Characteristic and flavor profiles of specialty Arabica coffee derived from fermentation with protease, at different conditions

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Abstract. Coffee is one of the most popular beverages in the world. Development of coffee beans with specific flavor profiles can enhance the quality and value of the product. In this study, the flavor profiles of Arabica coffee (*Coffea arabica L.*) beans treated with protease, at different enzyme loading, time, and pH, were investigated using gas chromatography tandem mass spectrometry (GC-MS/MS). The coffee beverages were evaluated for their sensory characteristics by Q-Grader (professional coffee taster). The coffee beans obtained from protease hydrolysis exhibited specific and unique volatile compounds or flavor profiles than the control (coffee beans without enzyme loading). Specific volatile profiles in the enzyme-treated coffee beans, such as isopropenyl acetate, 2-acetyl-5-methylfuran, and delta-dodecalactone were identified. Coffee beverages obtained from roasted coffee beans from protease fermentation with 0.84% protease, pH 6.2 at 41 h (R-FP5), and 1.00% protease, pH 5.0 at 30 h (R-FP8) were evaluated as specialty coffees (a score of 79.75 points), showing their specific aroma profiles as fruity, spice, mango, dry fruit, ripe fruit, nutty and tamarind notes. The present work revealed that enzymatic fermentation of the green Arabica coffee beans is a promising method for the modulating and improving of specialty coffee with specific acceptable flavors and aromas.

1 Introduction

Coffee is one of the most important crops with an enormous economic impact [1]. The International Coffee Organization (2021) reported that coffee consumption had significantly increased at an average growth rate of 1.9% per year over the past 5 years. The consumption of coffee beverages in Thailand has also increased by about 15% in volume yearly. The demand for coffee beans in Thailand was around 86,701 tons per year (Office of Agricultural Economics, 2021). Thailand relies mainly on the import of coffee beans because the domestic production cannot meet the above huge demand (Office of Agricultural Economics, 2021). According to the Department of Agriculture (2021), the general purchase price of coffee beans in Thailand falls around 66 Bahts/Kg, while the specialty coffee price is around 160–180 Bahts/Kg. Consequently, development of regular coffee to the higher quality coffee as specialty coffee would benefit the value-adding of regular coffee beans.

In the coffee market, “specialty coffee” refers to a beverage with a higher quality in unique flavors and characteristics than the general coffee available for commodity prices [2]. There is a great trend for specialty coffees over regular coffees in the international market [3], and the global markets driving the search for new processes to facilitate the production of specialty coffee [4]. The flavors and aromas in the green and roasted coffee beans are influenced by many factors, such as growing regions, cultivars, and post-harvest handling [5]. Well-ripe coffee cherries are processed using three different methods: semi-dry, dry/natural, and washed process [6, 7] while the most popular method for processing Arabica coffee is the washed process. Coffee beans are mechanically removed of the skin and pulp, leaving the mucilage adherent with the beans. Next, the depulped coffee beans are placed in water tanks to start a 24-48 h. fermentation process. After that, the beans will be removed from the tanks and sun-dried till the moisture content reaches 10-12% [8].

During coffee fermentation, the mucilage surrounding the seed is solubilized by microbial activity, resulting in various metabolic end-products that diffuse into the beans [7, 9]. The main components of green beans are carbohydrates, lipids, proteins, amino acids,

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organic acids, peptides, alkaloids, and phenolic compounds. Interestingly, more than 900 volatile compounds (e.g., acids, alcohols, ketones, esters, aldehydes, and furans) that contribute to the flavor and aroma of coffee are formed by roasting and released in the coffee beverage [10]. Recently, enzymatic treatments have been successfully applied to enhance the aroma quality of the roasted coffee beans [11]. Beta-glucosidase treatment could strengthen the natural peach-like aroma, fruity and floral juice aroma, which might be related to the release of bound aroma through enzymatic hydrolysis. Beta-glucosidase was also used to enhance the aroma compounds such as esters, aldehydes, and alcohols of orange juice and citrus juice during thermal processing [12].

Enzymatic technology uses pectinase and cellulase to digest the coffee pulp [13]. However, to the best of our knowledge, there are no reports on using protease enzyme to modify flavor profiles for coffee beans. The use of protease for bean fermentation was expected to produce specific and interesting flavor profiles. Therefore, this study aimed to investigate the effects of protease application during the washed process fermentation of green Arabica coffee beans, at different conditions, on the color, appearance, and flavor profiles of the roasted coffee beans. The quality of the flavor and aroma of specialty coffee in each sample was also evaluated by the professional certified Q-graders for coffee quality.

2 Material and methods

2.1 Coffee samples

Ripe coffee fruits (cherry coffee) of *Coffea arabica L.* were obtained from Pangkhon Village which is a very famous cultivation area of high-quality coffee beans in Chiang Rai Province at an altitude of 1,200-1,400 m in Northern Thailand. Ripe coffee cherries were selectively hand-picked (Fig.1A, 1B) and washed with water, and defects such as dried fruits, bad grains, leaves, sticks, and impurities were removed. The washed process was applied to the whole coffee cherries. The skin and pulp of the coffee cherries were removed mechanically, leaving the mucilage adherent to the beans (Fig.1C). Clean water was added to the de-pulped coffee beans until they were fully submerged in the water.

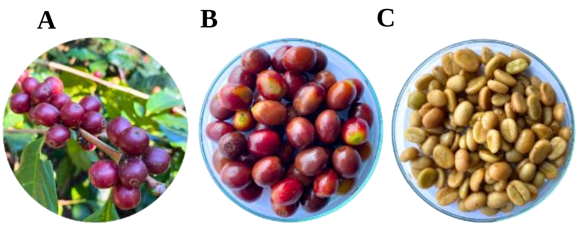

Fig. 1. The color and appearance of coffee beans before the fermentation with protease; A: Ripe coffee fruits (coffee cherries), B: Coffee cherries after harvesting, and C: Depulped beans covered with a mucilaginous layer.

2.2 Chemicals

Food grade protease (80,000 IU/mL) activity was obtained from Reach Biotechnology Co., Ltd. (Thailand), while food-grade tri-sodium citrate (99%) and citric acid (99.5%) were purchased from Krungthepchemi Co., Ltd. (Thailand). Methanol (99.9%) and HPLC grade acetonitrile (99.9%) were bought from RCI Labscan Co., Ltd. (Thailand). All other chemicals and regents were analytical grade.

2.3 Fermentation of green coffee beans

Coffee beans (1 Kg) from the washed process were soaked in 500 mL of citrate buffer at a solid-to-liquid ratio of 1: 0.5, with different pH, protease enzyme loading, and incubation time at room temperature. Central composite design (CCD) for 3 parameters with 16 experiments (Table 1) was used to examine the effects of each parameter on the physical properties and flavor profiles of the fermented coffee beans. The fermentation liquid and coffee beans were agitated daily. Then, the fermentation liquid was drained and the enzyme reaction was stopped by blanching at 100 °C for 5 min. The green coffee beans were immediately solar-dried until their moisture content reached around 12% (wet weight basis, wwb).

2.4 Roasting of green coffee beans

All of the protease-treated coffee beans and the control (without protease loading) were roasted with a final temperature of the coffee beans at 220 °C for 8 min using a L100 Plus Lab Roaster (Roest: L100 Plus, Norway) by the Professional Coffee Roaster at Passion Brew Co., Ltd. (Thailand).

Table 1. Central composite design for enzymatic fermentation of green coffee beans by protease.

Run	Enzyme loading (%)	pH	Time (h)
1	0.60	5.0	48
2	0.36	3.8	41
3	0.84	3.8	41
4	0.36	6.2	41
5	0.84	6.2	41
6	0.60	5.0	30
7	0.20	5.0	30
8	1.00	5.0	30
9	0.60	3.0	30
10	0.60	7.0	30
11	0.36	3.8	19
12	0.84	3.8	19
13	0.36	6.2	19
14	0.84	6.2	19
15	0.60	5.0	12
16 (Control)	Fermentation for 48 h without enzyme loading		

2.5 Determination of the CIE-LAB color of green and roasted coffee beans

The green beans fermented with 0.84% protease at pH 6.2, 41 h (Run 5) were named G-FP5, while G-FP8 was the green coffee beans from 1.00% protease at pH 5.0, 30 h (Run 8). The green beans fermented without protease were named as G-Control. The roasted green beans of G-Control, G-FP5, and G-FP8 were named as R-Control, R-FP5, and R-FP8. The CIE-LAB color parameters of R-Control, R-FP5, and R-FP8 were measured using Color Quest XE (USA). Color analysis was performed following Dong et al, 2017 [14]. All experiments were done in triplicate. Color parameters; L*[white (100) and black (0)], a*[redness (+) and greenness (-)], and b* [yellowness (+) and blueness (-)] were determined in the CIE L* a* b* space under the illuminant D65/10 ºC.
Total color differences *(ΔE)* compared the color between $L*_0$ $a*_0$ $b*0$ of the control and $L*_1$ $a*_1$ $b*_1$ of the enzyme-treated coffee beans, which were calculated using the following equations.

$$\Delta E* = [(L*_0 - L*_1)^2 + (a*_0 - a*_1)^2 + (b*_0 - b*_1)^2]^{\frac{1}{2}} \quad (1)$$

2.6 Aroma and flavor profiles of roasted coffees

The coffee beverages prepared from the roasted coffee beans of each treatment were evaluated by two Q-Graders (one male and one female) following the Fine Arabica Cupping Protocol (ICO, 2010). Samples of ground-roasted coffee (8.25 g, 70%–75% particles with size <841 μm) were mixed with 150 mL of hot water (93 °C) and tasted according to SCAA protocols. The same Q-Grader evaluated each coffee beverage five times. The evaluation attributes were fragrance/aroma, flavor, bitterness/sweetness, mouthfeel, salinity/acidity, and aftertaste, with scores ranging 6-10 points. Then, each Q-Grader gave an individual final score ranging from 0-100 points for eight sensory attributes evaluated (SCAA, 2013). The final score for each sensory attribute was the average of both Q-Graders. The roasted coffee beans, R-FP5 and R-FP8, presented the highest fragrance scores (7.5 points) were further identified for their volatiles and flavor profiles.

2.7 Analysis of volatile compounds in roasted coffees

Volatile compounds were analyzed using a headspace solid-phase microextraction (HS-SPME) gas chromatography tandem mass spectrometry (GC-MS/MS). The roasted coffee beans, R-Control, R-FP5, and R-FP8 were ground by a Knife Grinder (Electrolux, E3TB1-210K grinder, China). Two grams of the coffee powder were placed in a 20 mL of hermetically sealed flask. After equilibration at 60 ºC for 10 min, the volatile compounds were extracted at 60 ºC for 30 min, before the injection into the GC column. The headspace solid-phase microextraction (HS-SPME) with a Teflon-lined septa, 50/30 μm divinylbenzene/carboxen/ polydimethylsiloxane SPME fiber (Thermo Fisher Scientific, US) was used. The fiber exposition was 2 min before the injections. A GC-MS/MS (TRACETM 1300 Gas Chromatograph Thermo Fisher Scientific, US) equipped with mass spectrometry (MS) and a silica capillary TG-WAXMS 30 M (30 m × 0.25 mm x 0.25 μm) column was used for GC-MS/MS analysis. The oven temperature was programmed as follows: held at 50 ºC for 5 min then increased to 60 °C at the rate of 5 ºC/min, and rose to 230 °C at 30 °C/min, The carrier gas (helium) was used at a 1.2 mL/min flow rate. The volatile compounds were identified by comparing the mass spectra to the NIST11 library. In addition, an alkane series (C10–C40) was used to calculate each compound's retention index (RI) and compare them to the scholarly literature's RI values [15].

2.8 Statistical analysis

Analysis of variance (ANOVA, $p < 0.05$) and Tukey's test were performed by Minitab 2018 (Origin Lab Corporation, Northampton, USA).

3 Results and Discussion

3.1 Color and appearance of green coffee beans and roasted coffee beans

Color is one of the main indicators to evaluate coffee quality and is used to optimize drying processes [16, 17, 14]. The color and appearance of green coffee beans and roasted coffee beans after fermentation with protease at different conditions are demonstrated in Table 2. Results showed that color parameters were affected by the protease fermentation significantly (p < 0.05).

The results showed that color was affected by the protease fermentation. The green coffee beans of G-FP8 were brighter than G-Control, and G-FP5, with L* values of 35.3±0.0, 33.6±0.0, and 31.1±0.0, respectively. The L* values of roasted coffee beans, R-FP5 (24.4±0.0) and R-FP8 (23.5±0.0), were brighter than the R-Control (23.1±0.0). G-FP8 and G-FP5 exhibited a more greenish and yellowish shade (lower a* and b* values) than G-Control. The values of ΔE decreased with increased enzyme loading for the green bean fermentation. The enzyme could accelerate the hydrolysis rate and increase sugar yields related to pigment oxidation and affect color stability [18]. Different conditions for fermentation, including time and pH would lead to different intermediates and products, which contribute to specific characteristics and flavors [19].

The main components of green beans are carbohydrates, lipids, proteins, amino acids, peptides, and the coffee flavor and aroma can be enhanced by roasting, which releases over 900 volatile compounds [10]. These flavor/aroma precursors are transformed through thermal reactions during the roasting process. Proteins involved in Maillard reaction during the heating at high temperature, affecting the color and aroma of the coffee brews [20, 21].

Table 2. Color and appearance of green and roasted coffee beans after fermentation with/without protease.

	48 h, Fermentation without protease loading (G-Control)	0.84% Protease at pH 6.2, 41 h (G-FP5)	1.00% Protease at pH 5.0, 30 h (G-FP8)
L*	33.6 ± 0.02^{b}	31.1 ± 0.01^{c}	35.3 ± 0.04^{a}
a*	8.6 ± 0.10^{a}	6.3 ± 0.01^{c}	6.8 ± 0.01^{b}
b*	15.2 ± 0.01^{a}	9.08 ± 0.02^{c}	12.8 ± 0.01^{b}
ΔE		6.98 ± 0.08^{a}	3.48 ± 0.15^{b}

	48 h, Fermentation without protease loading (R-Control)	0.84% Protease at pH 6.2, 41 h (R-FP5)	1.00% Protease at pH 5.0, 30 h (R-FP8)
L*	23.1 ± 0.02^{c}	24.4 ± 0.01^{a}	23.5 ± 0.01^{b}
a*	4.7 ± 0.01^{b}	3.9 ± 0.01^{c}	5.0 ± 0.04^{a}
b*	4.2 ± 0.01^{b}	3.2 ± 0.02^{c}	5.0 ± 0.03^{a}
ΔE		1.79 ± 0.02^{a}	0.92 ± 0.03^{b}

Means in the same row column followed by the same letter are not significantly different at $p < 0.05$

Table 3 Aroma and flavor profiles of roasted coffees from the fermentation with protease at different conditions (evaluated by Q-Graders).

Run	Fragrance/Aroma	Fragrance score	Flavor
1	Fruity, dry fruit, spice, nutty	7.25	Mild sweet, sour aftertaste
2	Fruity, nutty, spice	7.25	Bitter, acidity
3	Nutty, caramel, ripe fruit, spice	7.25	Nutty, spice, dry
4	Nutty, caramel, almond, ripe fruit	7.50	Low body, short aftertaste, bland taste
5	Fruity, spice, mango, dry fruit	7.50	Ferment, strong sweet, sour
6	Nutty, fruity, low intent	7.25	Strong acidity, astringent, medium body
7	Nutty, fruity	7.25	Spice, pepper, sweet, astringent
8	Fruity, spice, nutty, ripe fruit, coconut	7.50	Tamarind, sour, dry
9	Fruity, spice, nutty, ripe fruit taste	7.20	Bland taste
10	Fruity, ferment, papaya	7.25	Ferment, plastic-like
11	Fruity, spice, ferment	7.25	Sour, earthy, short after taste
12	Fruity, spice	7.25	Sour, bitter, astringent after taste
13	Fruity, spice	7.00	Sweet, sour, fresh fruity, plastic-like
14	Nutty, ferment, fruit	7.25	Fruity, strong nutty
15	Nutty, fruity	7.25	Ferment, fruity, ripe fruit
16	Ferment, fruity	7.25	Plastic-like

3.2 Aroma and flavor profiles of roasted coffees

Table 3 illustrates the aroma and flavor profiles perceived by two Q-Graders. The green beans fermented with 0.36% protease at pH 6.2, 41 h (R-FP4), 0.84% protease at pH 6.2, 41 h (R-FP5), and 1.00% protease at pH 5.0, 30 h (R-FP8) presented the highest fragrance scores (7.5 points). The fragrance/aroma profiles of R-FP4 were characterized as nutty, caramel, almond, and ripe fruit. R-FP5 was distinguished as fruity, spice, mango, and dry fruit, and the aroma profiles of R-FP8 were characterized as fruity, spice, nutty, ripe fruit, and coconut. R-Control showed a lower aroma score (7.2 points) and presented as fruity, fermented, and plastic-like. The taste attributes, including sweetness and acidity, of R-FP5 and R-FP8 surpassed the other roasted coffees, but R-FP4 showed a short aftertaste, low body, and bland taste.

The beans fermented with protease at all conditions exhibited more unique flavor profiles than the control. The aroma or fragrance, flavor profiles, and scores of the roasted beans fermented with 0.84% protease at pH 6.2, 41 h (R-FP5) and 1.00% protease at pH 5.0, 30 h (R-FP8) were higher than the other conditions. Consequently, these two protease fermented coffee beans were selected for the evaluation of their color, appearance, volatile and sensory profiles. However, the coffee beans obtained from several conditions in this study also obtained evaluation scores over 7.0, revealing unique coffee flavors and indicating their potential for further study and development.

The final score and sensory characteristics are some of the crucial purchasing decisions by consumers for specialty coffees. The effects of protease fermentations on the flavor profiles of coffee brews prepared from roasted coffees are described in Figure 2. The fermentation with protease could differentiate and enhance the sensory descriptions of the roasted coffees. The fermented coffee beans, R-FP5 and R-FP8, received the highest sensory scores (79.5 points). The taste

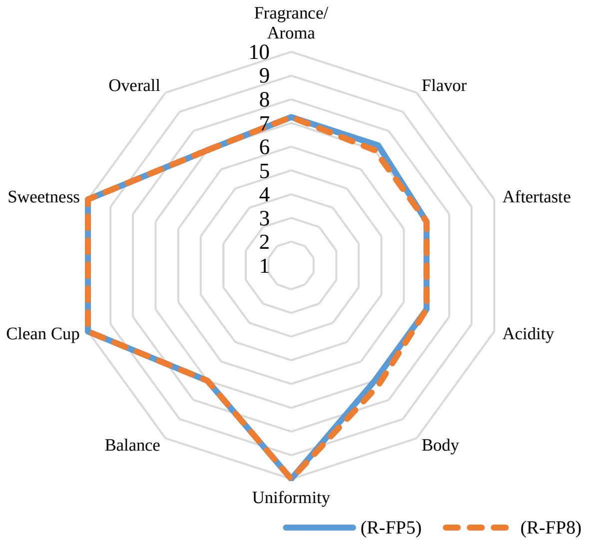

Fig. 2. Main sensory characteristics of the roasted coffee beans from protease fermentation, described by the Q-Graders.

attributes of R-FP5 and R-FP8 were strong sweet tastes. The fragrance score of the control was comparatively low thus it was not further evaluated as specialty coffee.

3.3 Volatile profiles of roasted coffees after fermentation with protease

Volatile profiles of three roasted coffees, including R-Control, R-FP5 and R-FP8 are presented in Table. 4, 5 and 6. Volatile analysis of R-Control roasted coffee identified 3 aldehyde compounds, 5 alcohol compounds, 6 acid compounds, 2 ketone compounds, 2 ester compounds, 2 ether compounds, 1 furans compounds, 1 pyrrole compounds, 6 pyridine compounds, 5 pyrazine compounds, 3 amine compounds, 3 phenol compounds, 2 terpene, 1 lactone, 2 alkane, 3 fatty acid, 4 benzene and 12 other compounds (Table 4).

The main route of coffee aroma formation is the Maillard reaction and Strecker degradation, which are responsible for the formation of various compounds, such as pyrazines, pyrroles, thiols, furans, furanones, pyridines, and thio-phenes [22, 23]. In ground and roasted coffee, more than 800 chemical compounds have been identified. Aldehydes, alcohols, acids, pyrazines, pyridines, pyrroles, thio-phenes, furans, sulfur compounds, and phenolic compounds are the most commonly detected classes of compounds in the headspace, resulting in an inherent aroma complexity [24].

In the R-Control, the majority of the volatile compounds were acids, pyridines, alcohol, and pyrazine. Acetic acid is one of the compounds in a mature coffee cherry as well as formic, lactic, glycolic, and acetic acid, that are degraded during the fermentation by microorganisms [25, 26]. In moderate concentrations, acetic acid also contributes to fruity, wine, and fermented aromas, while lactic acid can give buttery notes [27]. Pyrazines are considered as the most significant coffee flavor, with a very low sensory threshold. Generally, pyrazines give nutty, earthy, roasty, and green aromas [28].

The compound 2-furanmethanol contributes to the perception of roasted aromas. However, at high concentrations, it could generate an undesirable burnt and bitter smell [29]. Maillard reaction and thermal processes, such as thermal oxidation of lipids, thermal degradation of sugar polymers and D-glucose, thiamine, and, nucleotide degradation are involved in the synthesis of 2-furanmethanol [30]. Phenol derivatives are important contributors to the aroma of roasted coffee, which are formed by the thermal degradation of chlorogenic acids during roasting [31]. Phenol contributes to the perception of a spicy, phenolic aroma and plastic-like [32].

Volatile analysis of R-FP5 identified 3 aldehyde compounds, 4 alcohol compounds, 3 acid compounds, 2 ketone compounds, 2 ester compounds, 2 ether compounds, 1 furans compound, 1 pyrrole compounds, 5 pyridine compounds, 7 pyrazine compounds, 3 amine compounds, 3 phenol compounds, 1 lactone, 1 alkane, 6 benzene, and 12 other compounds (Table 5).

Table 4 Volatile compounds of the roasted coffee beans of R-Control.

No	Volatile compounds	Aroma perception	RT (min)	Peak Area	*
Aldehyde compounds (3)					
1	2-Ethyl butanal	a	1.594	16,238,253	
2	3-Furaldehyde	a	14.975	86,119,404	
3	Retinal (Retinaldehyde)	a	19.314	5,946,197	
Alcohol compounds (5)					
4	1,2-Ethanediol	a	15.112	31,315,907	
5	1,2:5,6-Dianhydrogalactitol	a	17.054	11,598,604	
6	2,3-Butanediol	Fruity, creamy, buttery	17.912	39,114,265	[33]
7	2-Furanmethanol	Caramellic, burnt, smoky	19.965	96,677,632	[34]
8	Phenylethyl alcohol	Flora, rose-like, honey	25.364	8,888,725	[35]
Acid compounds (6)					
9	Propanoic acid	Pungent, acidic, cheesy, vinegar	10.830	14,040,598	[33]
10	Acetic acid	Pungent,vinegar	14.720	80,108,996	[33]
11	4-Methyloctanoic acid	a	16.910	8,465,301	
12	Butanoic acid	Sour	20.196	24,357,150	[31]
13	Phosphonic acid	a	27.678	72,750,195	
14	L-Lactic acid	a	30.830	3,460,363	
Ketone compounds (2)					
15	2-Propanone	a	10.186	21,392,873	
16	2-Cyclopenten-1-one	a	23.654	27,759,304	
Ester compounds (2)					
17	Ethyl 2-methyl nicotinate	a	19.214	15,061,527	
18	Methyl salicylate	Wintergreen, mint-like	22.339	6,718,690	[31]
Ether compounds (2)					
19	Allyl ethyl ether	a	2.030	21,297,008	
20	Diethylene glycol hexyl ether	a	28.486	6,807,372	
Furans compounds (1)					
21	Methyl 2-furoate	a	27.249	7,279,559	
Pyrrole compounds (1)					
22	1H-pyrrole-2-carboxaldehyde	Musty, beefy, coffee	18.878	18,504	[33]
Pyridine compounds (6)					
23	Pyridine	Bitter, astringent, roasted, burnt	6.537	17,356,868	[24]

No	Volatile compounds	Aroma perception	RT (min)	Peak Area	*
24	Boron,trihydro(pyridine)-, (T-4)-	a	6.949	390,716	
25	4-Methyl-pyrimidine	a	9.076	38,108,704	
26	5-Methyl-7-amino-s-triazolo *(1,5*-a) pyrimidine	a	16.061	7,499,936	
27	4,6-Dimethyl pyrimidine	a	18.959	29,557,503	
28	2(1H)-Pyridinone	a	34.892	5,395,464	
Pyrazine compounds (5)					
29	2,5-Dimethyl pyrazine	Nutty, roasty, earthy, cocoa-like	10.729	17,016,351	[31]
30	2,3-Dimethyl pyrazine	Nutty, coffee-like, caramellic, cocoa-like	11.430	10,372,485	[8]
31	2-Ethyl-5-methylpyrazine	Coffee-like	12.563	17,762,448	[33]
32	2-Ethyl-3-methyl pyrazine	Nutty, peanut	13.097	29,003,648	[33]
33	2,5-Dimethyl-3-propyl-pyrazine	a	19.666	21,995,760	
Amine compounds (3)					
34	3-Methoxy-benzenamine	a	18.516	6,417,935	
35	4,5-Dimethyl-ortho-phenylenediamine	a	20.280	1,788,355	
36	1,3-Benzenediamine	a	22.805	7,438,772	
Phenol compounds (3)					
37	2-Methyl-6-(2-propenyl)-phenol	a	22.762	5,905,412	
38	Phenol	Rubbery, plastic-like	26.622	24,444,677	[32]
39	p-Tert-butyl-phenol	a	30.975	43,548,815	
Terpene (2)					
40	Linalool	Citrus, orange, lemon	17.188	6,134,625	[32]
41	p-cymene	a	18.177	6,448,714	
Lactone (1)					
42	2(3H)-Furanone, γ-crotonolactone (GCL)	a	27.795	25,449,681	
Alkane (2)					
43	2,4-Dimethyl-hexane	a	1.812	5,646,988	
44	Hexadecane	a	10.373	1,151,390	
Fatty acid (3)					
45	Ricinoleic acid	a	22.899	448,254	
46	Undecylenic acid	a	22.953	453,684	
47	11-Bromoun decanoic acid	a	23.211	2,817,733	
Benzene (4)					
48	(Fluoromethyl)-benzene	a	17.745	73,163,373	
49	1,2,4,5-Tetramethyl-Benzene	a	20.840	14,530,704	
50	1-Propenyl-benzene	a	25.743	9,931,380	
51	1,3,5-Benzenetriol	a	36.290	5,448,927	
Others (12)					
52	Carbon dioxide	a	1.218	8,457,285	
53	4a,7-Methano-4aH-naphth [1,8ab]oxirene	a	21.853	1,710,981	
54	2-Hexene	a	21.950	12,998,871	
55	Inosine	a	23.053	29,356,102	
56	2(1H)-quinoxalinone		26.913	19,655,890	
57	Eseridine	a	28.067	7,994,539	
58	1,4-Methanoazulen-7(1H)one		28.144	14,722,173	
59	1(2H)-Naphthalenone	a	28.620	8,685,939	
60	2,4-Dimethoxy-toluene	a	31.397	7,057,504	
61	p-Pentylace-tophenone	a	31.806	23,429,016	
62	Trehalose	a	32.225	13,099,649	
63	Caffeine	bitter	47.592	11,839,974	[31]

LRI and MS were used as the identification methods.
a means no odor detected or no report on their odor description/odor profile in coffee.

Table 5 Volatile compounds of R-FP5 (the roasted beans fermented with 0.84% protease at pH 6.2, 41 h).

No	Volatile compounds	Aroma perception	RT (min)	Peak Area	*
Aldehyde compounds (3)					
1	2-Ethyl-butanal	a	1.591	19,396,167	
2	3-Furaldehyde	a	14.995	70,468,180	
3	Retinal (Retinaldehyde)	a	19.321	5,134,275	
Alcohol compounds (4)					
4	1,2-Ethanediol	a	15.136	32,934,054	
5	2,3-Butanediol	Fruity, creamy, buttery	17.923	44,538,535	[33]
6	2-Furanmethanol	Caramellic, burnt, smoky	19.978	132,757,313	[34]
7	Phenylethyl Alcohol	Flora, rose-like, honey	25.371	8,012,223	[35]
Acid compound (3)					
8	Propanoic acid	Pungent, acidic, cheesy, vinegar	10.873	16,293,303	[33]
9	Acetic acid	Pungent,vinegar	14.730	91,254,371	[33]
10	Butanoic acid	Sour	20.206	23,164,583	[31]
Ketone compounds (2)					
11	2-Propanone	a	10.233	28,028,176	
12	2-Cyclopenten-1-one	a	23.661	26,400,375	
Ester compounds (2)					
13	Ethyl 2-methyl nicotinate	a	19.234	14,128,081	
14	Methyl salicylate	Wintergreen, mint-like	22.343	7,917,365	[31]
Ether compounds (2)					
15	Allyl ethyl ether	a	2.043	22,360,496	
16	Diethylene glycol hexyl ether	a	28.496	9,371,506	
Furans compounds (1)					
17	Methyl 2-furoate	a	27.259	7,086,827	
Pyrrole compounds (1)					
18	1H-Pyrrole-*2*-carboxaldehyde	Musty, beefy, coffee	18.821	33,882,941	[33]
Pyridine compounds (5)					
19	Pyridine	Bitter, astringent, roasted, burnt	6.614	7,031,535	[24]
20	4-Methyl pyrimidine	a	9.126	43,236,772	
21	5-Methyl-7-amino-s-triazolo (1,5-a)pyrimidine	a	16.088	7,452,592	
22	4,6-Dimethyl pyrimidine	a	18.976	33,573,830	
23	2(1H)-Pyridinone	a	34.905	9,275,490	
Pyrazine compounds (7)					
24	2,5-Dimethyl pyrazine	Nutty, roasty, earthy, cocoa-like	10.773	20,751,225	[31]
25	2,3-Dimethyl pyrazine	Nutty, coffee-like, caramellic, cocoa-like	11.474	11,816,828	[8]
26	2-Ethyl-5-methyl pyrazine	Coffee-like	12.604	22,676,824	[33]
27	2-Ethyl-3-methyl pyrazine	Nutty, peanut	13.134	38,331,735	[33]
28	2,3-Diethylpyrazine	Nutty, woody, roasty, cocoa-like	14.267	47,700,699	[32]
29	2,5-Dimethyl-3-propyl-pyrazine	a	19.683	23,020,096	
30	3-Methylpyridazine	a	27.688	55,863,959	
Amine compounds (3)					
31	4,5-Dimethyl-ortho-phenylenediamine	a	14.683	5,679,992	
32	1,3-Benzenediamine	a	16.407	5,342,270	
33	3,4-Dimethyl-o-phenylenediamine	a	20.287	15,468,240	
Phenol compounds (3)					
34	2,4,5-Trimethyl-phenol	a	20.619	8,397,781	
35	Phenol	Rubbery, plastic-like	26.632	29,402,863	[32]
36	p-Tert-butyl-phenol	a	30.981	63,510,053	

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No	Volatile compounds	Aroma perception	RT (min)	Peak Area	*
Lactone (1)					
37	2(3H)-Furanone, γ-crotonolactone (GCL)	a	27.802	19,163,437	
Alkane (1)					
38	Hexadecane	a	10.417	1,485,814	
Benzene (6)					
39	(Fluoromethyl) benzene	a	17.765	65,129,432	
40	4-Ethyl-1,2-dimethyl-benzene	a	18.194	5,082,207	
41	1,2,4,5-Tetramethyl-Benzene	a	18.677	12,546,131	
42	1-Propenyl-benzene	a	25.753	11,569,102	
43	1,3,5-Benzenetriol	a	26.437	62,256,012	
44	2,4-Diisocyanato-1-methyl benzene	a	28.919	5,018,290	
Others (12)					
45	Adenosine		13.999	6,552,383	
46	Levodopa	a	18.536	5,607,350	
47	4a,7-Methano-4aH-naphth [1,8a-b]oxirene		21.876	5,937,591	
48	Azelaoyl chloride	a	21.957	8,660,443	
49	Inosine	a	23.064	22,860,022	
50	2(1H)-Quinoxalinone	a	26.920	22,036,079	
51	Eseridine	a	28.074	10,478,976	
52	1,4-Methanoazulen-7(1H)-one	a	28.151	20,965,813	
53	1(2H)-Naphtha lenone	a	28.624	9,809,811	
54	2,4 Dimethoxy toluene	a	31.407	13,257,086	
55	Trehalose	a	32.236	15,321,395	
56	Caffeine	bitter	47.602	18,485,782	[31]

LRI and MS were used as the identification methods.
a means no odor detected or no report on their odor description/odor profile in coffee.

Volatile analysis of R-FP8 roasted coffee identified 2 aldehyde compounds, 3 alcohol compounds, 7 acid compounds, 2 ketone compounds, 2 ester compounds, 1 ether compounds, 2 furans compounds, 1 pyrrole compounds, 4 pyridine compounds, 7 pyrazine compounds, 1 amine compounds, 2 phenol compounds, 3 lactone, 1 alkane, 1 fitty acid, 4 benzene, and 12 other compounds (Table 6).

Isopropenyl acetate indicates ethereal acetic, fruity, sweet, and berry grape skin attributes, while 2-acetyl-5-methylfuran indicates nutty notes. Volatile furan brings sensorial characteristics of malty and sweet roasted aromas [28]. Furans were formed through thermal degradation of carbohydrates, ascorbic acid, or unsaturated fatty acids during roasting [36 , 37]. Delta-dodecalactone which exhibits coconut-fruity scent, buttery flavor was detected in R-FP8 but not in R-Control and R-FP5. Detecting different volatile compounds in protease-fermented coffee beans may indicate that the enzymatic digestion produced different precursors or intermediates during the reaction.

During coffee processing, yeasts and mesophilic bacteria hydrolyze pulp carbohydrates, resulting in the production of non-volatile and volatile compounds and pH changes that contribute to coffee quality [38 , 39]. Cheese aroma modulation with enzymes to improve insufficient taste and imbalanced aroma can release crucial flavor precursors such as free amino acid (FAA)

Table 6 Volatile compounds of R-FP8 (the roasted beans fermented with 1.00% protease at pH 5.0, 30 h).

No	Volatile compounds	Aroma perception	RT (min)	Peak Area	*
Aldehyde compounds (2)					
1	3-Furaldehyde	a	14.995	108,685,922	
2	Retinal (Retinaldehyde)	a	*26.330*	807,968	
Alcohol compounds (3)					
3	1,2-Ethanediol	a	15.136	40,747,987	
4	2,3-Butanediol	Fruity, creamy, buttery	17.919	10,090,834	[33]
5	2-Furanmethanol	Caramellic, burnt, smoky	19.975	159,630,577	[34]
Acid compound (7)					
6	Acetic acid	Pungent, vinegar	14.700	121,697,507	[33]
7	N,N-Bis(2-hydroxyethyl)-2-aminoethanesulfonic acid	a	16.762	6,877,736	
8	Propanoic acid	Pungent, acidic, cheesy, vinegar	17.057	5,335,447	[33]
9	Butanoic acid	Sour	20.203	22,790,537	[31]
10	Clofibric Acid	a	24.224	8,110,130	
11	Octadecanoic acid	a	32.044	3,986,782	
12	o-Acetoxycinnamic acid	a	*26.917*	16,118,456	
Ketone compounds (2)					
13	2-Propanone	a	10.236	58,859,047	
14	2-Cyclopenten-1-one	a	23.650	27,153,629	
Ester compounds (2)					
15	Isopropenyl acetate	Ethereal acetic, fruity, sweet, berry, grape skin	1.597	24,415,859	
16	Methyl salicylate	Wintergreen, mint-like	22.366	6,799,329	[31]
Ether compounds (1)					
17	Allyl ethyl ether	a	2.047	32,161,323	
Furans compounds (2)					
18	2-Acetyl-5-methylfuran	nutty notes	24.442	8,195,054	
19	Methyl 2-furoate	a	27.256	9,567,021	
Pyrrole compounds (1)					
20	1H-Pyrrole-2-carboxaldehyde	Musty, beefy, coffee	18.821	17,391,443	[33]
Pyridine compounds (4)					
21	Pyridine	Bitter, astringent, roasted, burnt	6.655	10,059,564	[24]
22	4-Methyl pyrimidine	a	9.129	52,843,161	
23	4,6-Dimethyl-pyrimidine	a	18.979	31,174,372	
24	2(1H)-Pyridinone	a	34.902	8,383,852	
Pyrazine compounds (7)					
25	2,5-Dimethyl-pyrazine	Nutty, roasty, earthy, cocoa-like	10.773	38,977,998	[31]
26	2,3-Dimethyl pyrazine	Nutty, coffee-like, caramellic, cocoa-like	11.470	13,609,115	[8]
27	2-Ethyl-5-methyl pyrazine	Nutty, roasted, somewhat "grassy"	12.755	31,164,580	[33]
28	2-Ethyl-3-methyl pyrazine	Coffee-like	13.124	37,182,626	[33]
29	2,3-Diethylpyrazine	Nutty, woody, roasty, cocoa-like	14.267	39,735,911	[32]
30	2,5-Dimethyl-3 propylpyrazine	a	19.676	1,899,277	
31	2(1H)-Pyridinone	a	34.902	8,383,852	
Amine compounds (1)					
32	*4,5*-Dimethyl-ortho-phenylenediamine	a	20.498	28,187,515	

No	Volatile compounds	Aroma perception	RT (min)	Peak Area	*
Phenol compounds (2)					
33	Phenol	Rubbery, plastic-like	26.632	27,388,621	[32]
34	p-Tert-butyl-phenol	a	30.985	70,346,976	
Lactone (3)					
35	Oxacyclododecan-2-one	a	25.019	7,080,985	
36	Delta-dodecalactone	Coconut-fruity scent, buttery flavor	27.799	12,162,293	
37	2(3H)-Furanone, γ-crotonolactone (GCL)	a	27.899	25,868,815	
Alkane (1)					
38	Hexadecane	a	10.427	722,194	
Fatty acid (1)					
39	Undecylenic acid	a	23.070	9,891,078	
Benzene (4)					
40	1,2,4,5-Tetramethyl-benzene	a	18.680	8,903,383	
41	1,2,3,5-Tetramethyl-benzene	a	20.850	7,444,840	
42	1-Propenyl-benzene	a	25.753	13,311,802	
43	1,3,5-Benzenetriol	a	26.434	37,661,742	
Others (12)					
44	Boron, trihydro(pyridine)-, (T-4)-	a	7.017	463,142	
45	Oxirane	a	12.382	5,183,248	
46	1H-Pyrazole	a	17.758	102,769,430	
47	Inosine	a	20.287	17,607,607	
48	Azelaoyl chloride	a	21.954	13,240,916	
49	2H-2,4a-Methanonaphthalen-8(5H)-one	a	26.823	1,799,690	
50	4a,7-Methano-4aH-naphth[1,8a-b]oxirene	a	27.028	1,971,219	
51	1(2H)-Naphthalenone	a	28.627	8,151,688	
52	2,4-Dimethoxy toluene	a	31.404	9,543,723	
53	Trehalose	a	32.229	30,110,903	
54	4H-Pyran-4-one	a	32.625	10,051,657	
55	Caffeine	Bitter	47.595	18,441,070	[31]

LRI and MS were used as the identification methods.
a means no odor detected or no report on their odor description/odor profile in coffee.

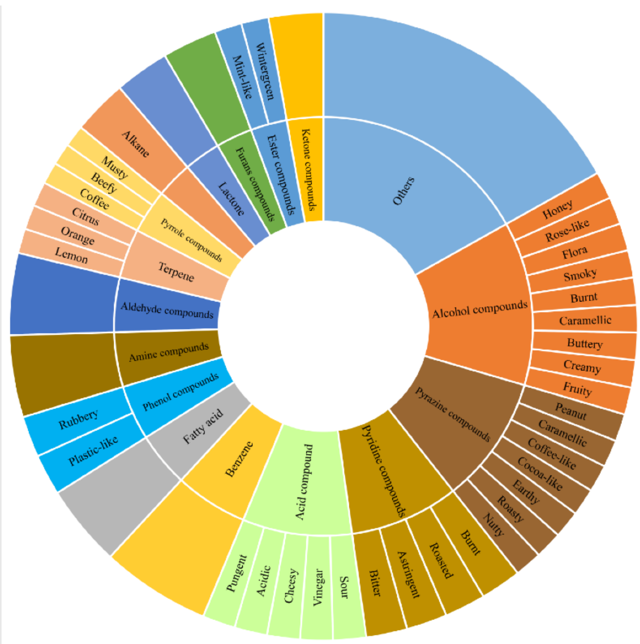

Fig. 3. Flavor wheel of coffee brews prepared form R-Control, the roasted beans fermented without protease.

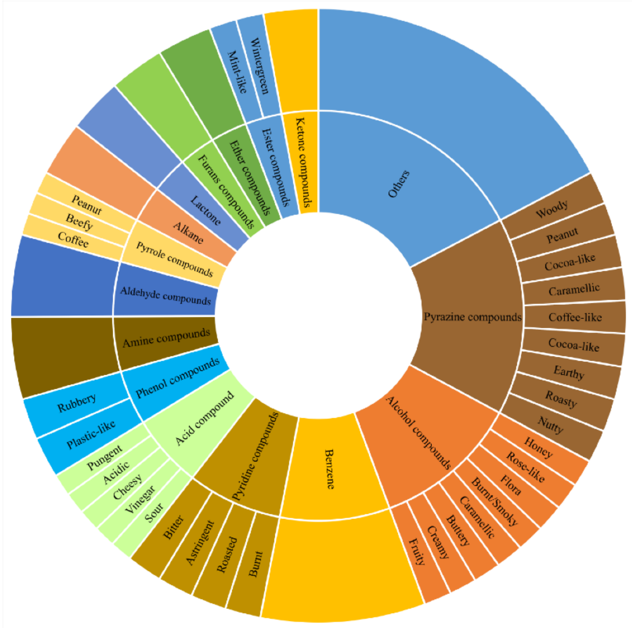

Fig. 4. Flavor wheel of coffee brews prepared from R-FP5, the roasted beans fermented with 0.84% protease at pH 6.2, 41 h.

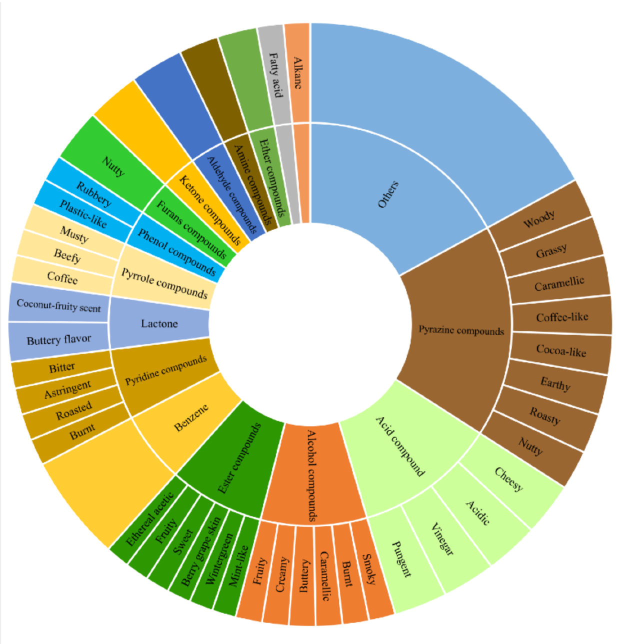

Fig. 5. Flavor wheel of coffee brews prepared from R-FP8, the roasted beans fermented with 1.00% protease at pH 5.0, 30 h.

and free fatty acid (FFA) flavor precursors better than the control without enzyme [40]. Coffee aroma is one of the most complex aroma profiles, which is formed by several volatile compounds [41]. In green coffee beans, proteins, amino acids, sugars, and phenolic compounds constitute important precursors of coffee aroma. Caramelization products are created during roasting when simple sugars and polysaccharides are thermally degraded [42]. Cellulase and pectinase hydrolyze pectin and cellulose, liberate carotenoids from the cell wall, and increase extraction efficiency. The high enzyme loadings resulted in faster rates of hydrolysis

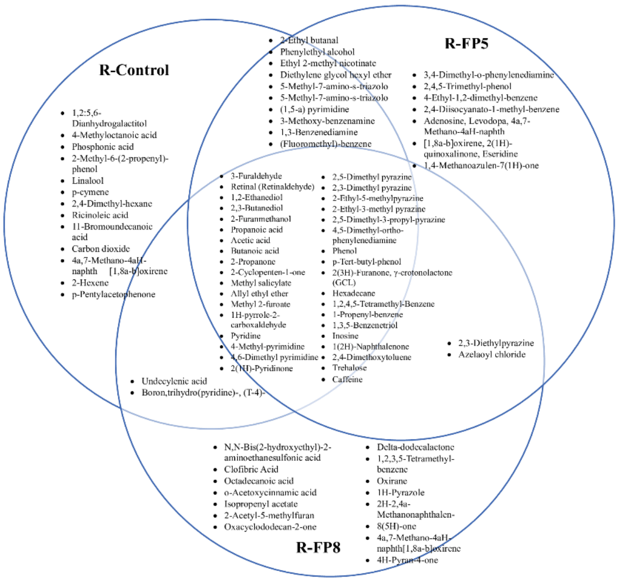

Fig. 6. Flavor profiles of roasted coffee beans after fermentation with protease (R-FP5 and R-FP8) compared to the R-Control.

and increased sugar yields [4]. The difference between the identified volatile compounds and flavor profiles were detected in the treatments with and without enzymatic fermentation in our study (Fig. 3, 4, 5 and 6).

4 Conclusion

This study demonstrated that enzymatic fermentation can modulate Arabica coffee beans' volatile profiles and flavor profiles. Different enzyme loading, fermentation time, and pH treatments of the green coffee beans resulted in roasted coffees with different colors and appearances. The roasted coffees fermented with protease (0.84% protease, pH 6.2 at 41 h, R-FP5), and 1.00% protease, pH 5.0 at 30 h, R-FP8) presented the best aroma scores (7.5 points), and were distinguished by fruity, spice, mango, dry fruit, nutty, ripe fruit, and coconut attributes while the control showed low aroma scores (7.2 points). Specific volatile compounds were detected in protease fermentation and may contribute to the beverage quality. Isopropenyl acetate, 2-acetyl-5-methylfuran, and delta-dodecalactone were detected in the coffee beans from protease fermentation. Coffee fermentation with protease was classified as premium (70.0–79.75), and the beverage was characterized by fruity, spice, mango, dry fruit, ripe fruit, nutty, and tamarind notes. The present work shows that enzyme fermentation of Arabica coffee beans can potentially modulate and improve the flavor and aroma of specialty coffee.

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