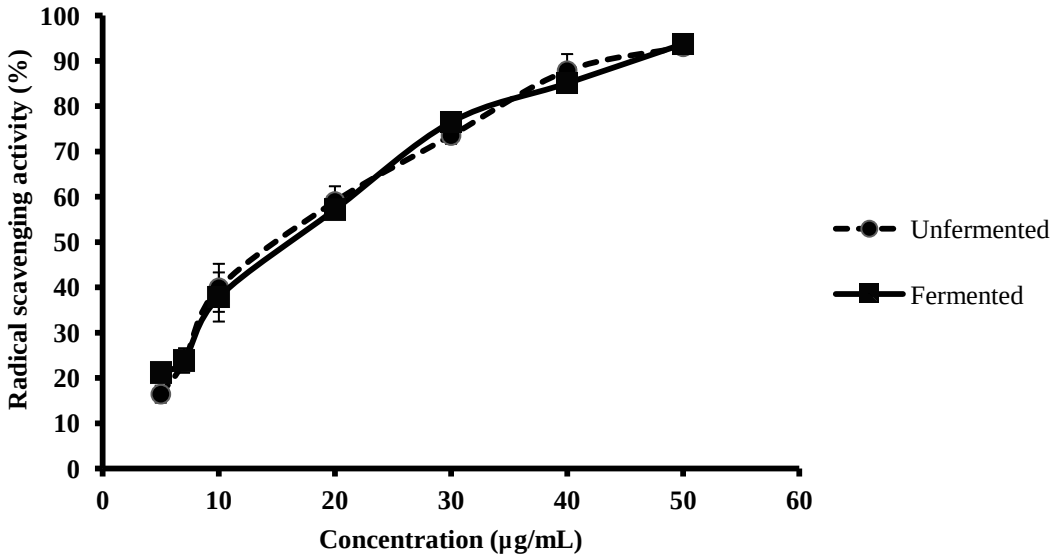


Figure 4. ABTS radical scavenging activity of fermented coffee compared to unfermented coffee

6. Sensory evaluation of co-fermented coffee with dang-qui extract and stevioside

To improve the flavor of fermented coffee, dang-qui extract and stevioside were added into green coffee bean during fermentation. Because of specific aroma during the fungi fermentation, an overall acceptability was decreased compared to regular coffee. Therefore, Co-fermentation masked the specific aroma. It also advanced flavor preference. Co-fermented coffee with the extracts has better taste and aroma than regular fermented coffee.

Table 7 shows the result of sensory evaluation. When the dang-qui extract added, sourness increased than regular fermented coffee. When the stevioside added, sweetness was fortified and bitterness was moderated than regular fermented coffee. In this result, 0.1% (w/v) dang-qui extract and stevioside are appropriate content for co-fermentation.

Table 8. Sensory evaluation of fermented coffee with dang-qui extract and stevioside

	Aroma	Flavor	Sweetness	Sourness	Bitterness	Overall
Rhizopus oryzae fermented coffee	3.2±1.5	4.1±1.8	2.4±1.2	4.1±2.0	5.5±1.2	2.9±1.4
D 0.05%	3.6±0.9	4.4±0.8	3.5±1.0	4.5±1.3	4.2±1.5	3.6±1.4
D 0.1%	4.0±1.3	4.5±1.4	3.3±1.4	5.0±1.5	4.1±1.3	3.6±1.6
D 0.5%	3.2±1.2	4.5±1.6	3.1±1.3	5.9±0.8	4.1±1.6	3.2±1.3
D 1%	3.7±1.3	4.0±1.7	2.6±1.3	6.1±1.1	3.6±1.3	3.6±1.8
D 2%	3.5±1.0	4.0±1.0	3.8±1.0	5.0±1.3	4.4±1.0	3.7±1.0
S 0.05%	3.7±1.3	4.0±1.1	3.8±1.0	3.1±1.4	3.9±1.3	3.9±1.1
S 0.1%	3.9±1.3	4.3±1.7	3.9±1.2	2.9±1.2	3.4±1.2	4.7±1.3
S 0.5%	3.8±1.5	4.1±1.5	6.2±0.6	2.6±1.2	2.8±1.1	3.6±1.7
S 1%	3.4±1.5	4.1±1.5	6.3±0.8	2.1±0.9	2.2±1.0	3.4±1.6
S 2%	3.5±1.2	3.6±1.8	6.8±0.4	2.1±0.9	1.7±0.7	2.7±1.8

(D: fermented coffee adding dang-qui extract S: fermented coffee adding stevioside)

%: Dang-qui extract and stevioside concentration (w/v) into green coffee bean

7. Functionality of co-fermented coffee with herbal extracts

To improve the functional compounds, herbal extracts were added during the fermentation. Especially, fermented coffee with dang-qui extract increased both chlorgenic acid and trigonelline. Caffeine was slightly decreased or similar with unfermented coffee. As a result, antioxidant activity improved over 16%. During the co-fermentation, L-carnitine content slightly decreased or still remained. Likewise, co-fermentation affects functionality of coffee.

Table 9. L-carnitine, total phenol content and major components content of co-fermented coffee with herbal extracts

	L-carnitine (mg/100g green coffee)	Total phenol content (mg GAE/g)	DPPH radical scavenging activity (μg/mL, SC50)	HPLC-UV analysis (g/100g coffee)		
				CGA	Caffeine	Trigonelline
Unfermented	ND	19.41±0.79	43±4.35	0.24 ± 0.01	0.71±0.01	0.30±0.01
Fermented	0.79 ± 0.004	20.78±1.52	26±7.6	0.91 ± 0.04	0.57±0.01	0.54±0.04
Fermented with dang-qui	0.56 ± 0.05	20.53±0.70	36±2.12	1.11 ± 0.13	0.55±0.08	0.51±0.10
Fermented with huang-qi	0.27 ± 0.09	19.63±1.06	44±5.22	0.81 ± 0.07	0.49±0.10	0.45±0.05

Mean and standard deviation values in triplicate. GAE: Gallic Acid Equivalent

Conclusion

Green coffee bean was fermented with *Rhizopus oryzae* from a soybean product. Compared to unfermented coffee, the fermented coffee produced 1.0 mg L-carnitine in 100 g coffee. In an analysis of HPLC, the amount of chlorogenic acid, caffeine and trigonelline were almost same in both unfermented and fermented coffee. Fermented coffee bean adding herbal extracts has more functional compounds than unfermented coffee. Furthermore, it improved the flavor and overall acceptability in sensory test. The herbal extracts such as dang-qui and huang-qi could mask the specific aroma from fungi fermentation. L-carnitine fortified coffee has the potential to be utilized as a functional beverage.

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국문 초록

본 연구에서는 발효 커피를 이용하여 최초로 L-카르니틴이 함유된 커피를 생산하였다. *Rhizopus oryzae* 를 이용해 커피 생두를 발효하였다. 생두 자체에는 L-카르니틴의 합성에 필요한 리신과 메티오닌이 매우 적음에도 발효 커피에서는 L-카르니틴이 확인되었다.

일반커피와 발효커피 간의 비교를 위해, LC/MS 를 이용하여 L-카르니틴을 분석하였다. 일반 생두에는 없었던 L-카르니틴이 발효 생두에는 100 g 당 1.03 mg 함유되어 있었으며, 로스팅 과정을 거친 발효커피에도 30% (w/w) 남아 있었다. 총 페놀, 총 플라보노이드 함량, 항산화 효과 (DPPH, ABTS), HPLC 를 통한 트리고넬린, 카페인, 클로로겐산을 분석하였다. 일반커피 대비 발효커피의 경우, 총 페놀함량 1.2 배가 증가하였다. 총 플라보노이드 함량과 성분 분석 결과에서는 유의미한 차이가 나타나지 않았다.

발효커피의 맛을 증가시키기 위해 당귀, 황기 추출물을 커피 생두 발효에 추가하여 혼합 발효를 진행하였다. 관능평가의 경우, 일반 커피보다 전체적인 선호도가 높아졌으며, 기능성 성분 또한 증가하였다. 카르니틴이 강화된 발효커피는 기능성 식품 시장에서 긍정적인 영향을 나타낼 것으로 기대한다.