Improvement of Robusta Coffee Aroma with L-leucine Powder

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Abstract

L-leucine powder (LP) were added to improve the aroma of Robusta coffee beans. Treatment was a short soaking (M1) or spraying procedure (M2), then LP was added at varying levels up to 3% (w/w). All samples were roasted (240 °C/15 min) and extracted using an espresso machine. Volatile compounds were analysed by solid-phase microextraction–gas chromatography–mass selective detection. Thirty volatile compounds (6 pyrroles, 8 pyrazines, 3 phenols, 9 furans, 2 ketones, 2 aldehydes) were analysed. In 15 coffee samples, the levels of total volatile compounds (based on peak area ratios) ranged from 8.9 (M1-1) to 15.5 (non-treated Robusta: NTR). Robusta coffee has lower levels of bitter aroma compounds when pre-treated with LP. The sum of bitter volatiles (phenols, pyrroles, pyrazines) was lowest in M1-5 (3% LP), M2-1 (1% LP; both dried at 50 °C/15 min) and M2-7 (3% LP, dried at 70 °C/15 min) compared with NTR ($p < 0.05$).

1. Introduction

Coffee is one of the most popular beverages in the world, and the demand for coffee is steadily increasing. According to a 2018 report by the International Coffee Organization ¹, about 148 million cups of coffee are consumed a year, with a further increase to 168.39 million worldwide expected in 2020 ².

Green coffee beans have various chemical and physical characteristics. The volatile compounds, non-volatile compounds, colour, water contents and pH value are dependent on the plant species, growing conditions, roasting conditions, grinding size and brewing type ^{3–6}. Green coffee beans undergo various chemical reactions, especially during roasting. Two major chemical reactions occur, the Maillard reaction and caramelisation. The Maillard reaction involves the interaction between the carbonyl group of a reducing sugar and the amino group of amino acids, producing brown nitrogenous polymers and melanoidins ^{4,7}. Caramelisation results from heat treatment of sugars and is a type of non-enzymatic browning like the Maillard reaction ⁸. These reactions are responsible for the colours and flavours in coffee. The volatile compounds in coffee derive from the various chemical reactions, such as the Maillard reaction, caramelisation and Strecker degradation, during the roasting process, and the various chemical compounds, such as amino acids, lipid, sugar, trigonelline and chlorogenic acid, in green coffee beans ^{4,9}. The volatile compounds of coffee influence the aroma of coffee and, in turn, affect consumers' sensory experience when drinking coffee ^{10,11}.

More than 100 types of green coffee beans have been identified. Arabica (*Coffea arabica*) and Robusta (*C. canephora* syn. *Coffea robusta*) are the most popular species of coffee grown for consumption ¹². Arabica beans have a superior aroma, being sweeter and more harmonious in flavour than Robusta beans, while Robusta beans have a bitter flavour, including metallic and musty aroma, rather than sweet aroma ^{12–14}.

Moreover, roasted Robusta beans have not only less quality of colour but also lack in flavour to use single origin ^{12,15}. The composition of volatile compounds in coffee determines the quality of the coffee aroma ¹⁶. Robusta beans have more phenolic compounds, pyrazines and pyrroles in comparison to Arabica beans. Phenolic compounds are important aroma compounds in coffee, imparting the sensory characteristic of bitterness ^{12,16}, while pyrazines and pyrroles impart nutty, metallic, bitter and strong aroma ¹⁷.

It is necessary to enhance the flavour of Robusta as much as Arabica in the coffee industry. As a way to improve the flavour of Robusta, several compounds were applied to the coffee beans. In the study ¹³, sugar solutions were used to modify aroma precursors of Robusta coffee and pyrazines which are dominant aroma in Robusta coffee were significantly reduced. Another study ¹⁵ also found that pre-treatment with acetic acid could decrease pyrazines while increasing furans. In addition, the addition of L-cysteine to green coffee beans reduces the loss of 2-furfurylthiol ¹⁸.

Regarding to the leucine, it is one of the non-polar amino acids and has lower reactivity in the Maillard reaction than the other amino acids ^{19,20}. This study was conducted to reduce the bitter aroma (pyrazines, pyrroles and phenols) of Robusta beans by adding L-leucine powder (LP). The hypothesis of this study was that the addition of large amounts of specific amino acid such as LP, which is less-reactive in Maillard reaction, could induce competitive inhibition with presence amino acids in coffee beans. Treatment was a short soaking (M1) or spraying procedure (M2), then LP was added at varying levels up to 3% to affect the chemical reaction of volatiles during the roasting process.

2. Materials And Methods

2.1. Chemical standards and materials

Green coffee beans (*C. arabica* Sitio Chapada from Brazil and *C. robusta* from Vietnam) and edible 100% LP (Myprotein, UK) were purchased from a local vender, Trini Coffee Co., in Seoul, Korea. A statement on guidelines as experimental research and field studies on plants (either cultivated or wild), is comply with relevant institutional, national, and international guidelines and legislation. Studies were complying with local and national regulations –formal ethical approval is not required. Green coffee beans and LP were stored at room temperature.

Pyrazine, 1-furfurylpyrrole, 2-acetylpyrrole, 1-furfuryl-2-formylpyrrole, 2-methylpyrazine, 2,5-dimethylpyrazine, benzaldehyde, 1-methylpyrrole, 2-acetyl-1-methylpyrrole, furfuryl alcohol, 2,6-dimethylpyrazine, 5-methyl-2-furfural, 2-ethyl-3-methylpyrazine, furfuryl methyl ether, dihydro-2-methyl-3(2H)-furanone, furfural, furfuryl acetate, 2-acetyl-5-methylfuran, furfural acetone, 2-methoxy-4-vinylphenol, hydroxyacetone, acetoxyacetone and 2-phenyl-2-butenal were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Quinoxaline, 2-formyl-1-methylpyrrole, 2-ethylpyrazine, 2,3-dimethylpyrazine, 2-propylpyrazine, 2-acetylfuran, 2-methylphenol and 4-hydroxy-3-methylacetophenone were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). C7–C40 *n*-alkane standard and the Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS, 50 µm) solid-phase microextraction (SPME) fibre were purchased from Supelco, Inc. (Bellefonte, PA, USA). Ethyl acetate, hexane and HPLC-grade water were obtained from J.T. Baker (Philipsburg, NJ, USA).

2.2. Preparation of coffee samples

Green coffee beans (*C. robusta* from Vietnam) were treated with LP in two ways (Table 1). In Method 1 (M1), 100 g of green coffee beans (*C. robusta* from Vietnam) were soaked in filtered water for 1 min and then mixed with LP at 0.1, 0.5, 1, 2 and 3% (w/w). Before roasting, green coffee beans mixed with LP were dried at 50 °C for 15 min using a food dehydrator intended for home use (LD-918, L'EQUIP, Korea). In Method 2 (M2), 100 g of green coffee beans (*C. robusta* from Vietnam) were sprayed with filtered water using a sprayer and mixed with 1 and 3% (w/w) LP. Before roasting, green coffee beans mixed with LP were dried at 50 °C for 15 min, 50 °C for 30 min, 70 °C for 15 min and 70 °C for 30 min using the dryer (LD-918, L'EQUIP). All green coffee beans were roasted at 240 °C for 15 min using a coffee bean roaster (CBR-101A, Gene Café, Korea). Roasted coffee beans were ground for 30 s twice using a blender (SMKANB-4000, Poongnyun Co., Ltd., Korea). Finally, 25 g of coffee bean powder was extracted with 200 ml of water by an espresso coffee machine (BCC-480ES, Bean Cruise, Korea).

Table 1
Preparation of coffee beans by adding L-leucine

Sample	Method	Pre-treatment method	Concentration of leucine	Drying temperature & time	Roasting temperature & time	Brewing method
M1-1	Method I	Soaking, 1 min	0.1%	50 °C , 15 min	240 °C , 15 min	Espresso coffee
M1-2			0.5%			
M1-3			1%			
M1-4			2%			
M1-5			3%			
M2-1	Method II	Spraying leucine solution on the coffee bean	1%	50 °C, 15 min		
M2-2				50 °C, 30 min		
M2-3				70 °C ,15 min		
M2-4				70 °C, 30 min		
M2-5			3%	50 °C, 15 min		
M2-6				50 °C, 30 min		
M2-7				70 °C, 15 min		
M2-8				70 °C, 30 min		

2.3. Analysis of volatile compounds in coffee by GC-MS

Volatile compounds in coffee were analysed by headspace–solid-phase microextraction–gas chromatography–mass spectrometry (HS-SPME-GC-MS). Extracted coffee solution (10 ml) and sodium chloride (1 g) were added to 20-ml headspace vial, and then 10 µl of quinoxaline as the internal standard (1,000 µg/ml) and 20 µl of *n*-alkane standard (10 µg/ml) were spiked. The mixture was equilibrated at 70 °C for 10 min using a hot plate. For adsorption of the volatile compounds, the SPME fibre (DVB/CAR/PDMS) was exposed to the vial headspace at 70 °C for 40 min. The fibre was then inserted into the GC injection port for desorption of the volatile compounds at 230 °C for 10 min. Gas chromatography–mass spectrometry (GC-MS) was performed using an Agilent 7820A GC with 5977E MS detector. Chromatographic separation of the volatile compounds was achieved using a DB-WAX column (60 m × 250 µm × 0.25 µm). The GC oven was set at 44 °C for 5 min, increased to 170 °C at 3 °C/min and held for 10 min and, finally, increased to 240 °C at 8 °C/min and held for 5 min.

GC-MSD was performed for qualitative and quantitative analysis of the volatile compounds. For qualitative analysis, each peak in the total ionisation chromatogram (TIC) obtained by GC-MSD was identified by co-injection, retention index (RI) on the DB-WAX column and mass spectrum in the Wiley Mass Spectral database. The quantitative analysis was calculated to peak area ratio (peak area of each peak/peak area of internal standard) of each compound with quinoxaline as an internal standard.

2.4. pH and colour measurements

Green/roasted coffee bean powder (25 g) was extracted with 200 ml of filtered water using an espresso coffee machine (BCC-480ES, Bean Cruise). The pH of the coffee was measured using a pH meter (SevenEasy, Mettler Toledo Co., Ltd., USA) at room temperature in triplicate.

Colour measurement of coffee powder was conducted in reflection mode using a colour meter from Nipon Denshoku Industries Co., Ltd. (Tokyo, Japan). Lightness [white ($L^* = 100$) and black ($L^* = 0$)], a^* value [redness (+) and greenness (-)], b^* value [yellowness (+) and blueness (-)] and ΔE^* [total colour differences] (Eq. 1) were recorded.

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \text{ (1)}$$

2.5. Statistical analysis

All experimental analyses were conducted in triplicate, and the results were expressed as mean ± standard deviation (SD). Significant differences between treatments were evaluated by one-way ANOVA, followed by Duncan's multiple range test ($p < 0.05$) using IBM SPSS Statistics 23 (IBM, Armonk, NY, USA).

3. Results And Discussion

3.1. Qualification and quantification of volatile compounds in coffee

The volatile compounds of 15 coffee samples were analysed by SPME-GC-MSD. The profile contained 30 volatile compounds, including 6 pyrroles, 8 pyrazines, 9 furans, 3 phenols, 2 ketones and 2 aldehydes (Table 2). For qualitative analysis, the peaks were identified by Kováts RI, co-injection and the Wiley Mass Spectrum Library database. A quantitative analysis was performed by calculating the peak area ratio of each volatile compound and the internal standard. All values are represented as the peak area ratio (peak area of each peak/peak area of internal standard).

Table 2
Volatile compounds in the coffee samples

No.	Volatile compounds	R.I	Literature RI ¹⁾	Identification method ²⁾
Pyrroles				
1	1-Methylpyrrole	1146	1149 ^{a)}	MS, RI, CO
2	2-Formyl-1-methylpyrrole	1637	1651 ^{b)}	MS, RI, CO
3	2-Acetyl-1-methylpyrrole	1671	1683 ^{b)}	MS, RI, CO
4	1-Furfurylpyrrole	1836	1833 ^{c)}	MS, RI, CO
5	2-Acetylpyrrole	2001	2022 ^{b)}	MS, RI, CO
6	1-Furfuryl-2-formylpyrrole	2285	N.D.	MS, KRI
Pyrazines				
7	Pyrazine	1221	1231 ^{a)}	MS, RI, CO
8	2-Methylpyrazine	1278	1288 ^{a)}	MS, RI, CO
9	2,5-Dimethylpyrazine	1338	1347 ^{a)}	MS, RI, CO
10	2,6-Dimethylpyrazine	1344	1353 ^{a)}	MS, RI, CO
11	2-Ethylpyrazine	1349	1359 ^{a)}	MS, RI, CO
12	2,3-Dimethylpyrazine	1362	1372 ^{a)}	MS, RI, CO
13	2-Ethyl-3-methylpyrazine	1421	1432 ^{a)}	MS, RI, CO
14	2-Propylpyrazine	1434	1428 ^{c)}	MS, RI, CO
Phenols				
15	2-Methylphenol	2006	1988 ^{c)}	MS, RI, CO
16	4-Hydroxy-3-methylacetophenone	2021	2004 ^{c)}	MS, RI, CO
17	2-Methoxy-4-vinylphenol	2215	2225 ^{b)}	MS, RI, CO

Table 2
(Continued)

No.	Volatile compounds	R.I	Literature RI ¹⁾	Identification method ²⁾
Furans				
18	Furfuryl methyl ether	1246	1251 ^{a)}	MS, RI, CO
19	Dihydro-2-methyl-3(2H)-furanone	1274	1283 ^{a)}	MS, RI, CO
20	Furfural	1471	1482 ^{a)}	MS, RI
21	2-Acetylfuran	1530	1536 ^{a)}	MS, RI, CO
22	Furfuryl acetate	1542	1559 ^{a)}	MS, RI, CO
23	5-Methyl-2-furfural	1584	1596 ^{b)}	MS, RI, CO
24	2-Acetyl-5-methylfuran	1640	1650 ^{a)}	MS, RI, CO
25	Furfuryl alcohol	1665	1678 ^{b)}	MS, RI, CO
26	Furfural acetone	1919	N.D.	MS, RI
Ketones				
27	Hydroxyacetone	1308	1323 ^{a)}	MS, RI, CO
28	Acetoxyacetone	1467	1483 ^{a)}	MS, RI, CO
Aldehydes				
29	Benzaldehyde	1537	1546 ^{d)}	MS, RI, CO
30	2-Phenyl-2-butenal	1933	1927 ^{c)}	MS, RI, CO
1) Literature R.I (Kovats Retention Index) was taken from the literature.				
2) Identification method: MS = Comparison with mass spectrum in Wiley Library; RI = Kovats Retention Index obtained from standard and literature values on DB-WAX; CO = Co-injection with authentic chemicals.				
3) The N.D. means that R.I was not found in the reference.				

3.2. Analysis of volatile compounds in coffee by GC-MS

Table 3 shows the peak area ratio of the volatile compounds for all 15 coffee samples: 13 samples of green coffee beans treated with LP, including M1 samples (M1-1 to M1-5) and M2 samples (M2-1 to M2-8), and the non-treated coffee beans of both varieties (NTR and NTA). Thirty volatile compounds, including 6 pyrroles, 8 pyrazines, 3 phenols, 9 furans, 2 ketones and 2 aldehydes, were identified by quantitative and qualitative analysis.

Table 3
Concentration of volatile compounds in non-treated coffee

Volatile compounds	NON-TREATMENT		PRE-TREATMENT (Method I)					
	Robusta	Arabica	M1-1	M1-2	M1-3	M1-4	M1-5	M2-1
Pyrroles								
1-Methylpyrrole	0.089±0.004	0.046±0.003	0.064±0.001	0.065±0.002	0.059±0.004	0.054±0.003	0.034±0.001	0.048±0.001
2-Formyl-1-methylpyrrole	0.263±0.02	0.217±0.013	0.212±0.011	0.172±0.004	0.182±0.09	0.196±0.013	0.165±0.01	0.128±0.001
2-Acetyl-1-methylpyrrole	0.084±0.002	0.044±0.003	0.069±0.004	0.053±0.003	0.063±0.003	0.065±0.002	0.048±0.003	0.037±0.001
1-Furfurylpyrrole	0.597±0.045	0.334±0.029	0.624±0.049	0.515±0.009	0.551±0.02	0.594±0.028	0.485±0.019	0.295±0.01
2-Acetylpyrrole	0.279±0.01	0.194±0.002	0.207±0.013	0.208±0.002	0.196±0.004	0.219±0.003	0.190±0.005	0.143±0.001
1-Furfuryl-2-formylpyrrole	0.434±0.018	0.267±0.021	0.415±0.005	0.358±0.009	0.388±0.023	0.443±0.02	0.357±0.031	0.238±0.01
Total pyrroles	1.745±0.074 ^e	1.103±0.065 ^a	1.591±0.075 ^d	1.372±0.017 ^{b,c}	1.439±0.033 ^c	1.571±0.054 ^d	1.279±0.065 ^b	0.889±0.03
Pyrazines								
Pyrazine	0.053±0.030	0.024±0.002	0.045±0.001	0.039±0.001	0.041±0.001	0.043±0.002	0.034±0.001	0.036±0.001
2-Methylpyrazine	0.751±0.048	0.284±0.011	0.524±0.026	0.490±0.009	0.533±0.04	0.512±0.016	0.451±0.007	0.375±0.03
2,5-Dimethylpyrazine	0.487±0.031	0.214±0.012	0.336±0.017	0.331±0.008	0.353±0.021	0.326±0.011	0.293±0.008	0.261±0.01
2,6-Dimethylpyrazine	0.385±0.022	0.164±0.007	0.274±0.015	0.261±0.008	0.282±0.018	0.269±0.005	0.236±0.007	0.190±0.01
2-Ethylpyrazine	0.365±0.026	0.136±0.01	0.266±0.018	0.254±0.015	0.266±0.022	0.260±0.009	0.229±0.005	0.182±0.01
2,3-Dimethylpyrazine	0.066±0.003	0.045±0.002	0.042±0.002	0.041±0.002	0.049±0.005	0.042±0.001	0.034±0.001	0.034±0.001
2-Ethyl-3-methylpyrazine	0.471±0.034	0.171±0.008	0.324±0.003	0.315±0.001	0.346±0.013	0.315±0.009	0.289±0.006	0.226±0.01
2-Propylpyrazine	0.035±0.001	0.022±0.001	0.028±0.002	0.026±0.001	0.026±0.001	0.026±0.001	0.024±0.001	0.017±0.001
Total pyrazines	2.614±0.162 ^d	1.058±0.050 ^a	1.841±0.103 ^c	1.756±0.036 ^c	1.896±0.116 ^c	1.793±0.031 ^c	1.591±0.022 ^b	1.321±0.09
Phenols								
2-Methylphenol	0.020±0.001	0.025±0.002	0.023±0.002	0.016±0.001	0.019±0.001	0.021±0.000	0.017±0.001	0.009±0.001
4-Hydroxy-3-methylacetophenone	0.087±0.005	0.120±0.002	0.090±0.005	0.084±0.002	0.074±0.003	0.096±0.002	0.074±0.003	0.061±0.001
2-Methoxy-4-vinylphenol	5.060±0.065	2.296±0.100	4.293±0.106	4.913±0.022	4.535±0.209	4.612±0.025	4.221±0.123	3.620±0.20
Total phenols	5.168±0.065 ^s	2.442±0.102 ^a	4.407±0.108 ^b	5.013±0.020 ^s	4.628±0.210 ^c	4.729±0.026 ^c	4.312±0.127 ^b	3.690±0.20

Table 3
(Continued)

Volatile compounds	NON-TREATMENT		PRE-TREATMENT (Method I)					PRE-TREATMENT
	Robusta	Arabica	M1-1	M1-2	M1-3	M1-4	M1-5	
Furans								
Furfuryl methyl ether	0.016±0.001	0.013±0.001	0.013±0.001	0.009±0.000	0.009±0.001	0.014±0.001	0.009±0.001	0.006±0.000
Dihydro-2-methyl-3(2H)-furanone	0.034±0.002	0.052±0.004	0.028±0.001	0.023±0.001	0.026±0.002	0.027±0.002	0.020±0.001	0.019±0.000
Furfural	1.769±0.129	2.073±0.122	1.340±0.068	1.338±0.023	1.343±0.080	1.312±0.053	1.169±0.036	1.031±0.087
2-Acetylfuran	0.185±0.011	0.198±0.008	0.148±0.007	0.124±0.000	0.134±0.006	0.135±0.005	0.113±0.006	0.081±0.007
Furfuryl acetate	0.364±0.027	0.470±0.037	0.161±0.025	0.210±0.004	0.227±0.006	0.294±0.013	0.209±0.008	0.144±0.002
5-Methyl-2-furfural	1.916±0.106	2.121±0.105	1.635±0.086	1.355±0.005	1.373±0.078	1.553±0.039	1.245±0.022	0.959±0.074
2-Acetyl-5-methylfuran	0.011±0.001	0.011±0.000	0.011±0.001	0.008±0.000	0.010±0.001	0.010±0.001	0.009±0.000	0.004±0.000
Furfuryl alcohol	1.156±0.046	1.101±0.026	0.850±0.028	0.755±0.009	0.775±0.059	0.855±0.033	0.691±0.020	0.540±0.048
Furfural acetone	0.097±0.008	0.107±0.007	0.025±0.002	0.120±0.010	0.133±0.006	0.114±0.006	0.125±0.004	0.016±0.002
Total furans	5.547±0.310 ^c	6.146±0.280 ^d	4.210±0.200 ^b	3.942±0.031 ^{ab}	4.030±0.209 ^b	4.314±0.116 ^b	3.592±0.076 ^a	2.800±0.212 ^c
Ketones								
Hydroxyacetone	0.022±0.000	0.018±0.001	0.025±0.002	0.024±0.002	0.013±0.000	0.021±0.002	0.019±0.001	0.017±0.000
Acetoxyacetone	0.021±0.001	0.018±0.001	0.064±0.004	0.016±0.001	0.018±0.001	0.018±0.001	0.015±0.001	0.013±0.001
Total ketones	0.043±0.001 ^d	0.036±0.002 ^{bc}	0.089±0.005 ^e	0.041±0.002 ^d	0.030±0.001 ^a	0.039±0.002 ^{cd}	0.034±0.001 ^{ab}	0.030±0.001 ^a
Aldehydes								
Benzaldehyde	0.233±0.015	0.112±0.008	0.161±0.012	0.171±0.004	0.166±0.010	0.156±0.004	0.156±0.006	0.121±0.012
2-Phenyl-2-butenal	0.119±0.008	0.070±0.006	0.086±0.007	0.104±0.004	0.093±0.003	0.090±0.003	0.092±0.005	0.059±0.003
Total aldehydes	0.352±0.022 ^d	0.181±0.014 ^a	0.246±0.014 ^b	0.274±0.006 ^c	0.259±0.010 ^{bc}	0.246±0.006 ^b	0.248±0.011 ^b	0.180±0.014 ^a
M1-1: soaking 1 min and mixing with 0.1% leucine powder (LP); M1-5: soaking 1 min and mixing 0.5% LP; M1-3: soaking 1 min and mixing with 1% LP; M1-4: and mixed with 1% LP and drying at 50 °C, 15 min; M2-2: spraying with filtered water and mixed with 1% LP and drying at 50 °C, 30 min; M2-3: spraying with LP and drying at 70 °C, 30 min; M2-5: spraying with filtered water and mixed with 3% LP and drying at 50 °C, 15 min; M2-6: spraying with filtered water and n °C, 15 min; M2-8: spraying with filtered water and mixed with 3% LP and drying at 70 °C, 30 min								

As a result of analysis of the 15 coffee samples, the peak area ratio of total volatile compounds ranged from 8.908 ± 0.555 (M2-1) to 15.469 ± 0.624 (NTR) and those of the individual groups of volatile compounds, including pyrroles, pyrazines, furans, phenols, ketones and aldehydes, ranged from 0.889 ± 0.038 (M2-1) to 1.900 ± 0.065 (M2-6), from 1.058 ± 0.050 (NTA) to 2.614 ± 0.162 (NTR), from 2.800 ± 0.212 (M2-1) to 6.146 ± 0.280 (NTR), from 2.442 ± 0.102 (NTA) to 5.998 ± 0.171 (M2-6), from 0.030 ± 0.001 (M1-3) to 0.089 ± 0.005 (M1-1) and from 0.172 ± 0.003 (M2-4) to 0.352 ± 0.022 (NTR), respectively. Among the 6 groups of volatile compounds, furans and phenols were the most dominant. Heterocyclic compounds, including pyrroles, pyrazines, phenols and furans, were detected at higher concentrations than non-heterocyclic compounds, including ketones and aldehydes. In coffee, pyrroles, pyrazines and phenols impart bitter, woody and smoky aromas. NTR showed higher peak area ratios of pyrroles, pyrazines and phenolic compounds compared to NTA ($p < 0.05$).

Comparing NTR and M1 samples (M1-1 to M1-5), the sum of bitter volatiles (phenols, pyrazines, pyrroles) was lower in the M1 samples than in NTR. The peak area ratio of pyrroles in M1-2 and M1-5 was 21.38% and 26.70% lower, respectively, compared with NTR ($p < 0.05$). Furthermore, the peak area ratio of pyrazines and phenols was 39.14% and 16.56% lower, respectively, in M1-5 than in NTR ($p < 0.05$). Among the M1 samples, M1-5 (3% LP, dried at 50 °C for 15 min) had the lowest peak area ratio of pyrroles, pyrazines and phenolic compounds, respectively ($p < 0.05$).

Comparing NTR and M2 samples (M2-1 to M2-8), the sum of bitter volatiles (phenols, pyrazines, pyrroles) was lower in the M2 samples than in NTR, except for M2-6 ($p < 0.05$). Among them, M2-1 (1% LP, dried at 50 °C for 15 min) and M2-7 (3% LP, dried at 70 °C for 15 min) were 38.07% and 18.38% lower, respectively, compared with NTR ($p < 0.05$). The peak area ratio of the 6 groups of volatile compounds (pyrroles, pyrazines, phenols, furans, ketones and aldehydes) was the lowest in M2-1 among the treated coffee samples. In particular, the peak area ratio of pyrroles and pyrazines was 49.05% and 49.46% lower, respectively, compared with NTR. In coffee, furans impart favourable coffee flavours, such as sweet aroma and fruity aroma. Furans contributed the largest peak area ratio among the 6 groups of volatile compounds. Samples NTA (6.146 ± 0.280), NTR (5.547 ± 0.310) and M2-2 (4.391 ± 0.224) had the highest peak area ratio of furans ($p < 0.05$). Petisca et al. (2013) analysed espresso coffee using GC-MS and found that furans represented the major chemical compounds in Arabica beans.

Ketones and aldehydes contributed the lowest peak area ratios among the 6 groups of volatile compounds. Comparing NTR and NTA, the peak area ratio of NTR was detected higher in total ketones and total aldehydes. Comparing NTR and M1 samples, NTR had a higher peak area ratio for total ketones and aldehydes, except for M1-1 ($p < 0.05$). In M1-1, the levels of total ketones were detected at 0.089 ± 0.005 , more than twice that in NTR. Previously, Robusta beans were found to have more ketones than Arabica beans^{21,22}.

The aroma of non-treated and treated coffee is described in Fig. 1, in which the peak area ratio of 30 volatile compounds in non-treated and treated coffee is expressed relative to their concentrations in NTA (100%). Figure 1(a–e) shows the aroma profiles of NTR, NTA and M1 samples. Volatile compounds in M1-1 were significantly lower than those in NTR, excluding acetoxyacetone (Fig. 1a). The peak area ratio of acetoxyacetone, related to buttery odour in coffee, was 66.85% and 71.61% higher than those in NTR and NTA, respectively. Figure 1(b) reveals significant differences in 28 volatile compounds, excluding 2,3-dimethylpyrazine and acetoxyacetone, between M1-2 and NTA ($p < 0.05$). Significant differences were also observed between M1-3 and NTA, except for 2-acetylpyrrole and acetoxyacetone (Fig. 1c), and between M1-4 and NTA, except for 2-formyl-1-methylpyrrole, furfuryl methyl ether and acetoxyacetone (Fig. 1d; $p < 0.05$). Figure 1(e) highlights significant differences in 26 compounds, except for ketones (buttery odour), 2-acetyl-1-methylpyrrole and 2-acetylpyrrole (bitter odour) ($p < 0.05$). M1-5 had a 1.6-fold higher peak area ratio of total bitter aroma compounds (pyrazines, pyrroles, phenols) than that of NTA but a 24.61% lower peak area ratio of total bitter aroma compounds compared with NTR ($p < 0.05$).

Figure 1(A–H) shows the aroma profile of NTR, NTA and M2 samples. In Fig. 1(A), 24 volatile compounds in M2-1 showed significant differences compared with NTA ($p < 0.05$). The peak area ratio of volatile compounds in M2-1 was lower than that of NTA, except for 2-methoxy-4-vinylphenol and benzaldehyde. In addition, the peak area ratio of all volatiles in M2-1 was lower than that of NTR, especially the peak area ratio of pyrroles, pyrazines and phenols, which were 49.05%, 49.46% and 28.60% lower, respectively ($p < 0.05$). Figure 1(B) indicates the volatile compounds of M2-2, NTR and NTA. There were no significant differences between M2-2 and NTA in 2-formyl-1-methylpyrrole, furfuryl methyl ether and benzaldehyde. Although the peak area ratio of 1-furfuryl-2-formylpyrrole, 4-hydroxy-3-methylacetophenone and 2-methoxy-4-vinylphenol in M2-2 was higher than those of NTR and NTA, the remaining volatile compounds in M2-2 indicated a significant decrease compared with NTR ($p < 0.05$). Figure 1(C) displays the volatile compounds of M2-3, NTR and NTA. There were no significant differences in 1-methylpyrrole and furfuryl methyl ether between M2-3 and NTA. Figure 1(D) is the profile of volatile compounds of M2-4, NTR and NTA. There were no significant differences in 4 volatiles (2-formyl-1-methylpyrrole, 2,3-dimethylpyrazine, 2-acetyl-5-methylfuran and 4-hydroxy-methylacetophenone) between M2-4 and NTA. The peak area ratio of phenols, one of the bitter flavours, was 9.21% and 57.10% higher, respectively, in M2-4 than in NTR and NTA ($p < 0.05$). Figure 1(E) shows significant differences in 27 compounds, except for 2-formyl-1-methylpyrrole, 2-acetyl-5-methylfuran and 2-phenyl-2-butenal between M2-5 and NTA ($p < 0.05$). The peak area ratio of phenols in M2-5 was 10.88% and 57.89% higher than those in NTR and NTA, respectively, ($p < 0.05$). Figure 1(F) shows significant differences in 28 compounds, except for 2-formyl-1-methylpyrrole and benzaldehyde between M2-6 and NTA. The peak area ratio of total volatiles in M2-6 was the highest among the treated samples, and the sum of volatile compounds of bitter aroma (pyrazines, pyrroles and phenols) in M2-6 was the highest among all samples ($p < 0.05$). Figure 1(G) indicates significant differences in 25 volatiles between M2-7 and NTA. The peak area ratio of all volatile compounds in M2-7 was lower than that in NTR, especially the peak area ratio of pyrazines, one of the bitter flavours, which was lower by 42.92% ($p < 0.05$). Figure 1(H) shows the profiles of volatile compounds in M2-8, NTR and NTA. The peak area ratio of volatile compounds in M2-8 indicated significant differences in 28 volatile compounds compared with NTA. Furthermore, M2-8 displayed a 9.54% and 57.26% higher peak area ratio of phenols compared with NTR and NTA, respectively ($p < 0.05$).

3.3. pH and colour

Table 4 shows the pH and colour values in non-treated and treated coffee. The pH and colour of coffee are important sensory properties determining the quality of the beverage¹⁵. The pH values showed significant differences between NTR and treated coffee ($p < 0.05$). The pH of NTR was significantly higher than that of NTA ($p < 0.05$). Furthermore, the pH of treated coffee was significantly reduced compared with NTR ($p < 0.05$). Among the M1 samples, M1-5 (3% LP, dried at 50 °C for 15 min) had the lowest pH value. Among the M2 samples, M2-1 (1% LP, dried at 50 °C for 15 min) and M2-7 (3% LP, dried at 70 °C for 15 min) had the lowest pH values.

Table 4
pH and color values of non-treated coffee and treated coffee

	pH	Color values			
		L*	a*	b*	dE*
NTR	5.34±0.01 ^f	38.43±0.00 ^{d,e}	13.48±0.00 ^b	30.09±0.06 ^c	50.63±0.04 ^d
NTA	5.02±0.01 ^a	33.17±0.01 ^a	14.50±0.05 ^f	27.74±0.05 ^a	45.61±0.03 ^a
M1-1	5.28±0.01 ^c	37.55±0.01 ^c	13.73±0.04 ^d	30.04±0.02 ^c	50.01±0.02 ^c
M1-2	5.28±0.01 ^c	38.46±0.00 ^e	13.63±0.04 ^c	30.80±0.03 ^d	51.13±0.03 ^e
M1-3	5.31±0.01 ^e	40.70±0.09 ^f	13.35±0.04 ^a	31.59±0.13 ^f	53.22±0.15 ^g
M1-4	5.30±0.01 ^d	37.17±0.00 ^b	13.71±0.04 ^d	29.92±0.00 ^b	49.65±0.01 ^b
M1-5	5.27±0.01 ^b	38.39±0.00 ^d	13.89±0.04 ^e	31.02±0.03 ^e	51.28±0.02 ^f
M2-1	5.28±0.01 ^b	39.85±0.02 ^j	13.64±0.04 ^c	31.44±0.07 ^g	52.56±0.04 ⁱ
M2-2	5.33±0.01 ^e	37.15±0.00 ^e	13.63±0.00 ^{b,c}	29.67±0.03 ^c	49.46±0.02 ^d
M2-3	5.30±0.00 ^c	35.37±0.01 ^c	13.62±0.05 ^{b,c}	28.65±0.03 ^b	47.52±0.01 ^b
M2-4	5.32±0.01 ^d	39.04±0.01 ⁱ	13.48±0.04 ^a	30.63±0.02 ^f	51.42±0.01 ^h
M2-5	5.33±0.01 ^e	37.46±0.00 ^f	13.91±0.04 ^e	30.41±0.03 ^e	50.21±0.02 ^e
M2-6	5.32±0.01 ^d	35.31±0.01 ^b	13.72±0.05 ^d	28.68±0.02 ^b	47.52±0.01 ^b
M2-7	5.28±0.01 ^b	38.07±0.01 ^g	13.72±0.05 ^d	30.56±0.05 ^f	50.71±0.02 ^g
M2-8	5.31±0.01 ^c	35.51±0.01 ^d	13.57±0.05 ^b	28.70±0.02 ^b	47.64±0.01 ^c
All levels are represented as mean ± standard deviation (S.D.) (n=3) (p<0.05).					
M1-1: soaking 1 min and mixing with 0.1% leucine powder (LP); M1-5: soaking 1 min and mixing 0.5% LP; M1-3: soaking 1 min and mixing with 1% LP; M1-4: soaking 1 min and mixing with 2% LP; M1-5: soaking 1 min and mixing with 3% LP; M2-1: spraying with filtered water and mixed with 1% LP and drying at 50 °C, 15 min; M2-2: spraying with filtered water and mixed with 1% LP and drying at 50 °C, 30 min; M2-3: spraying with filtered water and mixed with 1% LP and drying at 70 °C, 15 min; M2-4: spraying with filtered water and mixed with 1% LP and drying at 70 °C, 30 min; M2-5: spraying with filtered water and mixed with 3% LP and drying at 50 °C, 15 min; M2-6: spraying with filtered water and mixed with 3% LP and drying at 50 °C, 30 min; M2-7: spraying with filtered water and mixed with 3% LP and drying at 70 °C, 15 min; M2-8: spraying with filtered water and mixed with 3% LP and drying at 70 °C, 30 min					

Colour measurement of coffee powder was conducted in reflection mode using a colour meter. There were significant differences in ΔE^* values between NTR and treated coffee ($p < 0.05$). The minimum colour difference that the naked eye can detect is $\Delta E^* = 3.0$ ¹³. The colour of NTR and NTA can be distinguished, while NTR and M1 samples are difficult to distinguish by the naked eye. The colour differences between NTR and M2-3 and M2-6 were both $\Delta E^* 3.11$ ($p < 0.05$). The colour of Arabica beans and Robusta beans can be distinguished, with Arabica beans displaying a higher L^* value than Robusta beans²³.

For evaluating the correlation among pH, L^* and volatile compounds, the results for all samples are shown in Table 5 ($*p < 0.05$ and $**p < 0.01$). The pH values increased significantly as the levels of phenolic compounds and pyrazines increased ($p < 0.01$). That is, M1-5 (3% LP, dried at 50 °C for 15 min), M2-1 (1% LP, dried at 50 °C for 15 min) and M2-7 (3% LP, dried at 70 °C for 15 min) with low levels of phenols and pyrazines also had low pH values ($p < 0.05$). The levels of pyrroles, phenolic compounds and pyrazines increased significantly as the levels of total volatile compounds increased ($p < 0.05$). The correlation between L^* values [white ($L^* = 100$) and black ($L^* = 0$)] and the levels of furans were also significant ($p < 0.05$).

Table 5
Correlation between volatile compounds, pH and L* values

Volatile compounds								pH	L*
	Pyrroles	Pyrazines	Phenols	Furans	Ketones	Aldehydes	total		
Pyrroles	1								
Pyrazines	.728**	1							
Phenols	.811**	.634*	1						
Furans	0.280	0.215	-0.211	1					
Ketones	.549*	0.275	0.309	0.115	1				
Aldehydes	0.189	.725**	0.059	0.303	-0.011	1			
total	.931**	.822**	.746**	0.466	0.385	0.398	1		
pH	.569*	.705**	.830**	-0.486	0.186	0.259	0.492	1	
L*	-0.199	0.300	0.157	-.540*	-0.224	0.387	-0.135	.536*	1
1) The values of correlation indicate the significant differences according to * $p < 0.05$ and ** $p < 0.01$.									
M1-1: soaking 1 min and mixing with 0.1% leucine powder (LP); M1-5: soaking 1 min and mixing 0.5% LP; M1-3: soaking 1 min and mixing with 1% LP; M1-4: soaking 1 min and mixing with 2% LP; M1-5: soaking 1 min and mixing with 3% LP; M2-1: spraying with filtered water and mixed with 1% LP and drying at 50 °C, 15 min; M2-2: spraying with filtered water and mixed with 1% LP and drying at 50 °C, 30 min; M2-3: spraying with filtered water and mixed with 1% LP and drying at 70 °C, 15 min; M2-4: spraying with filtered water and mixed with 1% LP and drying at 70 °C, 30 min; M2-5: spraying with filtered water and mixed with 3% LP and drying at 50 °C, 15 min; M2-6: spraying with filtered water and mixed with 3% LP and drying at 50 °C, 30 min; M2-7: spraying with filtered water and mixed with 3% LP and drying at 70 °C, 15 min; M2-8: spraying with filtered water and mixed with 3% LP and drying at 70 °C, 30 min									

4. Conclusion

In this study, LP was blended with green Robusta beans in two different ways to reduce the bitter aroma (pyrazines, pyrroles, phenols). The sum of bitter volatiles (pyrazines, pyrroles, phenols) in M1-5 (3% LP, dried at 50 °C for 15 min) was lower than that in NTR by 24.61%, and the sum was the lowest among the M1 samples ($p < 0.05$). The sum of bitter volatiles in M2-1 (1% LP, dried at 50 °C for 15 min) was the lowest among all treated samples. In particular, the peak area ratio of pyrazines in M2-1 was lower than that of NTR by 49.46% ($p < 0.05$). The sum of bitter volatiles in M2-7 (3% LP, dried at 70 °C for 15 min) was 18.38% lower than that of NTR by ($p < 0.05$).

This study shows that pre-treatment of Robusta beans with LP affects the chemical reactions responsible for the generation of volatiles during the roasting process. Pre-treatment with LP reduced the bitter aroma of Robusta beans. Unlike previous studies that soaked the beans in acetic acid or sugar solutions to improve the aroma, this study pre-treated the surface of green Robusta beans with LP. The results of this study can suggest a new manufacturing method for coffee.

Declarations

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Author Contributions

A. Jo: Formal analysis, Investigation, Methodology; H. Park: Formal analysis; J. Park: Validation; S. Ha: Methodology; Y. Kim: Methodology; K.-G. Lee: Supervision; Validation; Investigation; Project administration.

Conflicts of interest

None.

Data Availability:

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Figures

Figure 1

Volatile compounds comparison arabica (a smooth circle) and non-treated Robusta (a dotted line) with treated coffee samples: (a) M1-1, (b) M1-2, (c) M1-3, (d) M1-4, (e) M1-5, (A) M2-1, (B) M2-2, (C) M2-3, (D) M2-4, (E) M2-5, (F) M2-6, (G) M2-7 and (H) M2-8

All analysis of this study was conducted from triplicate to express as mean $\pm$ standard deviation (SD).

M1-1: soaking 1 min and mixing with 0.1% leucine powder (LP); M1-5: soaking 1 min and mixing 0.5% LP; M1-3: soaking 1 min and mixing with 1% LP; M1-4: soaking 1 min and mixing with 2% LP; M1-5: soaking 1 min and mixing with 3% LP; M2-1: spraying with filtered water and mixed with 1% LP and drying at 50

, 15 min; M2-2: spraying with filtered water and mixed with 1% LP and drying at 50

, 30 min; M2-3: spraying with filtered water and mixed with 1% LP and drying at 70

, 15 min; M2-4: spraying with filtered water and mixed with 1% LP and drying at 70

, 30 min; M2-5: spraying with filtered water and mixed with 3% LP and drying at 50

, 15 min; M2-6: spraying with filtered water and mixed with 3% LP and drying at 50

, 30 min; M2-7: spraying with filtered water and mixed with 3% LP and drying at 70

, 15 min; M2-8: spraying with filtered water and mixed with 3% LP and drying at 70

, 30 min