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Drying characteristics and phytochemicals retention of selected clones of Liberica coffee grown in Malaysia

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Abstract

Coffee is one of the world's most well-known and popular beverages, and the trees can be found in many countries, including Malaysia. Coffee beans have been used in a variety of foods and beverages since ancient times due to their unique and tasty flavour. Arabica and Robusta are the two most widely grown clones, with Liberica being the most underutilized. Liberica clones have recently gained popularity in Malaysia due to their distinct flavour profile. In view of this, the current study assessed the drying characteristics of selected clones of Liberica coffees and the influence of drying temperatures on phytochemicals retention was conducted. Coffee beans from selected clones of 211, 213, 222, 224, and MKL7 were harvested at optimum maturity and dried in a convective oven at 50°C, 60°C, and 70°C. The drying periods for all clones were observed at 15 hrs, 8 hrs, and 6.5 hrs when drying at 50°C, 60°C, and 70°C respectively. Clones 211, 213, and 224 showed high antioxidant activity when subjected to oven drying at 60°C with values ranging from 0.997 IC_{50} (mg/mL) to 1.622 IC_{50} (mg/mL) for DPPH method and 0.099 µmol/mg to 0.145 µmol/mg for FRAP method respectively. Clone 224 retained the most total phenolics content after drying at 60°C, with a final concentration of 21.94 mg GAE/g. Drying at 50°C and 60°C resulted in the highest retention of total flavonoid content for clones 211, 213, 222, and 224, with concentrations ranging from 5.11 mg RE/g to 10.77 mg/RE, whereas total flavonoid content for clone MKL 7 remained stable at 7.30 mg RE/g, 7.71 mg RE/g, and 8.43 mg RE/g for all drying temperatures. Clone 224 retained the most chlorogenic acid and caffeine content when dried at 60°C. The study demonstrated that drying at 60°C is the recommended temperature for preserving total phenolics, total flavonoids, total antioxidants, chlorogenic acid, and caffeine content in Liberica coffees.

1. Introduction

Coffee is an important commercial crop with a significant economic value in many countries. The crop is one of the most traded commodities, with Brazil, Vietnam, and Colombia producing the most coffee, while the European Union and the United States of America consume and import the most (Food and Agriculture Organization of The United Nations, 2021). Coffee beans have been processed into a variety of food products because of their distinctive flavour, and the brews have been consumed by people all over the world ever since ancient times (Insanu *et al.,* 2021). The two coffee species, Arabica and Robusta, produce the majority of the world's coffee. However, shortages of these two species were reported in global stocks in 2021 and 2022, causing prices to double, particularly for Arabica (Davis *et al*., 2022). The production insufficiency was brought on by the combined effects of the COVID-19 pandemic in Brazil and the direct effects of drought in other coffee-growing countries (International Coffee Organization (ICO), 2022). On account of this, new attention is being given to the idea of diversifying the coffee crop portfolio with new cultivars, hybrids, and underutilised and alternative species such as Liberica coffee (Davis *et al*., 2021).

Liberica coffee is currently receiving more attention and consideration, as evidenced by the rise in popularity of online articles with the steady expansion of retail availability especially via the online platform, as well as the adoption by farmers in Africa and Asia since 2018 (Davis *et al*., 2022). The production of Liberica coffee was observed in Cameroon, Malaysia, Sierra Leone, and

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Uganda from 2002 to 2022 (Davis *et al*., 2022). The crop could grow well in a warm, lowland environment (0–1000 m elevation) with higher humidity and evenly distributed annual rainfall (Burkill, 1935). The stakeholders in the coffee industry find these characteristics to be very attractive because the species is adaptable to warm climates with low elevations as opposed to the cool-tropical, high-elevation conditions needed for Arabica (McCook, 2014). Given this, Liberica coffee has great potential to be competitive in the global market, which would encourage Malaysia to increase its research and development efforts. MARDI has been conducting extensive research on all facets of the production system, including post-harvest handling methods, particularly drying processes. One of the most underutilised coffee species grown in Malaysia is Liberica coffee. However, little to no research has been conducted on production technology and processing techniques for berries and green coffee beans since the plant is a low-income crop in Malaysia and minimal attention has previously been paid to this species. Given this, studies on Malaysian Liberica coffee would help manufacturers and growers understand how to handle and process the beans for product development and quality maintenance. As we all know, harvested coffee berries undergo a lengthy process that includes fermentation, drying, and roasting before being transformed into a cup of coffee after brewing. Hence, drying is an essential step for the primary processing of coffee beans because it is one of the most intensive processes in the production phase. Furthermore, because most coffee products are sold in dried form, the process is critical in coffee processing. In view of this, the current study was conducted to evaluate the influence of drying temperatures on the physical and chemical attributes of Liberica coffee grown in Malaysia in order to establish the optimal process parameters for quality preservation.

2. Materials and methods

2.1 Harvesting of coffee

Liberica coffee at an optimum maturity of 51 weeks after planting was manually harvested at MARDI's farm in Kluang, Johor. The outer skin was mechanically separated from the berries before drying. On the day of harvest, the berries were delivered to the post-harvest lab for sample preparation for the drying experiment. Only unblemished, healthy-appearing beans with no disease development were chosen for this experiment in order to produce dried beans of high quality prior to product development.

2.2 Drying of coffee

The coffee beans were dried in a hot air oven at 50°C, 60°C and 70°C with a constant air flow of 2.5 m/s. The quality of dried beans was analysed immediately after drying treatments.

2.3 Determination of moisture content

The AOAC method (Association Official Analytical Chemists, 2019) was used to determine the moisture content of coffee by drying the sample in a 105°C oven for 24 hrs (ULM 400, Mermert GmbH, Germany). On a wet basis, the initial moisture content was recorded to be around 55%.

2.4 Determination of moisture ratio for mathematical modelling of drying characteristics

The moisture ratios of all Liberica coffee clones were averaged and fitted into mathematical models to describe the drying behaviours of the beans during drying. The moisture ratio of the beans was determined using the following equation (*Botelho et al*., 2011).

$$MR = \frac{M - Me}{Mo - Me} \quad (1)$$

Where M (g_w/g_{dm}) represents the moisture content at any given time t, M_o is the initial moisture content and M_e is the equilibrium moisture content.

2.5 Plant materials and preparation of extracts

The fresh beans were collected from MARDI's farm in Kluang, Johor. The beans were washed with running tap water to remove any surface contaminants for chemical analysis. Prior to extraction, the dried samples were grounded into a fine powder and sonicated for 1 hr while being extracted with 70% methanol (1:10). The samples were then centrifuged for 10 mins at 1000 rpm to separate the supernatant from the sediment. Under the same circumstances, the extraction was performed three times.

2.6 Determination of 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of the test samples was determined using our previous work by Mirfat *et al*., 2020. The extracts were prepared in methanol at varying concentrations to yield a final volume of 7 L. They were then combined with 280 L of a methanolic solution that contained DPPH radicals to yield a final concentration of 0.06 mM. After giving the reaction mixture a good shake, it was allowed to stand in the dark for 30 mins. A positive control was used, which was ascorbic acid (vitamin C). Methanol was used as a blank and all the

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reagents were present in the negative control. Using a microplate reader (Aeon Biotek, VT, USA), the absorbance at 517 nm was measured to ascertain the DPPH radical scavenging activity. Equation 2 was used to determine the inhibition percentage.

$$\text{Inhibition (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100 \quad (2)$$

The DPPH inhibition percentage of each sample was plotted against the sample concentration. IC50 values, which represent the inhibitory concentration at which DPPH radicals were scavenged by 50%, were used to express the results. Each procedure was carried out three times with the least amount of light exposure possible.

2.7 Determination of ferric reducing antioxidant power assay

The ferric reducing antioxidant power (FRAP) assay was quantified using minor modifications to the reduction of ferric-tripiridyltriazine (Fe^{3+}-TPTZ) to a blue-colored ferrous form (Fe^{2+}-TPTZ) (Benzie and Strain, 1996). The 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (Sigma, USA), and 20 mM $FeCl_3.6H_2O$ (Sigma, USA) solution were combined to prepare the working solution of the FRAP reagent. This mixture was then warmed at 37°C in a water bath before use. After that, 200 mL of FRAP reagent was mixed with 7 mL of sample and 20 mL of distilled water, and the mixture was then incubated at 37°C for 4 mins. Finally, the reaction mixture's absorbance was measured in comparison to a blank at 593 nm. A calibration curve was created using ferrous sulphate heptahydrate ($FeSO_4.7H_2O$) as a reference standard (100–1000 mM). The results were determined as the concentration of antioxidants having ferric reducing ability in Fe mol/mg.

2.8 Determination of total phenolic content

The Folin-Ciocalteu colourimetric method was used to estimate the total phenolic content of the test samples, as described in previous work by Mirfat *et al*. (2020). In the beginning, 50 µL of the test sample was mixed with 100 µL of Folin Ciocalteu's phenol reagent. After 3 mins, 100 µL of 10% Na_2CO_3 was added to the reaction mixture and left to stand in the dark for 60 mins. The analysis was carried out in triplicate under dim lighting. The resulting, blue-coloured complex was measured at 725 nm in comparison to a blank. Gallic acid was used as a reference standard, and total phenol content was expressed in milligrams per gramme samples as gallic acid equivalents (GAE) by using the calibration curve.

2.9 Determination of total flavonoid content

The total flavonoids were determined using the aluminium chloride method, as previously reported by Mirfat *et al*. (2020). A 30 µL aliquot of extract was diluted with 120 µL dH2O. Then, a 9 µL 5% NaNO2 solution was added and allowed to react for 5 mins. After that, a 9 µL from 10% of $AlCl_3$ solution was added and allowed to stand for 5 mins. Finally, 60 µL NaOH and 72 µL dH_2O were added to the mixture, which was thoroughly mixed with a vortex. All samples were tested in triplicate and the absorbance at 510 nm was measured in comparison to a blank. The total flavonoid content was calculated from the calibration curve using rutin as a standard reference with rutin equivalents (RE) used to express the data.

2.10 Identification of chlorogenic acid using high-pressure liquid chromatography

Chlorogenic acid was quantified using an Agilent 1100 series high-pressure liquid chromatography (HPLC) system (Agilent Technologies, Waldbronn, Germany) that included a binary pump, a vacuum degasser, an auto-sampler, and a column oven. The compound was separated chromatographically using a Kinetek-C18 Column (250 mm × 4.6 mm × 5 m) and kept at 40°C. The mobile phase of A and B with a linear binary gradient of methanol: water (20:80) (0.1% formic acid) and acetonitrile (0.1% formic acid) was used. The mobile phase composition was shifted during the run as follows: 0 min, 11% B; 10 mins, 12% B; 30.00 mins, 40% B; 35.00 mins, 90% B; 37.00 mins, 90% B; and 40.00 mins, 11% B. The injection volume was 1 µL and the flow rate was set to 0.6 mL/min. The amount of chlorogenic acid in the extract was calculated using the equation obtained from the calibration curve of its peak area to the peak area of the standard with known concentration.

3. Results and discussion

3.1 Drying kinetics of Liberica coffee at different temperatures

Figures 1 to 3 show that the drying time required to achieve the desired moisture content is highly dependent on drying temperature. The reduction in moisture content was observed as drying progressed for all tested temperatures. These are well-known facts that other researchers have observed for many other crops with varying results depending on the shape, dimension, drying methods, and parameter settings (Md Saleh *et al*., 2020). Drying the berries at 50°C, 60°C, and 70°C took 900, 480, and 330 mins, respectively, to achieve the desired moisture content for all clones of Liberica coffee. The findings indicate that as the temperature rises, the time required to reduce moisture to a safe level decrease significantly due to vast differences in steam pressure between the drying air and the product, making water

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removal easier and faster (Siqueira *et al*., 2012). There have also been other reported causes for the decrease in drying time associated with an increase in temperature. Elevated temperatures reduce water viscosity, which directly affects fluid runoff. The decrease in viscosity promotes water molecule diffusion while also increasing the kinetic energy of the water molecules, which contributes to an increase in drying rate and a shorter drying period (Corrêa *et al*., 2010).

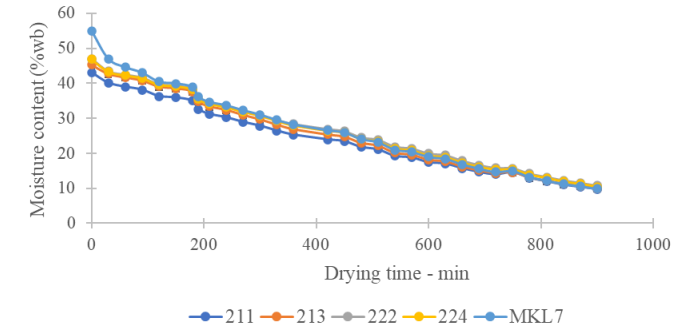

Figure 1. Drying of different clones of Liberica coffee at 50°C.

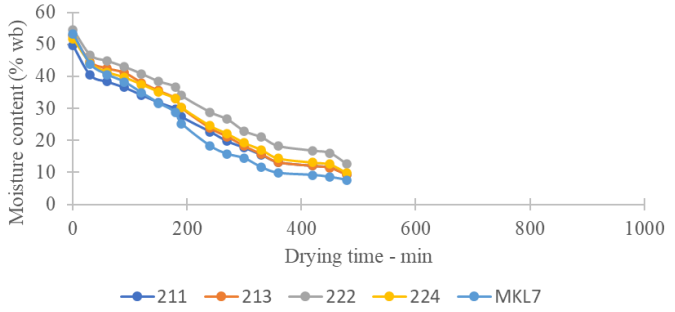

Figure 2. Drying of different clones of Liberica coffee at 60°C.

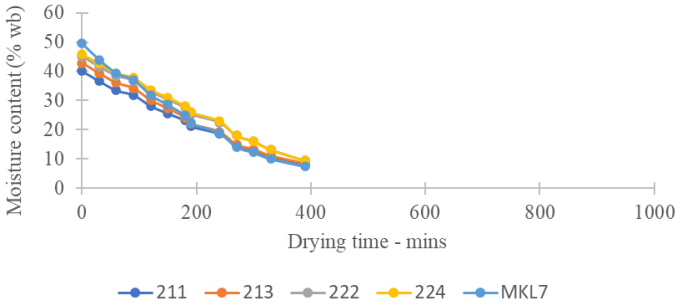

Figure 3. Drying of different clones of Liberica coffee at 70°C.

3.2 Mathematical modelling of drying kinetics of different clones of Liberica coffee

The results of Table 1 revealed that all mathematical models tested were suitable for fitting the drying kinetics of Liberica coffee during drying. Non-linear regression techniques were used to analyse the collected data during drying. All models fit well, with high values of R^2 and low values of Root Mean Square Error (RMSE). However, the best model for all drying temperatures is the Page equation, with R^2 values approaching 1.0 and RMSE less than 0.0005. The model can be used to predict the drying performance of Liberica coffee when exposed to different drying temperatures in the future. Aside from being an internationally recognised model, the Page model is mathematically more practical and simpler, with fewer parameters, making it easier and less complex to apply in drying simulations. Complex models are usually not preferred for drying simulations because measuring some variables during the drying process can be quite complicated (Suherman *et al*., 2020). The establishment of appropriate mathematical models for drying is advantageous for process control and optimisation, as well as for evaluation of dryer performance, which is critical in maintaining food safety and quality (Castro *et al*., 2018). Furthermore, selecting adequate mathematical models during drying is beneficial for process improvement and simulation by predicting the drying behaviours of coffee beans under various drying conditions.

3.3 Influence of drying temperatures on antioxidant activity of different clones of Liberica coffee

The results showed that different clones respond differently to heat treatment via the drying process. Figures 4 and 5 display the response of antioxidant activity for Liberica coffee during drying using two different assays. As we know, antioxidants react with free radicals via various chemical mechanisms, and the most common of which are hydrogen atom transfer (HAT), single electron transfer mechanism (SET), or a combination of both HAT and SET mechanisms (Munteanu and Apetrei, 2021; Siddeeg *et al*., 2021). As a result, two antioxidant assay methods based on different mechanisms were used in this study. When drying at 60° C, total antioxidant activity as measured by IC_{50} values for the DPPH assay, as shown in Figure 4, demonstrated stronger quenching capabilities towards free radicals for all clones except MKL7. Clones 211, 213, 222, and 224 have IC_{50} values of 1.622, 0.997, 1.083, and 1.152, respectively, compared to 4.728 for clone MKL7. The greater the antioxidant activity, the lower the IC_{50} value (Ahmed *et al*., 2021). Drying at 70°C reduced total

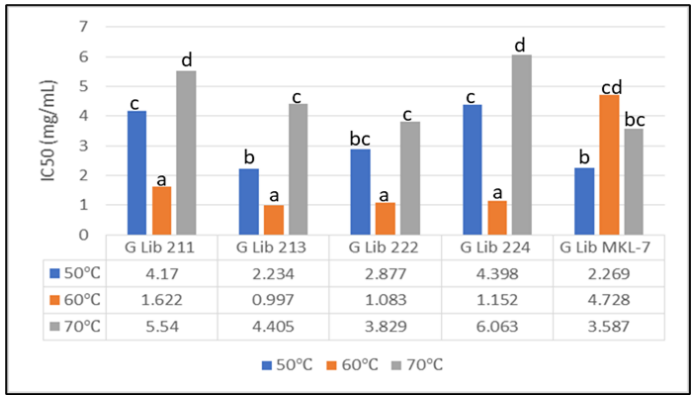

Figure 4. Antioxidant activity (DPPH assay) of different clones of *Liberica* coffee at different drying temperatures.

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Table 1. Mathematical modelling of drying kinetics of Liberica coffee during drying.

Model	Mathematical equation	Constant	Drying at 50°C	Drying at 60°C	Drying at 70°C
Exponential decay	$MR = a + e^{-c(t-b)}$	a	0	0	0.003706844
		b	0	0	7.328931729
		c	0.001555468	0.003294561	0.003649865
		R^2	0.996370704	0.990979036	0.990625394
		RMSE	0.000194912	0.000578241	0.000705578
Page	$MR = e^{-at^b}$	a	0.001221999	0.002229349	0.000740467
		b	1.038596479	1.070205902	1.286004695
		R^2	0.996809375	0.992172312	0.997633676
		RMSE	0.000179024	0.00052802	0.000168300
Diffusion approach	$MR = ae^{-kt} + (1-a)e^{-kbt}$	a	0.003551311	0.00796466	90648.03301
		b	0.01198091	0.015791716	1.000007852
		k	0.129138797	0.206346991	0.006727073
		R^2	0.996417948	0.991105531	0.997778274
		RMSE	0.000194123	0.000574858	0.000152540
Midili	$MR = ae^{-ktn} + bt$	a	0.97172044	0.972608664	1.045081725
		b	0.000129293	0.000215001	0
		k	0.034509748	0.05124534	0.029343179
		n	0.034509748	0.051245339	0.126066698
		R^2	0.998249948	0.993132269	0.990307798
		RMSE	9.31393E-05	0.000437117	0.000674370
Two-term	$MR = a_1 e^{-k1t} + a_2 e^{-k2t}$	a_1	0.047368508	1.212394961	1.045080193
		a_2	0.949685523	0.240825329	0
		k_1	0.001548915	0.002258257	0.003699194
		k_2	0.001548849	3.28502E-05	7.09091E-05
		R^2	0.996414415	0.993247941	0.990307824
		RMSE	0.000194206	0.000429777	0.00067437
Henderson - Pabis	$MR = ae^{-bt}$	a	0.997053706	0.995054901	1.045080986
		b	0.001548852	0.003272542	0.003699185
		R^2	0.996414411	0.991073371	0.990307879
		RMSE	0.000194206	0.000576103	0.000622495

antioxidant activity for all clones with the values of IC_{50} ranging from 3.587 mg/mL to 6.063 mg/ml. The decrease in total antioxidant activities must be attributed to the drying conditions associated with higher temperature and oxygen environments (Li *et al*., 2019). Nonetheless, the FRAP assay revealed varying levels of antioxidant activity for different clones of *Liberica* coffee (Figure 5). Clones 211 and 213 had high antioxidant activities at 0.144 µmol/mg and 0.145 µmol/mg when dried at 60°C, but clone 222 had the lowest activity when dried at the same temperature. The antioxidant activities of clones 224 and MKL7 were slightly lower, at 0.099 mol/mg and 0.173 mol/mg, respectively, but not significantly different from clones 211 and 213. The inconsistent results could be attributed to genetic diversification of different genotypes or cultivars which generates different levels of quality degradation since most of the chemical compounds showed significant differences between coffee cultivars (Toledo *et al*., 2016). Furthermore, post-harvest processing conditions (drying, storage, roasting, and grinding) influence the bioactive composition of coffee beans and subsequently affect the antioxidant capacity (Komes and Bušić, 2014).

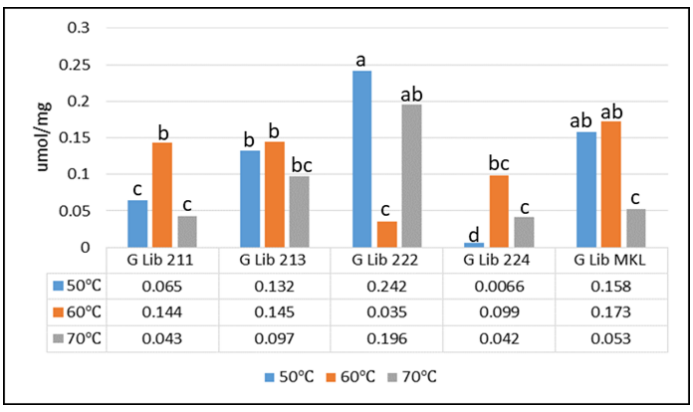

Figure 5. Antioxidant activity (FRAP assay) of different clones of Liberica coffee at different drying temperatures.

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3.4 Influence of drying temperatures on total phenolic and total flavonoid content for different clones of Liberica coffee

As shown in Figure 6, drying at 60°C retained the most total phenolic content for clone 224 (21.96 mg GAE/g), but the concentrations were significantly lower at 16.42 mg GAE/g and 15.75 mg GAE/g when drying at 50°C and 70°C, respectively. However, drying at all temperatures tested had no effect on the concentrations of total phenolic content for clone 222 and 213, with values ranging from 12.62 mg GAE/g to 19.75 mg GAE/g for clone 222 and 11.43 mg GAE/g to 13.67 mg GAE/g for clone 213. The opposite trend was observed for clone MKL7, which had the lowest total phenolic content when dried at a low temperature of 50°C but better retention of total phenolic content at 11.74 mg GAE/g and 19.19 mg GAE/g respectively at drying temperatures of 60°C and 70°C. This must be due to a longer exposure time to hot air at low temperatures, which may cause total phenolic degradation in clone MKL7. A similar observation was reported for Robusta coffee in terms of depletion of total phenolic content after a long drying period in ambient conditions. (Cheng *et al*., 2019). Tran *et al*. (2020) reported comparable results with high retention of total phenolic compound when drying a Robusta coffee at higher temperatures using both hot air and microwave dryers due to shorter drying time. Previous work by Ghanem Romdhane *et al*., (2015) found the same trend of reduction in total phenolic content of lemon when drying at a lower temperature of 40°C.

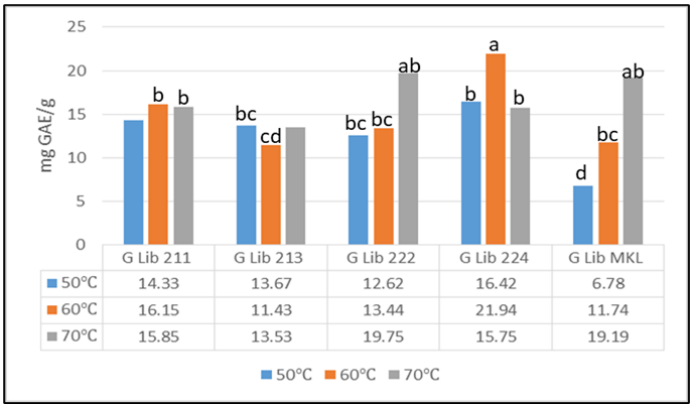

Figure 6. Total phenolic content of different clones of Liberica coffee at different drying temperatures.

Figure 7 depicts the fluctuations in total flavonoid retention for all clones of Liberica coffee when dried at various temperatures. The amount of total flavonoid content was significantly stable at $p<0.05$ for clones 222 and MKL7 when drying at 50°C, 60°C and 70°C with concentrations ranging from 6.22 mg GAE/g to 9.36 mg GAE/g for clone 222 and 7.30 mg GAE/g to 8.43 mg GAE/g for MKL7. The highest retention of total flavonoid content at 10.77 mg GAE/g was observed for clone 224 at a drying temperature of 60°C as well as for clone 222 at 9.36 mg GAE/g when drying at 50°C. However, drying at 70°C resulted in the lowest total flavonoid retention of 5.76 mg GAE/g for clone 211 and 4.09 mg GAE/g for clone 213, but significantly better retention of total flavonoid for clone 222 (6.22 mg GAE/g), 224 (7.10 mg GAE/g), and MKL7 (8.43 mg GAE/g). According to the findings, different clones demonstrated varying levels of quality retention at different drying temperatures, which is consistent with previous research by Tesfa (2019) on the drying of Arabica coffee, and a study by Pinar *et al*. (2021) on red pepper, in which different cultivars for both crops demonstrated heterogeneous retention of biochemical compositions in response to drying treatments.

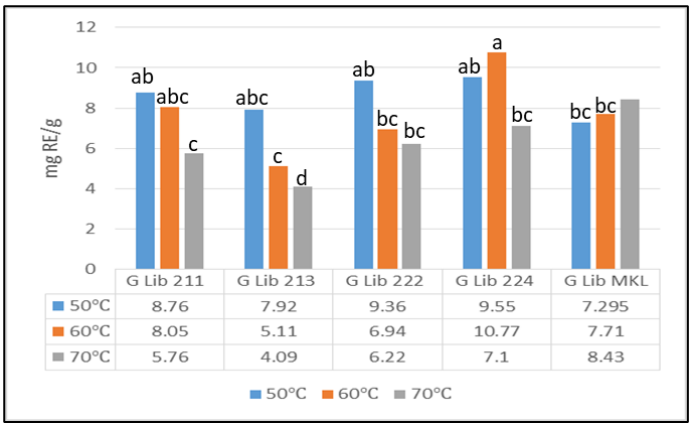

Figure 7. Total flavonoid content of different clones of Liberica coffee at different drying temperatures.

3.5 Influence of drying temperatures on chlorogenic acid and caffeine content for different clones of Liberica coffee

The study discovered that clone 224 of Liberica coffee retained more phytochemicals than others during drying, so the clone was chosen for detailed screening on chlorogenic acid and caffeine content because both compounds have a significant impact on the aroma and flavour of coffee (Cwiková *et al*., 2022; Richard *et al*., 2020). In view of this, the retention of both phytochemical compounds was compared to MKL7, a classic Malaysian variety of Liberica coffee which was introduced in 1998 by MARDI (Mohd Amirul Mukmin and Nor Amna Aliah, 2016). Figure 8 depicts both clones 224 and MKL7 had more caffeine content than chlorogenic acid when subjected to drying treatments. The greater stability with the concentrations remained unchanged for both chlorogenic acid and caffeine content was found for clone MKL7 at all drying temperatures. The values for chlorogenic acids are 6.066 mg/g (50°C), 6.797 mg/g (60°C) and 7.155 mg/g (70°C), while the amount of caffeine content ranges from 9.795 mg/g to 10.705 mg/g for drying temperatures between 50°C to 70°C. Clone 224, on the other hand, exhibited

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higher retention when drying at 50°C and 60°C, with the amount of chlorogenic acids remaining at 6.057 mg/g and 7.172 mg/g, respectively, and the caffeine content retained at 8.508 mg/g and 9.887 mg/g. The lowest retention for both caffeine content and chlorogenic acid was observed for clone 224 when drying at an elevated temperature of 70°C, indicating that drying at high temperatures may cause deterioration of these two compounds. The findings revealed that the stability of these two compounds is affected by clones/cultivars as well as processing conditions such as drying temperatures. (Toledo *et al*., 2016).

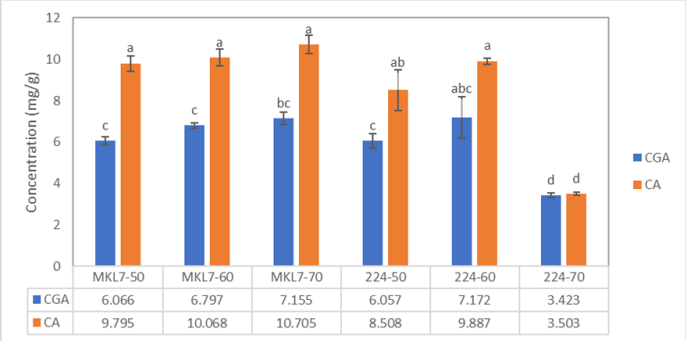

Figure 8. Chlorogenic acid (CGA) and caffeine (CA) content of different clones of Liberica coffee at different drying temperatures.

4. Conclusion

The study demonstrates the complex relationship between clones and drying temperatures on the quality retention of Liberica coffee grown in Malaysia. The best drying temperature for quality maintenance is 60°C, and clone 224 is the most heat-stable when thermally treated, with great commercialization potential towards the development of "speciality coffee" for economic benefits. Since quality determines the market value of coffee, and "speciality coffees" command higher prices than regular coffee, the production of high-quality coffee is dependent on various factors, including crop management, harvesting, processing, drying, and storage procedures. High moisture content in coffee (60%) makes it more susceptible to quality deterioration caused by microorganisms, resulting in phytochemical loss. As a result, technological treatment is required to achieve microbiological stability and thus increase shelf life while maintaining the quality of the final product. Therefore, it is critical to investigate new drying strategies for preserving the bioactive components of coffee beans in order to provide a theoretical foundation for the industrial production of functional food in the future.

Conflict of interest

The authors declare no conflict of interest.

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