

## Predictive model for moisture content of *Coffea liberica* seeds subjected to ambient air drying

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### Abstract

Determination of seed moisture content (MC) is a destructive method according to the Department of Standards Malaysia and the International Seed Testing Association (ISTA) where the seeds were dried at  $103\pm 2^\circ\text{C}$  for  $16\pm 1$  hrs in a convection oven to know the amount of water within seeds. The present work analysed the relationship between the weight and MC of *Coffea liberica* seeds so that the seeds with MC estimates could subsequently be applied as planting materials or for germplasm storage for future varietal improvement for the beverage sector. From the fully ripe fruits, single seeds, two seed units, three seed units, and four seed units were studied for suitability in developing a predictive model for MC. In each context, seeds of varying MCs for model building were obtained with air drying for 0, 2, 4, 6 and 8 days at the ambient temperature of  $28\pm 2^\circ\text{C}$  and relative humidity (RH) of  $55\pm 5\%$  in the laboratory. Prior to desiccation treatments, the seed units were tagged and noted for fresh weight (FW), length and breadth. After desiccation treatments, weight after desiccation (DesW) was recorded and the actual MC of the seeds was determined with the oven-drying method. Plots of MC - desiccation period showed that ambient air-drying reduced the MC of seeds progressively and consistently. With the initial MC of  $0.4 \text{ g H}_2\text{O g}^{-1} \text{ Wt}$  on a wet weight (Wt) basis, air drying of the seeds in the laboratory for up to 8 days reduced their MC to  $0.15 \text{ g H}_2\text{O g}^{-1} \text{ Wt}$ . Subsequent correlation analysis revealed that MC had a positive relationship with DesW, but was negatively associated with (Loss of water), which is the difference between FW and DesW. Stepwise linear regression analysis implied that the MC of *C. liberica* seeds could best be estimated as  $\text{MC} = 0.185 + 0.118 \text{ FW} - 0.412 (\text{Loss of water})$  using two seed units. The model was statistically significant ( $P < 0.001$ ) with appropriate  $R^2$  and  $\text{SE}_E$  values of 0.95 and 0.0187 g, respectively.

## 1. Introduction

*Coffea liberica* with its common name of Liberica coffee is a member of the Rubiaceae family. Originated from Liberia, West Africa, it has relatively larger berries with higher sphericity, volume, surface area, and mass, but a lower bulk density in comparison to Arabica and Robusta coffee (Ismail *et al.*, 2014; Hung *et al.*, 2021). Commercially, the seeds within the berries are roasted and valued as a beverage. However, the seed is rather sensitive to desiccation from the point of view of seed technology, and is difficult to store, thus impeding the conservation of its germplasm collection for future crop improvement (Muhamad Ghawas and Wan Rubiah, 1991; Hay *et al.*, 2021). Currently, the seeds are classified as intermediate after the introduction of this new category of seeds by Ellis (1991). Intermediate

seeds have some degree of desiccation tolerance but they do not survive very low moisture content (MC) or the combined effects of low MC and low-temperature storage (Yulianti *et al.*, 2020; Nadarajan *et al.*, 2023). Under tropical climatic environments, some work showed that *C. liberica* seeds could retain viability when air dried to MC of about  $0.15 \text{ g H}_2\text{O g}^{-1} \text{ Wt}$  on wet weight (Wt) basis but died at MC of  $0.1 \text{ g H}_2\text{O g}^{-1} \text{ Wt}$  (Tsan, unpublished data).

Determination of MC of seed is a destructive method because the seeds are dried at  $103\pm 2^\circ\text{C}$  for  $16\pm 1$  hrs in an oven in this procedure (Department of Standards Malaysia, 2018; International Seed Testing Association (ISTA), 2019). Preliminary work with some tropical recalcitrant seeds indicated that some mathematical

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equations were appropriate to calculate their estimates of MC (Hasmah *et al.*, 2012). Some seed attributes, especially the weight, were highly associated with MC, allowing the calculation of the amount of water lost from the seeds. However, more recent work noted that single seeds could be less reliable for developing a theoretical model for the MC of seeds (Tsan, unpublished data). The current study investigated the smallest number of seeds needed for realizing a predictive model for MC of *C. liberica* seeds. Single seeds, two seed units, three seed units and four seed units of varying MCs following ambient air-drying in the laboratory were applied in model building in the current work. Statistical assessment for the dependent variable - independent variable(s) relationships was performed to derive suitable mathematical formula for estimating the MC of seeds.

## 2. Materials and methods

### 2.1 Seed extraction and cleaning

A total of 300 fully ripe fruits were harvested from the field within the campus (N 2° 13' 16.446" E 102° 27' 8.262") in November 2022. The fruits were brought to the Plant Science Laboratory on the campus immediately. After sorting out a few damaged fruits, each fruit was cut carefully using a pair of secateurs. The two ovoid seeds in each fruit were taken out after removing the fruit pericarp.

The seeds were rubbed carefully with sand to remove the arils. Then, the seeds were cleaned with slow-running tap water followed by pat drying using a paper towel. The cleaned seeds were air-dried overnight in the laboratory to remove excessive water.

### 2.2 Seed grouping and labelling

On the following day, 555 intact seeds of uniform size and shape were selected. The seeds were randomly separated into 150 samples of single seeds, 45 samples of two seed units, 45 samples of three seed units, and 45 samples of four seed units according to Table 1. All samples were further randomly tagged for five desiccation treatments, each was replicated three, using

Table 1. Seed grouping.

Type	No. of desiccation treatments	No. of replicates	No. of samples per replicate
Single seed	5	3	10
Two seed unit	5	3	3
Three seed unit	5	3	3
Four seed unit	5	3	3

labelled aluminium boats placed in numbered Petri dishes. Lastly, each sample within the replicate was numbered accordingly. Thus, the seed units were tagged based on a completely randomized method. The size of the aluminium boats was made to ensure that the seeds could be laid as a single layer in the boats for even air drying for different periods to obtain seeds with varying MCs.

### 2.3 Measurement of fresh weight, length and breadth

Prior to desiccation treatments, each sample was recorded for fresh weight (FW) using an analytical balance. Then, a vernier calliper was used to measure the length and breadth of each seed in each sample. The length of the seed was its largest dimension. The breadth was that perpendicular to the length. As the ovoid seed has a rather flattened base opposite to the raphe, seed breadth was taken as the average value of the largest and smallest breadth measurements.

### 2.4 Seed desiccation

After measurements of FW and dimensions, the tagged samples were respectively subjected to air drying for 0, 2, 4, 6 and 8 days at ambient temperature in the laboratory. The average temperature in the laboratory was 28±2°C and the relative humidity was 55±5%. Seed desiccation was aimed to obtain seeds of varying MCs on a wet weight (Wt) basis, which ranged from the initial MC of approximately 0.4 g H<sub>2</sub>O g<sup>-1</sup> Wt to approximately 0.15 g H<sub>2</sub>O g<sup>-1</sup> Wt by the end of the air-drying process after eight days according to preliminary trials (Table 2).

Table 2. Desiccation treatment.

Air drying period (days)	Expected MC of seeds (g H <sub>2</sub> O g <sup>-1</sup> Wt)
0 (control)	0.40
2	0.30
4	0.25
6	0.20
8	0.15

### 2.5 Moisture loss and actual moisture content determination

After each desiccation treatment, the tagged samples were respectively measured for weight after desiccation (DesW) using the same analytical balance. (Loss of water) was calculated as the difference between FW and DesW as follows.

$$(\text{Loss of water}) = \text{FW} - \text{DesW}$$

In the next procedure, the actual MC of each sample was determined according to the Department of Standards Malaysia and the International Seed Testing Association (Department of Standards Malaysia, 2018;

ISTA, 2019). The samples in their aluminium boats were dried at  $103\pm 2^\circ\text{C}$  for  $16\pm 1$  hrs in a convection oven to remove all the water in the seeds. After oven drying, the samples were cooled to room temperature for 15 minutes in a desiccator. Then, the weight of each oven-dried sample (OvenW) was determined using the same analytical balance. Finally, the MC of each sample was calculated in a Microsoft Excel spreadsheet using the formula below.

$$\text{MC of seed (g H}_2\text{O g}^{-1}\text{ Wt)} = (\text{DesW} - \text{OvenW}) / \text{DesW}$$

### 2.6 Statistical analysis

Data analysis was carried out separately for single seeds, two seed units, three seed units and four seed units using SPSS version 28. In each context, the MC versus desiccation period was plotted. Correlation analysis was performed to determine the relationships between pairs of parameters for MC, Length, Breadth, FW, DesW and (Loss of water). It was followed by stepwise linear regression analysis as represented by:

$$Y = a + bX_1 + cX_2 + dX_3 + eX_4 + fX_5 + \epsilon$$

Where  $Y$  = Dependent variable, i.e. MC,  $X_1, X_2, X_3, X_4, X_5$  = Independent (explanatory) variables, i.e. Length, Breadth, FW, DesW, (Loss of water),  $a$  = Intercept,  $b, c, d, e, f$  = Slopes and  $\epsilon$  = Residual (error)

The smallest possible number of seeds for reliable modelling and the most appropriate predictive model for the MC of seeds were determined.

## 3. Results

### 3.1 Moisture content versus desiccation period

The data collected demonstrated that ambient air drying in the laboratory impacted the MC of *C. liberica* seeds in a linear trend over a period of eight days (Figure 1). The seeds were dried to MC of  $0.15 \text{ g H}_2\text{O g}^{-1}\text{ Wt}$  from the initial MC of  $0.4 \text{ g H}_2\text{O g}^{-1}\text{ Wt}$  as planned. Plots of MC - desiccation period for single seeds, two seed units, three seed units and four seed units confirmed the consistencies of the desiccation treatments (Figure 1). Likewise, the coefficients of determination ( $R^2$ ) of the desiccation rates, which ranged from 0.94 to 0.98, implied that the single seeds and all other seed units were appropriate for developing a probabilistic model that describes the MC of seeds (Figures 1a – 1d).

### 3.2 Correlation analysis

Subsequent correlation analysis of the parameters generally showed that MC was significantly dependent on DesW or (Loss of water) (Table 3). With two seed units, three seed units and four seed units, MC was positively related to DesW at correlation coefficients of  $>0.88$  and negatively associated with (Loss of water) at coefficients of  $<-0.9$ . Single seeds also demonstrated similar MC – DesW and MC – (Loss of water) associations but the interdependence was relatively weaker as compared to that established by the other seed units. On the other hand, there was no significant relationship between the length and breadth of seeds, which were  $18.23\pm 2.05$  mm and  $8.49\pm 0.84$  mm, respectively. These seed dimensions were also generally

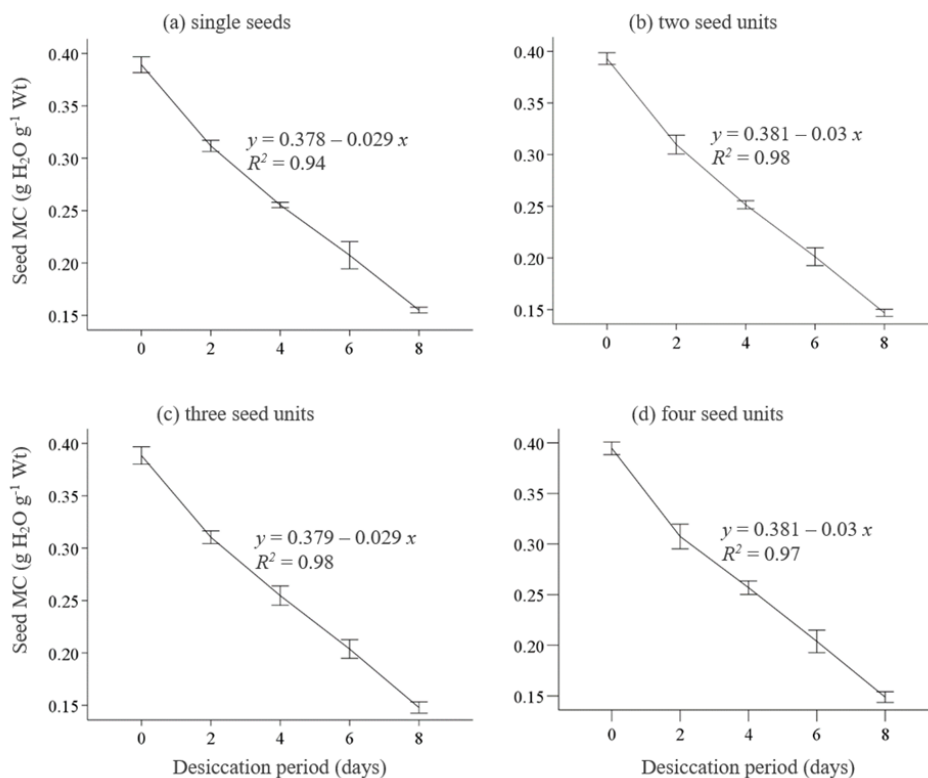


Figure 1. MC of seeds as affected by the desiccation period.

Table 3. Correlation matrix

<b>Single seed</b>						
	MC	Length	Breadth	FW	DesW	Loss of water
MC	1					
Length	-0.135 ns	1				
Breadth	0.031 ns	0.184*	1			
FW	-0.077 ns	0.502***	0.195*	1		
DesW	0.522***	0.299***	0.148 ns	0.723***	1	
Loss of water	-0.818***	0.245**	0.052 ns	0.314***	-0.429***	1
<b>Two seed unit</b>						
	MC	Length	Breadth	FW	DesW	Loss of water
MC	1					
Length	0.24 ns	1				
Breadth	0.038 ns	0.135 ns	1			
FW	0.249 ns	0.699***	0.651***	1		
DesW	0.916***	0.484***	0.257 ns	0.577***	1	
Loss of water	-0.963***	-0.157 ns	0.091 ns	-0.84 ns	-0.862***	1
<b>Three seed unit</b>						
	MC	Length	Breadth	FW	DesW	Loss of water
MC	1					
Length	0.323*	1				
Breadth	0.157 ns	0.006 ns	1			
FW	0.278 ns	0.521***	0.780***	1		
DesW	0.886***	0.498***	0.435**	0.636***	1	
Loss of water	-0.960***	-0.352*	-0.140 ns	-0.278 ns	-0.918***	1
<b>Four seed unit</b>						
	MC	Length	Breadth	FW	DesW	Loss of water
MC	1					
Length	0.229 ns	1				
Breadth	0.011 ns	-0.033 ns	1			
FW	0.376*	0.633***	0.476***	1		
DesW	0.969***	0.277 ns	0.087 ns	0.462***	1	
Loss of water	-0.909***	-0.002 ns	0.135 ns	-0.031 ns	-0.901***	1

ns: no significant relationship, \*significant relationship at 5%, \*\*significant relationship at 1%, \*\*\*significant relationship at 0.1%.

not correlated to MC.

### 3.3 Model development

The next procedure of stepwise linear regression analysis generated seven equations using single seeds, two seed units, three seed units and four seed units (Table 4). Each model was highly significant with a p-value of <0.001. Among the models, Model 4 established using two seed units was the most suitable model in terms of accuracy and simplicity for predicting the MC. This model required two predictors, namely FW and (Loss of water). In this mathematical equation, the FW of two seed units which weighed  $1.5786 \pm 0.1241$  g was taken as part of the intercept since FW was not significantly correlated to MC. Nonetheless, FW was required for calculating (Loss of water) which was highly and inversely correlated to MC and thus was the

determinant for MC. In other words, this model was appropriate only for desiccated seeds with known initial weight (FW). From another point of view, Model 4 had the highest  $R^2$  of 0.955 and the lowest standard error of estimate ( $SE_E$ ) of 0.0187 g among all the models, thus verifying the suitability of this model for determining MC estimate (Table 4). On the other hand, Models 1 and 2 generated with single seeds were the least reliable as they had the lowest  $R^2$  of 0.67 and 0.70, and the lowest  $SE_E$  values of 0.046 g and 0.048 g, respectively (Table 4).

## 4. Discussion

This study yielded results which supported the possibility of describing the MC of *C. liberica* seeds through weight changes. The stepwise linear regression

Table 4. Model summary following stepwise regression analysis.

<b>Single seed</b>						
No. Model		No. of predictors	P-value	$R^2$	$R^2_{adj.}$	SE <sub>E</sub>
1	MC = 0.359 - 0.709 (Loss of water)	1	<0.001	0.67	0.667	0.048
2	MC = 0.262 + 0.132 FW - 0.763 (Loss of water)	2	<0.001	0.71	0.701	0.046
<b>Two seed unit</b>						
No. Model		No. of predictors	P-value	$R^2$	$R^2_{adj.}$	SE <sub>E</sub>
3	MC = 0.374 - 0.418 (Loss of water)	1	<0.001	0.93	0.925	0.024
4	MC = 0.185 + 0.118 FW - 0.412 (Loss of water)	2	<0.001	0.96	0.953	0.019
<b>Three seed unit</b>						
No. Model		No. of predictors	P-value	$R^2$	$R^2_{adj.}$	SE <sub>E</sub>
5	MC = 0.371 - 0.284 (Loss of water)	1	<0.001	0.92	0.92	0.024
<b>Four seed unit</b>						
No. Model		No. of predictors	P-value	$R^2$	$R^2_{adj.}$	SE <sub>E</sub>
6	MC = - 0.345 + 0.223 DesW	1	<0.001	0.94	0.938	0.022
7	MC = - 0.217- 0.048 (Loss of water) + 0.184 DesW	2	<0.001	0.95	0.943	0.021

analysis was useful to generate a model for predicting the MC of seeds, as also noted earlier for some other recalcitrant tropical fruit seeds (Hasmah *et al.*, 2012; Soeswanto *et al.*, 2021). MC of *C. liberica* seed could simply be predicted with its most significant determinant in terms of weight loss due to desiccation, i.e. (Loss of water). The higher the (Loss of water) was, the lower the MC of seeds could be expected as shown by the model  $MC = 0.185 + 0.118 FW - 0.412$  (Loss of water), while FW appeared as part of the intercept since FW was not significantly correlated to MC. It was also encouraging to note that the MC of seeds could be predicted with two seed units, which is a convenient and small sample. However, this equation was probably a controlled model; the initial weight (FW) of the seed must be known for predicting the MC of the seed after desiccation treatment.

The non-destructive quantification of MC of seeds through a practical equation could be valuable for several applications with intermediate and recalcitrant seeds (Hong *et al.*, 1996; Lopes and Neto, 2020; Ellis, 2022; Trusiak *et al.*, 2023). With a reliable MC estimate, the seeds could subsequently be distinguished, stored and conserved with suitable techniques for future uses (Xu *et al.*, 2019; Wang *et al.*, 2021; Wang *et al.*, 2023). However, it could be unlikely to have a universal predictive model for all the intermediate and recalcitrant seeds since these desiccation-sensitive seeds differ considerably but generally have a narrow range for the lowest safe MC. At least, a species-specific equation for a defined moisture regime, especially around the lowest safe MC, would be advantageous. Despite the convenience of modelling for predicting the MC of seeds, caution should be taken concerning the estimates on seed viability as other factors of geographical origins, handling and storage conditions could also be crucial

beyond the MC - survival relationship of the seeds.

## 5. Conclusion

MC of *C. liberica* seeds could be predicted with two seed units, instead of the destructive oven drying method, allowing conservation potential for the future genetic improvement of this beverage crop. The model for MC estimation was as follows:  $MC = 0.185 + 0.118 FW - 0.412$  (Loss of water).

## Conflict of interest

The authors declare no conflict of interest.

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