



# The Effect of Drying Techniques on the Antioxidant Capacity, Flavonoids and Phenolic Content of Fermented Local Cocoa Bean

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## ABSTRACT

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The effect of drying techniques namely sun drying, freeze drying, and oven drying on the antioxidant capacity, total phenolic and flavonoid contents of 3 clones of fermented local cocoa bean (BR25, MCBC6, PA76) was determined. The highest cocoa bean extraction yield was found using freeze drying, followed by sun drying, and oven drying method. The highest total phenolic content was obtained from freeze drying of BR25 with value of  $1380.86 \pm 4.21$  mg GAE/g, and the lowest was found in oven drying of PA76 with total phenolic content of  $672.95 \pm 1.35$  mg GAE/g. For total flavonoid content, the highest value was found from freeze drying method of BR25 with value of  $4536 \pm 10.37$  mg QE/g and the lowest was in oven drying of PA76 with flavonoid content of  $2639 \pm 7.07$  mg QE/g. Lower IC<sub>50</sub> value indicated a stronger antioxidant activity of extracts. Results obtained show the same trend where the lowest IC<sub>50</sub> value was obtained in freeze dry followed by sun dry and oven dry method. The lowest IC<sub>50</sub> value was found in for freeze drying techniques of PA76 with value of  $4.70 \pm 0.37$  mg/ml and the highest was found in oven drying of MCBC6 with IC<sub>50</sub> value of  $369.17 \pm 1.29$  mg/ml. Freeze drying method was found to be the best method to preserve highest amount of total phenolic and flavonoid content.

### Keywords:

Drying methods, cocoa beans, antioxidant activity, polyphenols

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## 1. Introduction

Cocoa bean (*Theobroma cacao* L.) has become one of the leading commodity crops in Malaysia after oil palm and rubber. Statistics by Azhar and Lee [1] showed that up to 15,000 hectares of cocoa were planted around Malaysia, with 5,000 hectares planted in Sarawak, 5,500 hectares in Sabah and the remaining 4,500 hectares planted in Peninsular Malaysia. The demand for cocoa and cocoa-based products is high now and this high demand is expected to be continuously increased or maintain for a few more years. The export of Malaysia's cocoa-based products such as cocoa butter, cocoa powder, cocoa paste, and chocolates have also showed increasing trend from previous 1992 until last year 2017 [17]. These products are believed to consumed in great demand

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worldwide due to its unique flavour and aroma that cannot be replaced by product of other types of plant.

The discovery of the active phenolic compounds in cocoa has gained interested in research of its various beneficial effects on ageing, blood pressure regulation, oxidative stress, and atherosclerosis and has changed some negative perception of high fat content of cocoa products [9]. In recent years, research focusing on cocoa polyphenols has increased, mainly on flavonoid compounds due to known benefit as antioxidant source for health reasons.

Phenolic of polyphenols are one of the main secondary metabolites which generally found in edible and non-edible plants. Phenolic compounds in cocoa bean are responsible for its pungent together with its bitter taste [2]. Furthermore, phenolic compounds has antioxidant properties as it acts as catechol group acting as electron acceptor thus responsible for antioxidant activity [8].

Cocoa genotype, soil, climate, harvest condition, and processing method of cocoa such as fermentation, drying and roasting, causes important effects on the characteristics of cocoa [3]. Some researchers also reported that final quality of cocoa product is greatly influence by the drying methods of the cocoa. Commonly, there are few steps done by cocoa manufacturers to prepare the cocoa bean before it can be marketed. Cocoa beans are usually transformed into useful edible product by a complex process namely fermentation, drying, and roasting [21]. The efficiency of the processing and manufacturing procedure play an important role in maintaining a good quality of cocoa beans. Past studies articulated that different drying method seems to produces a great change in the quality of cocoa beans since it resulted in formation of cocoa flavour precursors that develop mainly during roasting of cocoa bean.

Malaysia's cocoa products vary in quality as different holders use different processing methods. There has also been a survey investigating various drying method used by smallholders from different regions in Malaysia, including Sabah, Sarawak, Perak, and Pahang. The survey showed that cocoa was dried mostly using natural technique (87.5%), others were artificially dried (6.3%) or dried using combined techniques (6.3%) and it is reported to contribute to a different in quality of cocoa beans [8]. Therefore, this study was done to determine the effect of different drying techniques namely freeze-drying, sun-drying, and oven-drying of different clones of local cocoa beans in correlation to their antioxidant capacity, flavonoid, and phenolic content.

## 2. Materials and Methods

### 2.1 Chemicals and Reagents

Aluminium chloride ( $\text{AlCl}_3$ ), sodium carbonate ( $\text{Na}_2\text{CO}_3$ ), ascorbic acid, 2,2-diphenyl-2-picrylhydrazyl (DPPH), Folin–Ciocalteu reagent, quercetin, sodium hydroxide (NaOH) were purchased from Sigma Aldrich (Subang Jaya, Malaysia); ethanol, gallic acid and sodium Nitrate ( $\text{NaNO}_2$ ) were from Merck (Petaling Jaya, Malaysia).

### 2.2 Sample Collection and Fermentation

All 3 of the cocoa bean clones (B225, MCBC6, PA76) were harvested directly from cocoa trees at Cocoa Biotechnology Research Centre (KKIP), Sabah. Fermentation process was done based on method provided by Lopez *et al.*, [16] with slight modification. Harvested cocoa beans fruits was first opened and the seeds were transferred into a plastic container for fermentation. The beans in the container was constantly turned for every 48 h and stopped after 5 days. The drainage of acidic liquid due to liquefaction of mucilaginous pulp and aeration of the cocoa beans was done by

pouring out the juices out of the plastics container. The fermentation process was carried out for 5 days.

### 2.3 Drying Procedure of Cocoa Beans

Each of the clones of fermented cocoa bean (B225, MCBC6, PA76) was divided into 3 portions for drying purpose. Each clone with 3 portions was dried using three different methods, which were sun-drying, oven-drying and freeze-drying.

### 2.4 Sun-Drying

Sun-drying method of the cocoa beans was performed according to method proposed by Prasanna *et al.*, [20]. Each clones of cocoa bean was dried under direct sun light. The beans were spread uniformly (not more than 4cm) on boards and left on the ground under the sunlight. The sample was only dried on a sunny day from 9:00 AM until 5:00 PM. The partly dried cocoa bean was wrapped in polyethylene cover and keep at room temperature. The process was repeated until the beans were fully dried. The beans were stirred occasionally to ensure even drying and protected from rain. The sun-drying lasted for 5 days.

### 2.5 Oven-Drying

Fermented cocoa beans were dried using oven based on method proposed by Santhanam *et al.*, (2017). Cocoa samples of each clone were placed in an oven and dried at 55°C. The beans were weighted every 2 hours and stopped after constant weight was obtained. The heating was stopped after 48 hours.

### 2.6 Freeze-Drying

Cocoa beans were dried using freeze dryer method. The cocoa bean was first being treated at -80 °C for 24 hours to allow the freezing of any moisture content of the samples. Then the samples were placed in the tubes of freeze-drier for 72 hours.

### 2.7 Preparation of Cocoa Bean Extract

Extraction of cocoa was conducted according to method by Velioglu *et al.*, [23] with modification. Grounded cocoa was extracted using a 70% ethanol aqueous solution for 30 minutes at 20 Hz using a sonicator. The extractions were conducted using a fixed 40 mg sample/mL solvent ratio. After extraction, the crude extract was filtered under vacuum condition through whatman no 1 filter paper to obtain a clear extract solution. These solutions were directly appropriate concentration for further analysis of DPPH scavenging, assay, total phenolic content, and flavonoid content.

### 2.8 Extraction Yield

After extracting the cocoa beans in 70% ethanol, the liquid sample was concentrated to using a rotary evaporator and further air-dried in fume hood at room temperature until constant weight.

The final weight of the dried crude extract was used for the determination of percentage yield using equation:

$$\text{Extraction yield (\%)} = \frac{\text{weight of dried crude extract (g)}}{\text{weight of grounded cocoa powder (g)}} \times 100 \quad (1)$$

### 2.9 Determination Total Phenolic Content

The phenolic content of the cocoa was evaluated according to method prepared by Singleton *et al.*, [22]. Ethanolic cocoa extract (0.4ml) was mixed with 10% Folin-Ciocalteu reagent of 1.6ml and left for 5 minutes before added with 4ml of 2% sodium carbonate. The mixture was then left for 60 minutes in dark room. After that, the mixture was taken for UV-VIS spectrophotometer reading at 765nm. The calibration curves of gallic acid diluted in water (0.31-0.5mg/ml) as standard was constructed and recorded. All results were expressed as milligrams of gallic acid equivalents (GAE) per gram dry weight (DW) of cocoa.

### 2.10 Determination of Flavonoid Content

The determination of total flavonoid content was carried out based on method by Metussin, *et al.*, [18] with slight modification. Aliquot of 2 ml cocoa extract was pipetted into a test tube and mixed with 0.6ml of  $\text{NaNO}_2$  (5% w/v) and left for 5 minutes. After that, 1.0ml of  $\text{AlCl}_3$  was added and  $\text{NaOH}$ (1.0 ml) was then added after another 6 minutes. The solution was shaken and left aside for 10 minutes before the absorbance was measured using UV-VIS spectrophotometer at 510 nm. Quercetin was used the standard and prepared by diluting it into various concentration of 0.10-0.30 mg/ml. The total flavonoid content was expressed in milligram quercetin equivalent per gram dry weight sample of cocoa (QE mg/g of sample).

### 2.11 2,2-Diphenyl-2-Picrylhydrazyl Radical Scavenging Assay

Antioxidant capacity was analyzed using DPPH radical scavenging assay based on method proposed by Othman *et al.*, [19] with slight modification. A DPPH (500  $\mu\text{M}$ ) solution was prepared by diluting it in 70% ethanol. An aliquot of 0.6ml cocoa extract mixed with 3.0 ml DPPH solution in test tube. The solution was shaken vigorously and left in a dark room for 20 minutes at room temperature. After 20 minutes, the absorbance was measured using a UV-VIS spectrophotometer. Duplicate measurement was carried out. Ascorbic acid was used as a standard compound with various concentrations (0.031-0.5 mg/ml). The scavenging activity of DPPH was calculated as follows:

$$\text{DPPH Scavenging (\%)} = 1 - \frac{\text{abs of sample}}{\text{abs of control}} \times 100 \quad (2)$$

The  $\text{IC}_{50}$  values of the crude extracts with respect to DPPH scavenging was also determined. The  $\text{IC}_{50}$  values of cocoa extracts were estimated from the DPPH scavenging for different extract concentrations being the concentration that inhibits 50% of DPPH activity. By plotting the percentage inhibitions against the extract concentrations, the  $\text{IC}_{50}$  value was determined through a nonlinear regression analysis using GraphPad Prism 6 trial version (GraphPad Software Inc, La Jolla, CA, USA). The results for  $\text{IC}_{50}$  were reported as mean of duplicate results.

### 3. Results and Discussion

#### 3.1 The Effect of Drying Techniques on Extraction Yield

Drying techniques are one of the main factors effecting extraction yield. Each drying methods have different effects on microstructure, therefore the quality of dehydrated products [7]. Table 1 shows the percentage extraction yield comparison between different drying techniques. The yield decreases from freeze drying>sun drying>oven drying for all 3 clones of cocoa, the highest yield was obtained from BR25 (12.49±1.36%) using freeze dry method, and the lowest was obtained from oven drying of PA76 cocoa beans with yield of (4.38±1.85%). The results proved that drying techniques does affect the amount of preserved compound in the samples. Kunal *et al.*, [13] reported that freeze drying method gives advantages such as reducing loss of volatile or heat-sensitive substances which leads to higher yield obtained using freeze dry methods.

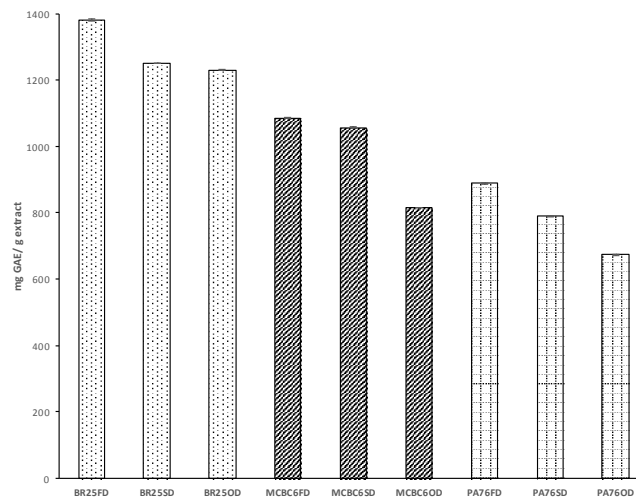
**Table 1**

Percentage yield of crude extracts

Crude extract	percentage yield of different drying techniques (%)		
	Freeze dry	Sun dry	Oven dry
BR25	12.49±1.36	10.8±2.42	5.51±2.76
MCBC6	8.39±2.53	6.85±2.85	6.12±3.52
PA76	10.06±1.43	6.16±1.42	4.38±1.85

#### 3.2 The Effect of Drying Techniques on Total Phenolic Content (TPC)

Several main phenolic compounds have been found and identified in cocoa beans which include epicatechin, galocatechin, epigallocatechin, flavone and some minor compounds [12]. In relation to this study, the TPC of different cocoa extracts was determined by spectrophotometry using Folin-Ciocalteu assay and expressed as gallic acid equivalent (GAE).



**Fig. 1.** Comparison between TPC of different clone of cocoa bean dried using freeze dry, sun dry and oven dry

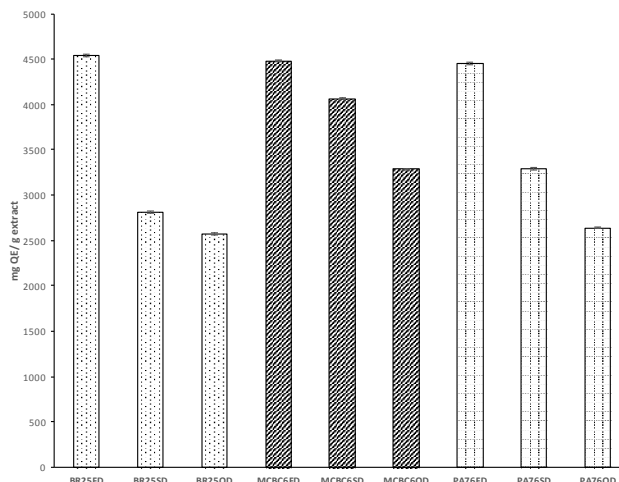
The effects of drying techniques on TPC of 3 different clones (BR25, MCBC6 and PA76) can be seen in Figure 1. Based on the results, it can be observed that every cocoa shows the highest TPC in freeze drying technique followed by sun drying and the lowest TPC was in oven drying of each

cocoa clones. TPC obtained from freeze drying of 3 clones are  $1380.86 \pm 4.21$  mg GAE/g for BR25,  $1084.26 \pm 1.52$  mg GAE/g for MCBC6, and  $888.55 \pm 1.35$  mg GAE/g for PA76. The obtained results of higher TPC in freeze drying is in accordance with that of Irondi *et al.*, [10] which also reported that freeze dry resulted the highest preservation of phenolic in Carica papaya seed compared with other drying techniques that includes oven drying. Similar result was also reported on different products, where polyphenol compounds such as catechin was retained by freeze drying process. Freeze drying techniques which dries the samples based on lyophilization principles is the gentlest drying technique in order to preserve temperature sensitive compounds (Youssef and Mokhtar, 2014). Lyophilization techniques work in such ways where it develops ice crystal within the tissue of the samples cocoa that allow high efficiency of extraction of phenolic compound. This can be explained by greater rupturing of cell structure of the cocoa causing by the ice crystal formations, that lead to better solvent access and more efficient extraction [10]. Another reason for higher TPC by freeze-drying is due to the inactive enzymatic activity occurs in the samples when freeze. This is because there is a very low temperature used to freeze the samples and dried under vacuum. The low temperature also has lead impeded browning process.

On the other hand, sun-drying method resulted in lower TPC, which are  $1249.71 \pm 1.52$  for BR25,  $1056.85 \pm 1.43$  mg GAE/g for MCBC6, and  $787.05 \pm 1.52$  mg GAE/g for PA76. While oven drying method, TPC obtained are  $1229.93 \pm 1.18$  mg GAE/g for BR25,  $815.10 \pm 1.01$  mg GAE/g for MCBC6, and  $672.95 \pm 1.35$  mg GAE/g for PA76. Besides sun drying, Lower TPC was also found in oven dry when compared to freeze dry. Youssef and Mokhtar [25] reported that drying process involving thermal energy in sun dry and oven dry can affect the phenolic content by thermal breakdown. This will damage the cell structure of the cocoa samples thus allow migration of component such as enzymes, light and also oxygen. The binding of phenolic compounds to migrated component such as protein, caused changes in chemical structures or low extraction efficiencies, and also may breakdown phenolic compound. This scenario therefore is related to the loss in total phenolic content value [11]. This results also supported by Lim and Murtijaya [15] where it was found that some phenolic compounds are easily decomposed in direct sunlight or if dried at elevated temperature.

### 3.3 The Effect of Drying Techniques on Total Flavonoid Content (TFC)

Total flavonoid content method was conducted by using spectrophotometric assay based on aluminium complex formation. The cocoa extracts from freeze drying technique exhibited the highest flavonoid content followed by sun drying and oven drying technique based on the flavonoid content assay. Overall, the effect of drying techniques on TFC showed similar trend with TPC. It may be because flavonoids are also categorized as phenolic compounds, hence, the similar trend was observed. The reduction of flavonoid content as observed in figure 2 was also reported by Vina *et al.*, [24] in which application of drying at temperature of  $50\text{ }^{\circ}\text{C}$  caused loss of flavonoid in Brassica oleracea L. gemmifera DC. In addition, it was also found that many flavonoid compounds are actually heat sensitive [5].



**Fig. 2.** Comparison between flavonoid content of different clone of cocoa bean dried using freeze dry, sun dry and oven dry

### 3.4 The Effect of Drying Technique on Antioxidant Capacity

The molecule of DPPH is characterised as a stable free radical by virtue of DPPH ability to capture free radicals caused by the delocalization of the unpaired electron all over the molecule. This assay determines the scavenging of stable radical species of DPPH by antioxidants. In antioxidant assay, the comparison was made based on  $IC_{50}$  values, which is the extract concentration needed to inhibit 50% of DPPH scavenging. Thus, a low  $IC_{50}$  value is preferable as it represents good activity of a tested extract [10].

**Table 2**

$IC_{50}$  of different clone of cocoa bean dried using freeze dry, sun dry and oven dry

Crude extract	DPPH $IC_{50}$ (mg/ml)		
	Freeze dry	Sun dry	Oven dry
BR25	6.47±0.03	17.65±0.36	133.47±1.11
MCBC6	16.55±0.21	47.29±0.99	369.17±1.29
PA76	4.70±0.37	67.40±0.53	145.56±1.27

The results showed that the lowest  $IC_{50}$  value was obtained via freeze drying, followed by sun drying and oven drying respectively. Similarly, freeze drying technique proved to retain the highest free radical scavenging effect with oven drying retain the lowest. This is due to the mild condition provided in freeze drying techniques that helps to reduce the degradation of bioactive compound that might contributed to the antioxidant activity of the cocoa bean.

For oven drying method,  $IC_{50}$  obtained are at higher values. This might be linked with the degradation of antioxidant related compounds, which caused lower antioxidant activity of cocoa extracts. Different clones of BR25, MCBC6 and PA76 of cocoa indicated different values of  $IC_{50}$ . However, all of the scavenging activity of the cocoa clones showed a similar trend, in which inhibition activity decreased from freeze drying to sun drying and oven drying (data not shown). This is in agreement with the fact that the antioxidant activity of cocoa bean does correlate with the content of phenolic compounds including flavonoids [9].

#### 4. Conclusion

It was found that the content of phenolic and flavonoids and its associated antioxidant capacity vary among different clone of cocoa in which the highest was obtained from cocoa BR25>MCBC6>PA76. Among the drying techniques used, freeze dry techniques was shown to produce highest TPC, TFC and antioxidant activity followed by sun dry and oven dry. This study has been beneficial in widening the knowledge on the effect of different drying techniques on the content of phenolic, flavonoids and antioxidant activity of cocoa bean.

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