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Cellulase and protease assisted sugar germination on quality improvement of Robusta coffee in comparison to Arabica coffee

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Abstract

Arabica and Robusta coffee are the two main coffee tree species that are cultivated around the world. Premium Arabica coffee is rich in flavour and aroma. Meanwhile, the cheaper Robusta coffee has a bitter taste, a muddy odour, and a weaker aroma and flavour. This study aimed to improve the quality of Robusta coffee through cellulase and protease-assisted germination. Germination results in producing flavour precursors and can be accelerated by degrading the endosperm of coffee beans through the activity of cellulase and protease. Quality parameters were based on morphological study (scanning electron microscope [SEM]), flavour profile analysis (gas chromatography-mass spectrometry [GC -MS]) and sensory evaluation. Results indicated that sugar-and enzyme-added germinated Robusta (R5) has similar morphological characteristics and pH values to Arabica. Flavour profile analysis indicated that 2-Furanmethanol of treated Robusta, which contributes to bitter taste, was decreased compared to untreated Robusta (control). In sensory evaluation, the final score of sensory properties of germinated Robusta (R2), enzyme-added Robusta (R3), and sugar-and enzyme-added germinated (R5) was improved, which indicated that there was no significant difference ($p>0.05$) compared to Arabica. These results showed that the proposed additional processing method of green coffee beans in this study was able to improve the quality of cheaper Robusta coffee which has some similar properties to premium Arabica.

1. Introduction

Coffee originated from the *Coffea* genus of plants. Among the many species of the *Coffea* genus, *Coffea arabica* (also known as Arabica) and *Coffea canephora* (also known as Robusta) are the two primary cultivated coffee tree species (Bastian *et al*., 2021). Arabica coffee is smooth, mild, rich in flavour, and has a strong aroma, while Robusta coffee shows a smaller flavour, an almost muddy odour, a bitter taste, and a weaker aroma. Despite its promising quality properties, Arabica coffee has several disadvantages. Arabica coffee must be cultivated in a high elevation area (900-1700 m) (Abubakar *et al*., 2020; Konieczka *et al*., 2020). Arabica coffee is also fragile, vulnerable to attack from pests, and easily damaged by poor handling. Current research findings also found that Arabica coffee is susceptible to changes in altitude, carbon dioxide, light exposure, nutrient management, temperature, climate change, and water stress (Ahmed *et al*., 2021), which leads to a higher susceptibility of Arabica to the highly destructive coffee leaf rust fungus (Castillo *et al*., 2020; Febrianto and Zhu, 2023).

The quality of coffee used for beverages depends upon the chemical composition of the roasted beans, which is influenced by the chemical composition of the green beans and the conditions of postharvest processing (Restuccia *et al*., 2019). Several researchers have attempted pre-treatment of coffee beans to increase quality in low-altitude Arabica coffee beans, such as fermentation (Bressani *et al*., 2018; da Mota *et al*., 2020). However, this method requires a large-scale steam-generating apparatus and the loss of a significant quantity of low molecular weight water-soluble flavour precursors such as saccharides and amino acid materials (Park, 2016). The flavour and taste of coffee can be improved by germinating green coffee beans. During the germination process, polymer materials such as proteins and polysaccharides undergo decomposition. Coffee beans create amino acids, which act as taste precursors as they continue their metabolic process (Bastian *et al*., 2021). However, the endosperm of coffee beans can inhibit or prevent germination by acting as a physical

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barrier to the emergence of radicles. Certain enzymes degrade the endosperm for germination to occur, thereby increasing the rate and proportion of seed germination (Mostafa *et al*., 2021). Therefore, this study aimed to improve the quality of Robusta green coffee beans through enzymatic-assisted germination.

2. Materials and methods

2.1 Sample preparation

Robusta and Arabica (Mandheling) green coffee beans were obtained from Coffeeland Sdn Bhd. Enzymes used in this study included cellulase from *Aspergillus niger* and protease from *Aspergillus oryzae*. Robusta green coffee beans were subjected to five different treatments, according to the method by Park (2016), as detailed below.

2.1.1 Control (R1)

Green coffee beans (200 g) were washed, placed on an aluminium tray, and dried at 55°C until the moisture level reached the range of 10%-12%.

2.1.2 Germination (R2)

Green coffee beans (200 g) were washed and placed into a zipper bag. A total of 100 mL of distilled water was poured into the zipper bag. In a sealed state, the zipper bag was subjected to a temperature of 55°C in an incubator for 24 hrs and pre-treated by occasionally opening the zipper bag to provide the required oxygen for germination.

2.1.3 Enzyme-addition (R3)

Green coffee beans (200 g) were washed and placed into a zipper bag. Then, 100 mL of distilled water containing cellulase and protease in an amount of each 0.1% relative to the total weight of the green coffee beans were poured into the zipper bag and mixed well.

2.1.4 Enzyme-added germination (R4)

Green coffee beans (200 g) were washed and placed into a zipper bag. Then, 100 mL of distilled water containing cellulase and protease in an amount of each 0.1% relative to the total weight of the green coffee beans were poured into the zipper bag and mixed well. In a sealed state, the zipper bag was subjected to a temperature of 55°C in an incubator for 24 hrs and pre-treated by occasionally opening the zipper bag to provide the required oxygen for germination.

2.1.5 Sugar-and enzyme-added germination (R5)

Green coffee beans (200 g) were washed and placed into a zipper bag. Then, 100 mL of distilled water containing cellulase and protease in an amount of each 0.1% relative to the total weight of the green coffee beans and flavour precursors (sucrose, glucose, and fructose in amounts of 6 g, 2 g, and 2 g, respectively) were poured into the zipper bag and mixed well. In a sealed state, the zipper bag was subjected to a temperature of 55°C in an incubator for 24 hrs and pre-treated by occasionally opening the zipper bag to provide the required oxygen for germination.

All treated Robusta coffee beans were dried using the same technique as R1 (control). Robusta and Arabica green coffee beans were roasted at 200°C for 10 mins. After roasting, all coffee beans were ground and stored until used for further analysis.

2.2 Morphological characteristic analysis

Morphological characteristic analysis was carried out using a scanning electron microscope (Hitachi TM3030Plus Tabletop SEM) at 1000× magnification. The images were observed using Secondary Electrons (SE) mode with a beam acceleration of 15 kV.

2.3 Flavour profile analysis

Flavour profile analysis was conducted using Solid Phase Microextraction (SPME) and Gas Chromatography-Mass Spectrometry (GC-MS) as the methods reported by Kim *et al*. (2019). Ground coffee (2 g) was added into SPME vials and closed with a lid. The SPME fibre (PDMS/DVB) was exposed to the headspace of the sample vial at 40°C for 20 mins while stirring in a temperature-controlled magnetic stirrer. The fibre was placed in the GC injector and heated to 250°C for 1 min. Chromatographic separation was performed using gas chromatography (Agilent 7890A). The GC injector was operated in splitless mode with a constant flow of 1.1 mL min^{-1}. The GC oven program was set as follows: 60°C for 3 mins; 180°C at a rate of 12°C min^{-1} for 4 mins; 40°C min^{-1} until the temperature reached 240°C for 4 mins. The separated compounds were analysed using mass spectrometry (Agilent 5975C), and compound identification was done by comparison of the mass spectra against a database (NIST).

2.4 pH value analysis

The pH value of coffee was measured using a digital pH meter (Hanna HI 2211). Ground coffee (8.25 g) was brewed in 150 mL of distilled water (93°C). After 20 mins, the pH value of the coffee was analyzed three times (Basheer *et al*., 2022).

2.5 Sensory analysis

Sensory analysis of coffee was carried out using the cupping test by 3 expert panellists from Coffeeland Sdn

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Bhd (Ribeiro *et al*., 2017). Parameters evaluated were fragrance/aroma, flavour, aftertaste, acidity, body, uniformity, balance, clean cup, sweetness, and overall quality which were scored on a scale of 6-10 points with 0.25 increments. The panellists were further asked to describe the characteristic flavour of each sample.

3. Results and discussion

3.1 Morphological characteristics

In the processing of coffee, roasting is the key unit operation that significantly impacts the sensory, structural, chemical, and physical transformations of green coffee beans. During the roasting process, the beans develop honeycomb-shaped porous structures, which serve as storage for flavour components within the various pores (Getaneh *et al*., 2020). Park (2016) reported that the green coffee beans that underwent low temperature and enzyme-added germination had a thinner wall, uniform pores, and an increased number of pores when roasted compared to the control group. The study showed that pre-treatment of green coffee beans affected the morphological characteristics of the pores of roasted coffee. Based on Figure 1, sugar-and enzyme-added germinated Robusta (R5) has morphological characteristics similar to Arabica in shape and wall of pores.

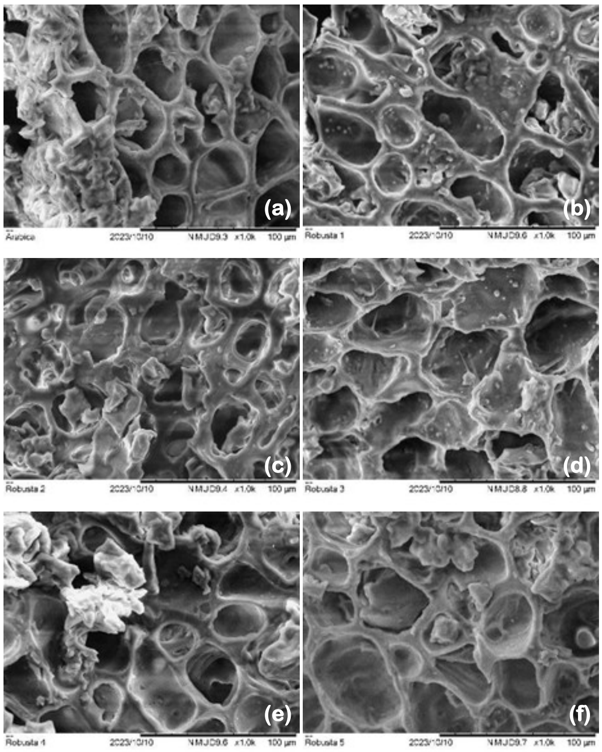

Figure 1. Scanning electron microscope of roasted coffee: (a) Arabica; (b) control (R1); (c) germinated robusta (R2); (d) enzyme-added robusta (R3); (e) enzyme-added germinated robusta (R4); (f) sugar-and enzyme-added germinated robusta (R5).

3.2 Flavour profile

Volatile compounds in roasted coffee define the sensory properties of coffee (Dippong *et al*., 2022). The volatile compounds found in all samples are listed in Table 1. Among them, the major identified volatile compounds were 2-Furanmethanol, 5-Methyl-2-furancarboxaldehyde, and Acetate 2-Furanmethanol. Each compound contributes to the specific flavour/aroma of coffee. In general, 2-Furanmethanol was the compound with the highest amount in coffee (Thammarat *et al*., 2018). This compound was reported as a negative factor in flavour and contributes to a burnt, sweet, caramel, and bitter taste. Table 1 shows that the amount of this compound decreased in the treated Robusta (R2-R5) compared to the control group (R1). 5-Methyl-2-furancarboxaldehyde gives an almond, burnt sugar, and caramel-tasting compound, and the acetate 2-Furanmethanol compound contributes to the fruity aroma. These compounds were reported as positive factors of flavour and found in high amounts in Arabica coffee (Park, 2016; Afriliana *et al*., 2019). In general, the results indicated that the amount of these compounds increased in the treated Robusta in comparison to the control group. However, their amount was still not close

Table 1. Selected volatile compounds of coffee.

Compound	Peak Area ($\times 10^7$)					
	Arabica	Control (R1)	Germination (R2)	Enzyme-addition (R3)	Enzyme-added germination (R4)	Sugar-and Enzyme-added germination (R5)
2-Furanmethanol	37.73	40.00	34.88	33.41	33.72	25.42
2-Furancarboxaldehyde, 5-methyl-	8.94	4.75	5.48	5.21	6.13	6.52
2-Furanmethanol, acetate	25.95	5.43	4.02	7.33	7.26	5.91
Ethanone, 1-(1H-pyrrol-2-yl)-	5.64	3.31	3.09	3.65	4.66	4.01
Cyclopentasiloxane, decamethyl-	1.53	1.21	0.72	1.37	1.62	1.74
Dodecane	2.44	1.42	1.35	1.63	2.12	1.95
Phenol, 4-ethyl-2-methoxy-	3.94	2.41	2.57	2.82	0.34	2.76
Furan, 2,2'-[oxybis(methylene)]bis-	1.73	0.93	1.03	1.26	1.62	1.37
2-Methoxy-4-vinylphenol	4.63	3.89	3.84	4.24	4.40	3.75
Tetradecane	1.00	0.11	0.77	1.02	1.22	1.05
Hexadecane	0.07	0.12	0.09	0.14	0.17	0.13
Hexadecanoic acid, methyl ester	0.17	0.24	0.15	0.20	0.19	0.13

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to Arabica coffee.

3.3 pH value

The pH value is related to the acidity of coffee. At its peak, acidity contributes to the liveness, sweetness, and fresh-fruit character of coffee. Arabica coffee has a lower pH compared to Robusta coffee (Nur *et al*., 2023). Referring to Table 2, the pH value of treated Robusta (R2-R5) was lower than the control group (R1). However, only Robusta with sugar-enzyme added-germination (R5) has a non-significant pH value compared to Arabica. According to Yeager *et al*. (2023) and Rune *et al*. (2023), sucrose acts as the primary precursor to formic, acetic, glycolic, and lactic acids, which are formed during roasting. A difference in the content of sucrose in green coffee will ultimately result in different final contents of acids. Increasing the content of any acid will lower the pH of coffee.

Table 2. pH value of coffee.

Sample	pH value
Arabica	5.44±0.02^{c}
Control (R1)	5.61±0.02^{a}
Germination (R2)	5.57±0.01^{b}
Enzyme-addition (R3)	5.56±0.01^{b}
Enzyme-added germination (R4)	5.57±0.01^{b}
Sugar-and Enzyme-added germination (R5)	5.42±0.01^{c}

Values are presented as mean±SD of triplicates. Values with different superscripts are statistically significantly different at 5% significance level based on Analysis of Variance (ANOVA) and post hoc test using Duncan.

3.4 Sensory properties

Coffee provides a complex mixture of different flavours, which together produce a range of sensory properties. Sensory properties were determined using the ‘cup score’ method of the Specialty Coffee Association of America (SCAA). Table 3 shows that the Arabica sample has the highest final score, while sample R1, the Robusta sample without treatment (control), has the lowest final score. According to the analysis of variance (ANOVA) and post hoc test using Duncan ($\alpha = 0.05$), the final score of sensory properties of some treated Robusta samples (R2, R3, and R5) was improved, which indicated that there was no significant difference compared to Arabica. Furthermore, a non-significant score for the balance parameter was found in samples that involved germination (R2, R4, and R5). Balance is an important parameter that includes a synergistic combination of flavour, aftertaste, acidity, and body that complements or contrasts with each other (Chiriboga *et al*., 2022).

The sensory evaluation result of coffee was further described and showed that every sample has a different sensory profile. Arabica sample has a unit profile of fruity note, particularly green capsicum, mild in intensity, smooth, and mild acid. Meanwhile, Robusta samples have a different intensity, profile, aroma, and taste. Sample R1 has a burn profile, R2 has a dark chocolate profile, R3 has an ash profile, and R4 and R5 have a smoky profile. Generally, Robusta samples have high intensity, and the level of intensity was reduced from sample R1 to R5. Furthermore, a little sweet note, close to fruity, was found in sample R5.

4. Conclusion

Sugar-and enzyme-added germination (R5) obtained Robusta coffee with similar morphological characteristics to Arabica coffee regarding pore shape and wall. In chemical properties, the 2-Furanmethanol

Table 3. Sensory evaluation of coffee.

Compound	Score					
	Arabica	Control (R1)	Germination (R2)	Enzyme-addition (R3)	Enzyme-added germination (R4)	Sugar-and Enzyme-added germination (R5)
Fragrance/Aroma	7.83±0.29^{a}	6.67±0.29^{b}	6.92±0.38^{b}	7.25±0.43^{ab}	7.17±0.58^{ab}	6.92±0.52^{b}
Flavour	8.50±0.50^{a}	6.92±0.14^{b}	7.33±0.58^{b}	7.00±0.50^{b}	7.17±0.52^{b}	6.83±1.04^{b}
Aftertaste	8.17±0.29^{a}	7.00±0.25^{a}	7.42±0.52^{a}	7.17±1.04^{a}	7.08±0.63^{a}	7.33±0.76^{a}
Acidity	7.50±0.00^{a}	6.83±0.76^{a}	6.92±0.52^{a}	7.75±1.15^{a}	7.33±1.53^{a}	7.42±1.51^{a}
Body	8.00±0.50^{a}	7.17±0.76^{a}	6.83±0.63^{a}	7.50±0.50^{a}	7.17±0.76^{a}	7.83±1.26^{a}
Uniformity	8.33±0.58^{a}	6.42±0.52^{b}	7.33±0.58^{ab}	7.00±1.00^{b}	6.67±0.58^{b}	7.33±0.58^{ab}
Balance	8.00±0.00^{a}	6.50±0.50^{b}	7.50±0.50^{ab}	6.75±0.66^{b}	7.00±0.75^{ab}	7.50±0.50^{ab}
Clean Cup	8.33±0.58^{a}	6.33±0.58^{b}	6.67±0.58^{b}	7.00±1.00^{ab}	7.00±1.00^{ab}	7.00±1.00^{ab}
Sweetness	8.00±0.00^{a}	6.33±0.58^{b}	7.17±0.29^{ab}	7.00±1.00^{ab}	6.67±0.58^{b}	6.67±0.58^{b}
Overall	8.17±0.29^{a}	6.67±0.29^{b}	7.17±0.29^{ab}	7.42±0.63^{ab}	6.83±0.88^{b}	7.17±0.76^{ab}
Final Score	80.83±2.08^{a}	66.83±2.92^{b}	71.25±2.14^{ab}	71.83±7.15^{ab}	70.08±6.77^{b}	72.00±6.16^{ab}

Values are presented as mean±SD of triplicates. Values with different superscripts are statistically significantly different at 5% significance level based on Analysis of Variance (ANOVA) and post hoc test using Duncan.

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peak area, which contributes to negative factor flavour, and pH value of treated Robusta (R2-R5) were lower than untreated Robusta/control (R1). Sugar-and enzyme-added germination (R5) showed the best improvement, which was indicated by a non-significant difference ($p>0.05$) pH value compared to Arabica coffee. In sensory evaluation, the final score of germinated Robusta (R2), enzyme-added Robusta (R3), and sugar-and enzyme-added germinated (R5) was not significantly different ($p>0.05$) with Arabica coffee. Therefore, this additional processing method is able to improve the quality of Robusta coffee.

Conflict of interest

The authors declare no conflict of interest.

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