EFFECTS OF BLANCHING AND DRYING ON THE PRODUCTION OF POLYPHENOLS RICH COCOA BEANS AND PRODUCT QUALITY

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"To my beloved parents"

DECLARATION

I hereby declare that the present work is prepared solely by me during the course of my doctoral studies at the University of Nottingham Malaysia Campus (UNMC). It has not been submitted anywhere for any awards. Works of other people are fully acknowledged according to standard referencing.

This thesis complies fully with the regulations set by the University of Nottingham.

Abhay S. Menon
19th October 2016

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ABSTRACT

The high potential of health beneficial polyphenols and antioxidants in cocoa beans has been a major topic for research in recent years. The large-scale application of cocoa beans for health beneficial compounds is relatively unexplored and it needs to be widely utilized by pharmaceutical and nutraceutical industries. Processing methods such as fermentation and drying are major deterrents for recovering high polyphenols in cocoa beans.

Hence, it was the intention of this work to introduce the application of hot water blanching pre-treatment and various drying methods for producing polyphenols rich cocoa beans by using unfermented beans. The studies incorporated the application of various drying methods such as oven, adsorption, vacuum, freeze and sun drying methods on cocoa beans. The studies compared the ability of these drying methods to preserve the bioactive capacities namely, total polyphenolic contents and antioxidants activity after hot water blanching. The potential of adsorption, vacuum and freeze drying methods for recovering high polyphenols content are useful in comparing it with the conventional cocoa drying methods such as oven and sun drying methods.

For the studies on oven drying of cocoa beans, the drying parameters (T= 60°C, 70°C and 80°C) used were similar to the conventional hot air drying parameters used in industries. The total polyphenolic contents of fermented cocoa beans dried at 70°C was found to be the highest. The polyphenols degradation kinetics for oven drying method of cocoa

beans was determined using first-order reaction kinetics model based on various drying temperatures and durations of drying.

The studies on drying kinetics of fresh cocoa beans dried using oven, vacuum, adsorption and sun drying methods were successfully analysed. It was found that adsorption drying and vacuum drying methods dried cocoa beans faster than oven and sun drying methods. Two respective falling rate periods were recorded by adsorption and vacuum drying. The effective diffusivities were determined and were found to be in accordance to that of published literatures.

Hot water blanching pre-treatment were performed for fresh and fermented cocoa beans (whole beans and half cut). Blanching pre-treatment method was found to show significantly higher total polyphenolic contents when compared with unblanched cocoa samples. The optimal blanching parameter (90°C for 5 min) obtained for fresh beans were subsequently used for experiments involving fresh cocoa beans. The total polyphenolic contents and antioxidant activity of blanched and unblanched cocoa beans were analysed. Results showed that both adsorption and vacuum drying methods showed high recovery of polyphenolic compounds and antioxidants on comparison with freeze dried cocoa samples, which was used as a benchmark in quality analysis of food products. High polyphenols contents were achieved after the blanching and drying treatments and were noted to be significantly higher on comparison with published literatures.

Sensory analysis of both blanched and unblanched cocoa beans were analysed after drying using various drying methods. The results for unfermented cocoa beans showed

high astringency flavour attributes which further confirmed the high contents of polyphenols in cocoa beans. The cocoa and acidic flavour attributes were recorded to be less owing to the unfermented nature of cocoa beans.

The results obtained provides a gateway towards the use of advanced drying technology in cocoa industry. The potential of blanching pre-treatment to mediate high recovery of cocoa polyphenols after drying has been proven through this work. The processing methods used in the current study can be implemented in on-farm cocoa processing, making it a more sustainable farming option.

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CHAPTER 1

INTRODUCTION

1.1. Background

1.1.1. Cocoa

Cocoa tree belongs to the genus *Theobroma*, a group of small trees which has its origin set in the rainforests of Amazon basin and other tropical areas of South and Central America. Cocoa beans are grown in a tropical belt straddling the equator (10° and 20° north to south) in the area called the “cocoa belt” [1]. The trees can be 12 meters tall and starts bearing fruits after 5 years and takes 10 years to reach maximum yield.

The fruit is called a pod or cabosside and contains about 20 to 40 seeds (cocoa beans) with colour ranges from brownish yellow to purple. Each plant produces 20 to 50 pods a year; where approximately 10 pods produce about 1 to 1.5 kg of dry cocoa beans. The three main groups of cocoa which can be found worldwide are Criollo, Forastero and Trinitario [2]. About 71 % of world cocoa beans production is from African countries; where Ivory Coast is the highest producer of cocoa with 33% of total global supply [3]. Cocoa production in Asia-Oceania region is considerably lower with an overall global production share of 10.5 %. In Asia-Oceania, Indonesia is the highest producer of cocoa followed by Papua New Guinea and Malaysia according to 2014/2015 statistics by World Cocoa Foundation [4].

1.1.2. Cocoa industry in Malaysia

Malaysian cocoa industry was established in 1950 and the first commercial cultivation site was in Jerangau, Terengganu. According to the 2015 Malaysian Cocoa Board statistics, 18150 hectares of land were used for cultivation and produced 1729 tonnes of dried cocoa. However, there has been a substantial decrease in the overall production of cocoa from the year 2000 onwards. In 2000, the overall production of cocoa beans was approximately 70,262 tonnes [4]. The reasons for the sudden decline in cocoa production are due to (i) the overall price decrease of cocoa per tonne in global market (ii) long growth period to attain maturity to bear fruits and (iii) farmers shift to more viable commercial agricultural products such as oil palm [5].

From a recent study, 95 % of cocoa producers are smallholders and hence they are the dominant producers of cocoa in Malaysia [4]. Although the production of cocoa in Malaysia has declined over the years, Malaysia is a major exporter of cocoa products such as cocoa paste, cocoa butter and cocoa powder. The raw cocoa beans in such instances are mainly sourced from import markets and the overall turnover was estimated to be RM 4.2 billion in 2015 [4]. Cocoa powder and cocoa butter are the main products used for manufacturing of various food products (e.g. chocolates, beverages and confectioneries) and non-food products (e.g. toiletries, pharmaceuticals and cosmetics) globally [6].

1.1.3. Processing of cocoa beans

During harvesting season, cocoa pods are harvested from the plantation and the beans are subjected to fermentation and drying processes before they are stored. Various

methods are used for fermentation and drying which are still very primitive and only involves low levels of technical expertise. Most smallholders process cocoa by fermenting fresh cocoa beans in large wooden boxes with gunny sack lining and practice traditional sun drying to dry the beans [7,8]. Microorganisms such as yeast and bacteria (from the surrounding air), naturally carry out fermentation. The quality of dried cocoa produced significantly depends on the drying method used. Drying process not only helps in removing moisture from cocoa beans for long storage period, but also to preserve the quality attributes necessary for chocolate production [9]. The current large scale drying (both artificial and natural) of cocoa beans are relatively crude and inefficient. For example, wood furnaces are commonly used to supply hot air for drying. The smoke emitted from the furnace could contaminate the beans and give it a smoky flavour attribute [10] mostly due to the presence of polycyclic aromatic hydrocarbons [11]. In such artificial drying method, the drying temperature used is usually above 60°C. The high drying temperatures results in high drying rates and shortens drying duration. This is not suitable for flavour development precursor enzymes which give cocoa beans the distinct cocoa flavour during the roasting process. The fast drying time leads to the presence of highly acidic compounds in the beans which is undesired due to the presence of volatile compounds from insufficient evaporation.

1.1.4. Cocoa Polyphenols

The high polyphenolic contents of cocoa beans have been major focus of research in recent years. The dietary flavonoids in cocoa are proven to be higher than red wine or green tea per serving [12]. Various benefits of cocoa polyphenolic compounds include

anti-carcinogenic, anti-thrombotic, anti-ulcer, anti-atherogenic, anti-inflammatory, immune modulating, anti-microbial, vasodilatory and analgesic effects [13]. Browning of cocoa beans is a process that leads to a substantial degradation of cocoa polyphenols due to the activity of polyphenol oxidases. This reaction produces the typical brown colour in cocoa beans desirable for chocolate manufacturer. It is reported that fermentation, drying temperature and humidity has a significant role in polyphenol based browning process [14]. Although, cocoa beans with high polyphenolic contents have higher astringency and bitterness scores which are found to be undesirable traits for conventional consumption [15], but consumption of polyphenol rich chocolates with high antioxidant activity is proven to have immense health benefits [16]. This has paved ways to the development of huge demand for polyphenol rich cocoa in the current generation of consumers who are health conscious.

1.2. Problem Statement

Fresh cocoa beans which are not fermented have high polyphenolic contents and antioxidant capacity. With the health benefits potential of cocoa kept in view, pharmaceutical and nutraceutical industries start to look into the adaptation of cocoa for the production of supplements or similar cocoa based products with high medicinal value. This would not only establish a high demand for cocoa from the current situation but will also lead to a significant increment in the present market value of cocoa beans in Malaysia. Thus, mass production of polyphenol rich cocoa beans based on a convenient and cost effective pre-treatment and drying method should be envisaged.

Reported research on the effects of drying on cocoa bean quality were primarily focused on the development of cocoa flavour attribute which was beneficial for the chocolate industry [17]. The majority of the research focus was on conventional hot air and sun drying methods. Since conventional hot-air drying process is usually carried out at high temperatures, this drying method is not optimal for polyphenolic recovery from the dried cocoa beans. Non-conventional advanced drying method such as heat pump drying has been used by Hii, *et al*. [18] and showed high recovery of cocoa polyphenolic compounds after drying. The authors reasoned the mild drying conditions namely low temperature and relative humidity for this activity. Since the application of advanced artificial drying technologies in cocoa industry is low, it is important to look into new alternatives for drying.

The current study focusses on the application of adsorption, vacuum, freeze, oven and sun drying methods for the production of polyphenol rich cocoa beans. The application of adsorption drying for cocoa drying has not been reported in any literature. Adsorption drying is an advanced drying method which uses a hygroscopic adsorbent to reduce the inlet air-moisture content by adsorption process. This leads to faster drying of the sample at low temperature condition. The use of adsorption drying on agricultural products was proven to retain higher bioactive compounds such as polyphenols after drying [19,20]. The application of vacuum drying employs the use of low pressure conditions (<200mbar) which considerably reduces the oxygen level in the drying chamber. Studies have shown that lack of oxygen decreases the enzymatic activity of bioactive compounds in many agricultural products [21–23]. For cocoa drying,

the use of vacuum drying technology for the production of high quality beans . Oven drying (hot air) has been widely used for the drying kinetics analysis and quality evaluation of fermented cocoa beans [8,24,25]. Freeze drying method for cocoa processing has been used as a benchmark for quality assessment. Besides, it is the most efficient drying method to retain the highest amount of bioactive compounds [26,27]. Sun drying has been extensively used to dry food products since ancient times and particularly effective for small scale drying of cocoa beans. However, sun drying has many disadvantages pertaining to long drying time, exposure to contaminations, dependency on weather, pest and insect attack and high labour/land area requirements.

In the past, incorporation of pre-treatments for quality improvement of cocoa beans were only focused on cocoa pulp preconditioning for enhancing cocoa flavour in the dried product [28,29]. In the current study, the application of hot water pre-treatment on cocoa beans before drying (adsorption, vacuum, freeze, oven and sun) were utilised for the production of polyphenol rich cocoa beans. The thesis is divided into 8 Chapters and the following flowchart (Figure 1.1) provides information on the thesis outline.

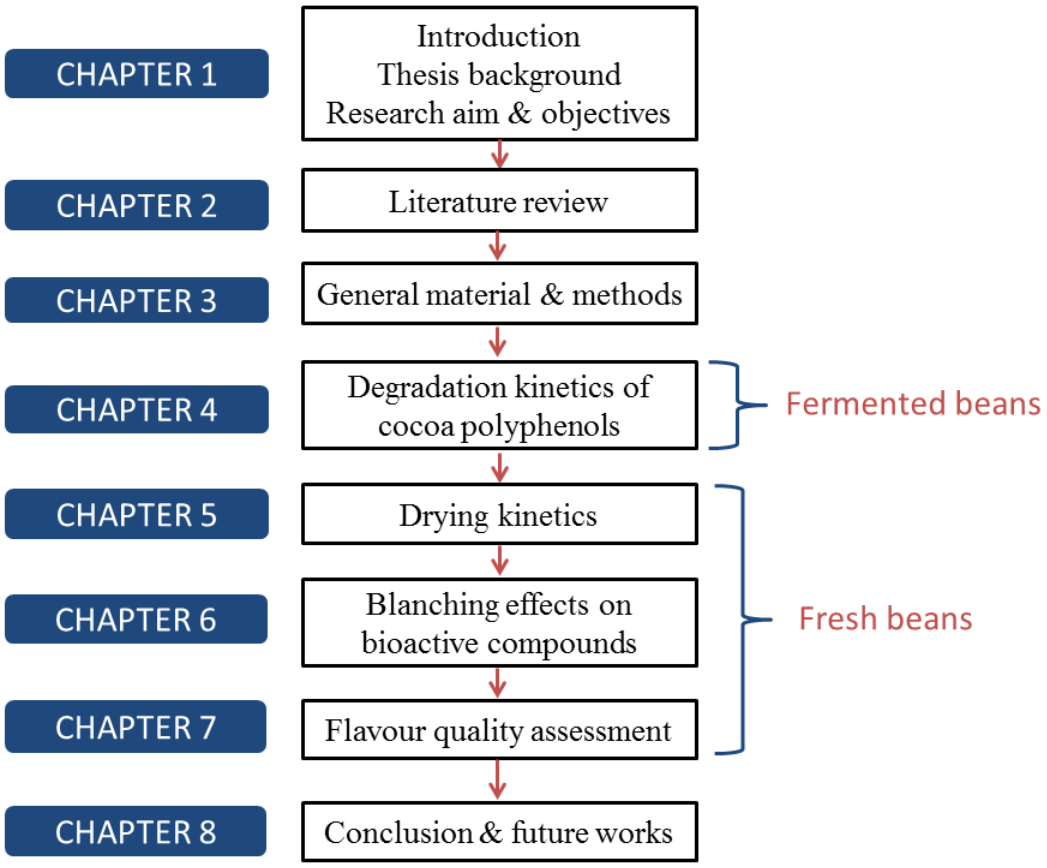


Figure 1.1: Flow chart representing the overall thesis

1.3. Research Objectives

Overall objective for this research was to develop a processing method combining hot water blanching pre-treatment and an optimum drying method to produce cocoa beans containing high polyphenolic contents and increased antioxidant activity. The main drive to develop such processing method was to explore the potential of producing dried cocoa beans with high polyphenols content which can be implemented in current on-farm processing. Specific objectives of this research were as follows:

- To evaluate the effects of drying temperature on the degradation kinetics of cocoa polyphenols during hot air drying.
- To evaluate the drying kinetics of fresh cocoa beans under various drying methods.
- To evaluate the effects of hot water blanching on the total polyphenol content and antioxidant activity of fresh cocoa beans.
- To evaluate the flavour quality of blanched and unblanched fresh cocoa beans after drying.

1.4. Research scopes

1.4.1. Degradation kinetics of cocoa polyphenols during conventional cocoa drying practices

Fermented cocoa beans were dried in a hot air oven at three temperature settings; 60°C, 70°C and 80°C for drying exposure times of 12 h, 24 h, 32 h and 40 h. The polyphenol degradation mechanisms due to temperature and exposure time were analysed. The drying kinetics of cocoa beans were examined based on the drying curves and rates of drying. This Chapter focusses on the polyphenolic degradation of cocoa beans, using the drying conditions prevalent in conventional cocoa processing methods (oven drying in temperatures above 60°C) used commercially.

1.4.2. Drying kinetics of fresh cocoa bean

Fresh cocoa beans were dried using adsorption drying (ca. 60°C for 24h), vacuum drying (60°C, P=150 mbar for 24 h), oven drying (70°C for 30 h) and sun drying (ambient air condition, 36 h) methods. The moisture reduction processes were monitored

throughout drying. The drying air temperature and bean temperatures were recorded. The drying kinetics was examined and compared based on the drying curves and rates of drying.

1.4.3. Effects of blanching on total polyphenolic content of fresh cocoa beans

The effects of blanching pre-treatment on polyphenol activity of cocoa beans (whole bean and half cut) cocoa beans after oven drying were analysed. The optimal blanching parameter observed for maximum polyphenol recovery of fresh beans was used for the subsequent drying methods (adsorption, vacuum, freeze, and sun). The total polyphenol content and antioxidant capacity were examined after blanching pre-treatment and drying application.

1.4.4. Flavour evaluation

Sensory evaluation was carried out by examining the cocoa liquor of fresh cocoa beans processed after blanching pre-treatment and drying (adsorption, vacuum, freeze, oven and sun). The flavour attributes examined were cocoa flavour, astringency, bitterness and sourness. Comparisons were benchmarked against commercial cocoa samples of from Ghana.

1.5. Contribution of Research

The cocoa industry over the years have solely focused on supplying cocoa for production of various food products (chocolates, beverages and confectioneries) and non-food products (toiletries, pharmaceuticals and cosmetics). The proposed methods adopted in current research will provide an alternative method for the cocoa industry in producing

high quality polyphenols rich dried cocoa beans. Polyphenol rich cocoa beans would have a high market demand not only in food industries but also in pharmaceutical and nutraceutical industries. This will also help in generating lucrative revenue for the cocoa farmers and making cocoa a more sustainable farming option that will benefit both the upstream and downstream of cocoa industries.

CHAPTER 2

LITERATURE REVIEW

2.1. Cocoa processing

The two major steps involved in cocoa processing are fermentation and drying. In majority of cocoa industries, fermentation process is usually carried out near the cocoa farms. Fermentation is required to develop acidity surrounding the beans and increase in temperature that would eventually lead to the degradation of the beans. This process is important to develop the flavour precursors necessary in chocolate manufacturing. Fermentation gradually ends at the onset of drying process. During drying, enzymatic activity occurs which develops flavour attributes necessary in chocolate manufacturing. Figure 2.1 shows the pictures of cocoa tree, pods, fresh cocoa beans and dried cocoa beans.

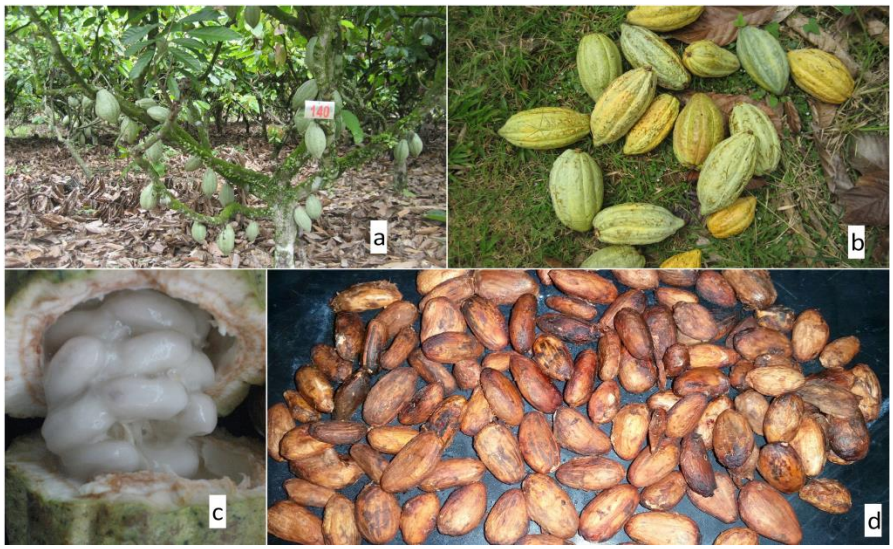


Figure 2.1: Pictorial representations (a) cocoa tree (b) cocoa pods (c) fresh cocoa beans and (d) dried cocoa beans.

2.1.1. Cocoa fermentation

In general, cocoa pods that are still attached to cocoa trees are sterile in nature and immune to any microbial interactions. When the pods are opened after harvesting, the microbes present in the air, the harvester's body and container used to transport beans gets inoculated into the bean. The fresh beans have mucilaginous outer pulp residues which are rich in sugars. The pulp provides an optimal condition for the growth of inoculated microorganisms. In pulp body of the beans, microbial activities of a succession of yeast, lactic acid bacteria (LAB) and acetic acid bacteria (AAB) results in the formation of lactic acid and acetic acid. Consequently the depectinization and liquefaction of the pulp substrate occurs [30]. This process is known as external pulp fermentation of beans and occurs within the first 3 days of fermentation [10]. As the acid constituents and heat of fermentation increases, the embryo of cocoa beans degrades. This leads to death of the bean and triggers the internal fermentation of cocoa beans [31].

During internal fermentation process, several chemical processes occur inside the bean cotyledons. The proteins and sucrose inside the beans degrades to form enzymes, which serve as major flavour precursors for cocoa [31]. This degradation process is also known as anaerobic hydrolytic phase of internal fermentation process [32]. Upon degradation of cocoa cell walls, oxygen diffuses into the cotyledon tissues and oxidizes. The oxidation occurs due to the activation of oxidases enzymes present within the vacuoles of cocoa cell body. The formation of high molecular weight tannis (brown pigments) occurs when the cocoa polyphenols are oxidized by the activity of polyphenol oxidases

enzymes [31]. The oxidation of polyphenol constituents are reported to continue throughout drying process [33]. The other processes which may reduce polyphenol contents during fermentation are non-enzymatic degradation of polyphenols (Maillard reaction) and diffusion of soluble polyphenols into the fermentation sweating [34].

Various fermentation methods are employed by farmers but heap and box fermentation processes (Figure 2.2) are commonly used by cocoa producers [10]. The box fermentation method is further categorized into deep and shallow box depending on the box size. The shallow fermentation box as shown in Figure 2.3 has a depth of 0.3 m. Shallow fermentation box method is more efficient than deep box fermentation (depth of ca. 1 m) because fermentation is more uniform owing to the lesser fermentation mass within the box. Both fermentation processes take about 5 to 7 days for completion.

Figure 2.2: Heap and box methods of cocoa fermentation

Figure 2.3: Shallow fermentation box

In heap fermentation, cocoa beans of mass ranging from 6 to 12 kg are heaped on the floor and covered with banana leaves. This method is mainly practiced in West African countries such as Ghana and produces cocoa beans with the best flavour quality [35].

2.1.2. Cocoa drying

After fermentation, approximately 50 % of cocoa proteins are denatured. Thus, drying process is necessary to prevent deterioration of beans by enzymes and microbial activity [36]. In general, cocoa beans need to be dried below 7.5 % (wet basis) for prolonged storage and transportation purposes. As mentioned earlier, browning of polyphenols which begins in the final stages of internal fermentation continues during drying [37]. The enzymatic reactions that occur during drying help in browning reaction where initially epicathecin is oxidized into O-quinones while anthocyanins are hydrolyzed into precursors of the browning process. In subsequent reactions, condensation of quinones with amino acids occurs and follows by polymerization of condensation products. The

final brown (melanin) pigments produced confirms the end of enzymatic reactions during drying [38]. The oxygen required for browning process during initial drying is obtained from the dissolved oxygen present in moisture embedded within the bean body. After evaporation of this moisture, the gaseous oxygen obtained from the voids developing in cocoa cotyledons provide oxygen for an increased oxidation process [17]. The rate of polyphenol oxidation decreases significantly when the moisture content of the dried beans reaches around 20 % [34]. The cross sectional views of fresh, fermented and dried cocoa beans are shown in Figure 2.4.

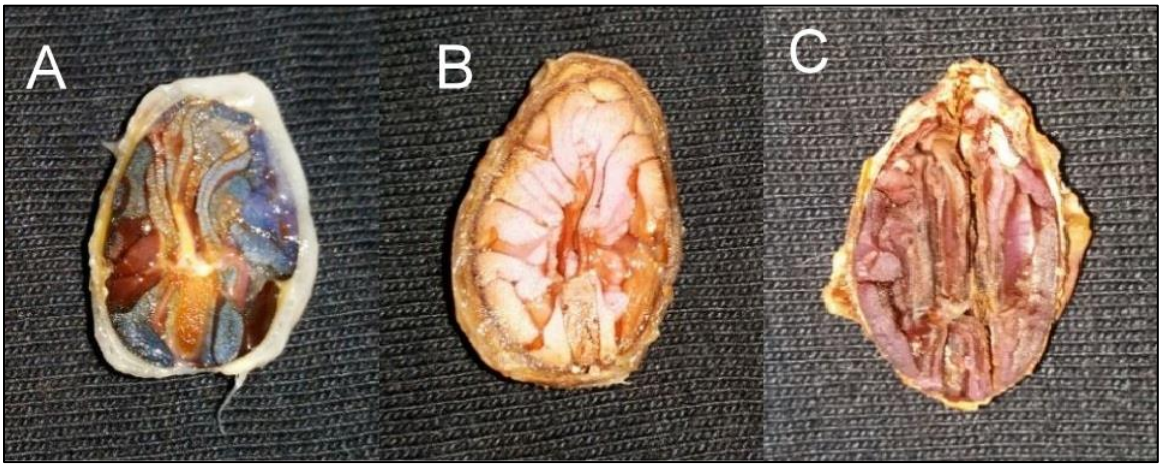


Figure 2.4: Cross sectional views of (A) fresh cocoa bean, (B) fermented cocoa bean and (C) dried cocoa fermented bean

The fresh beans before fermentation show a white thick layer of mucilaginous coating. The sugars present inside degrades during external fermentation due to microbes and liquefies into fermentation mass. The fermented beans show presence of brown pigments which are caused by the browning of polyphenolic compounds that occurs during internal fermentation. The embryo of the bean is killed after this process. The wet outer layer of the bean which forms the testa and the moist cotyledons are

detached after drying and can be easily separated during de-shelling process. The extend of browning also increases after drying due to the high degradation of polyphenol compounds into high molecular weight tannins [33]. The high purple colouration in fresh cocoa beans symbolizes the existence of high polyphenol compounds. Studies by Misnawi *et al.* [39] reports that there is an inverse relationship between oxidation and hydrolysis of polyphenol compounds to the purple colour retained after fermentation process.

2.2. Drying methods

The two main cocoa drying techniques are natural and artificial drying methods. In natural method, sunlight and wind energy are used while in artificial drying method hot air produced from a heat source (usually furnaces) are used. Since majority of cocoa plantations in the world are owned by small scale farmers, natural sun drying method is used due to the small drying mass, simple operational procedures involved and low cost. Artificial hot air drying methods are used by large cocoa producers to dry large quantities of cocoa in a time efficient manner.

2.2.1. Natural drying

Natural drying is the most traditional technique followed from ancient times. It refers to sun drying method where, cocoa are dried in raised platforms, trays or cement floors [40]. This method involves very low level technical influence and hence can be constructed easily. Besides, it is cheap since it utilizes energy sources such as sunlight and wind which are abundant and renewable. The major disadvantages of using this

method are the factors related to weather conditions which can influence the drying time. Furthermore, sun drying is highly labour intensive as the beans need to be monitored and regularly turned or raked for even drying and aeration of cocoa beans [8]. Figure 2.5 shows the typical setup of sun drying operation in conventional cocoa drying.

Figure 2.5: Sun drying of cocoa beans on cement floors

Cement floor drying (Figure 2.5) methods involve spreading of cocoa beans on a cement surface under sunlight. The major drawback of this method is overheating of cocoa beans due to the hot cement surfaces. The small-scale cocoa producer's dry cocoa beans in a bamboo mat supported by wooden framework. This method requires

constant mixing of cocoa and the microbes are inoculated into the cocoa bean through the bamboo mat and the surrounding air.

Research has been performed on the application of direct solar dryers which are sun drying equipment covered with transparent cover to negate the effects of rainfall. This method of drying is suitable in regions where cocoa harvesting season coincides with rainy season. The cocoa beans dried using this method has good quality attributes in comparison to direct sun drying [40]. However, there is a limitation in the amounts of cocoa beans which can be dried since large masses of cocoa can lead to uneven drying and development of acidity within the beans.

2.2.2. Artificial drying

Artificial drying of cocoa beans involves direct or indirect contact with heat sources to generate heated air. The drying process which involves mass transfer of moisture from beans is due to convection. Convective drying for cocoa are further categorized into natural and forced convection system.

Samoan drying (Figure 2.6) is an example of natural convection drying process, where the beans are dried in a raised concrete chamber. The beans are dried in perforated metal sheet below which consists of a heating tube. The heat source is obtained by burning firewood and the heat generated within the heating tube is transferred above into the layer of cocoa beans. This method is inefficient as the drying can be quite uneven and the exhaust gases from the fire could lead development of an undesirable smoky flavour in the dried cocoa beans [41]. There is also a need for consistent turning of cocoa beans within the drying chamber to prevent overheating.

Figure 2.6: Picture of Samoan cocoa drying method

In forced air convection system, the heated air is forced and channeled through the beans by a blower to facilitate drying. Depending on the construction of dryer, it can be classified as circular, rotary and flatbed dryers. The drying temperatures used in such cases ranges from 60°C to 80°C. High temperature based drying leads to insufficient development of flavour which is important in chocolate manufacturing. Besides, there is a major decline in polyphenol content of cocoa beans after drying at such high temperatures [37]. To overcome the major disadvantages related to artificial hot air drying in commercial productions, several researches have been performed in lab-scale hot air based drying methods [8,24,25,42]. Majority of the published work focusses on the effects of varying drying parameters such as temperature and relative humidity and comparing it with results obtained from natural drying method. Several literatures

looked into the effects of polyphenol degradation during hot air drying of cocoa beans [8,24]. Research till date suggests that polyphenol degradation is dependent on the drying temperature and exposure time, where high temperature and long duration of drying shows very low level of cocoa polyphenols recovery [24]. The development of advanced non-conventional drying technology for cocoa drying is relatively less in use. Hii *et al.* [18] used heat pump drying for improving the quality of cocoa beans. The low temperature and de-humidified air conditions used for drying is reported to enhance the quality of dried cocoa. The sensory scores were reported to match Ghanaian reference sample and polyphenolic contents measured were reported to show a significant improvement up to 73 % in comparison with freeze dried cocoa bean.

2.2.2.1. Adsorption drying

Adsorption drying uses desiccants to dehumidify the air that are channeled into drying chamber to provide dry air circulation for drying. Adsorption drying is a relatively low temperature drying process. This drying method is useful for the production of high quality, high value agricultural products with minimal flavour loss and degradation reactions (protein denaturation, browning and enzymatic reactions) [45]. The adsorption process is usually facilitated using hygroscopic desiccants with high thermal stabilities such as, zeolite (molecular sieves), silica gel, activated charcoal, calcium sulphate and montmorillonite clay [45]. Zeolite based adsorption drying have been used in various studies for the drying of agricultural products [46–48]. Zeolite has high affinity towards water and helps in significantly reducing the humidity of air subjected to drying

the sample. This helps the samples in acquiring equilibrium moisture content at a faster rate [49]. Research by Kusumo *et al.* [19], shows that tea leaves dried by zeolite based adsorption drying shows high levels of catechins in the dried product in comparison to traditional sun dried tea sample. Another research on onion bulb using adsorption drying method at 60°C for 2 h, showed high antioxidants (IC_{10} value of 163.05) on comparison reference samples (not dried, IC_{10} value of 170.32) [50]. The basic layout of an adsorption dryer is shown in Figure 2.7.

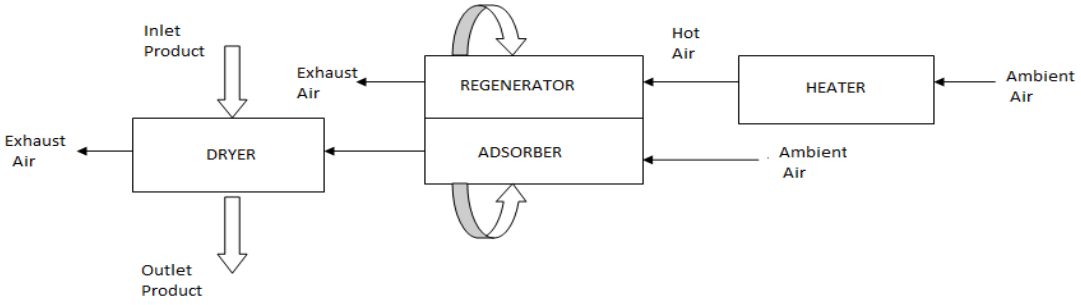


Figure 2.7: Basic layout of adsorption dryer

2.2.2.2. Vacuum drying

Vacuum drying is an advanced drying process where moisture from the drying sample is evaporated at lower boiling point of water by means of creating a vacuum. It is extensively used in chemical process industries like food and pharmaceuticals. Since the boiling point of water reduces significantly (due to low pressure), the rate of water evaporation increases and hence, the sample dries faster. The reduced relative humidity of the drying chamber also paves way to fast drying rates of samples in vacuum drying

[23]. Several literatures have noted high bioactive compound recovery for agricultural products dried using vacuum drying. The concentrations of bioactive compounds recovered after vacuum drying were similar to that obtained after freeze drying method [21,23,51,52]. Freeze dried samples are reported to recover the maximum bioactive active compounds and a benchmark used for comparative research of bioactive compounds in food products [27]. For example research by Hossain *et al.* [53] reported that rosemary when dried in vacuum oven at 60°C at 600 mbar pressure showed no significant difference in antioxidant capacity using ORAC (39.6 g Trolox g^{-1} dw) in comparison to samples dried using freeze drying method (40.2 g Trolox g^{-1} dw). The percentage difference in total polyphenol content for rosemary extracts after vacuum and freeze drying methods were reported to be non-significant. The authors reasoned this activity due to the lack of oxidation of polyphenol degradation enzymes (polyphenol oxidases) to the vacuum conditions prevalent in both drying methods.

2.2.2.3. Freeze drying

Freeze drying or lyophilization is a drying method where perishable agricultural products are dried to preserve their active bioactive compounds. The freeze drying process occurs by solidifying the samples by freezing and then reducing the surrounding pressure to allow the frozen water in the sample to sublimate directly from solid phase to gas phase. Since sublimation occurs at very low temperatures, the thermal degradation of enzymes and proteins does not occur. Thus, the dried sample are expected to be rich in bioactive compounds [54]. In cocoa processing, freeze drying

methods have been used as benchmark for quality aspects such as total polyphenol content and antioxidant activity [37]. The polyphenol contents retained by fermented cocoa beans after freeze drying were reported to be as high as 101.4 mg GAE g^{-1} [37]. For fresh cocoa beans the polyphenol recovery after freeze drying for 24 h at 0.015 mbar pressure was recorded to be 181.7 mg GAE g^{-1} [24].

2.3. Mechanism of cocoa drying

A well fermented cocoa bean consists of two main parts; the outer testa and inner cotyledons. It should be noted that fresh cocoa beans have a significant amount of mucilaginous pulp which liquefies and drains off during fermentation [28]. The moisture content of the bean testa surface is about 300 % (dry basis) and inner cotyledon about 50 % (dry basis) [55]. In general, the typical drying rate curves of cocoa beans would consist of both constant rate period and falling rate period [36]. The falling rate periods are further classified into first falling rate and second falling rate (Figure 2.8).

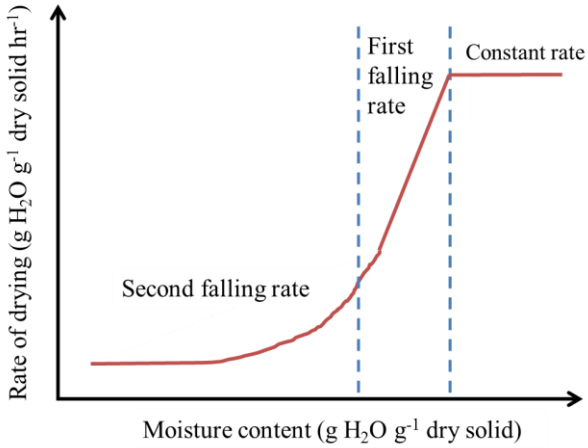


Figure 2.8: Typical drying rate curves of cocoa beans

The constant rate periods are usually found in the initial phase of drying. This is due to the presence of a consistent layer of moisture prevalent in the testa surface during the initial 2 hours of drying [25]. Generally in artificial drying methods, only falling rate periods are noticed because of the high temperature conditions prevalent during drying and the moisture from testa surface are removed at a rapid rate [37]. Hii *et al.* [18] reported the existence of constant rate periods for cocoa dried using heat pump dryer in step down ambient air (30.7°C) conditions. The authors explained low drying temperature leads to the lower rates of moisture evaporation from bean surface. The onset of falling rate period is noted by the movement of vapourized moisture from within the cotyledons of cocoa bean to outside. The movement of residual moisture from inside the bean to the bean surface where evaporation occurs is mediated through conduction process [8]. Temperature plays a major role in such condition where the driving force required for the moisture transfer from the inner region of beans to outside is higher. The dependence of temperature on rate of drying is evident from the drying rates of sun drying process. The rates of sun drying (ambient condition) is much lower in comparison to hot air drying (above 60°C) where low temperature in sun drying takes longer time in acquiring equilibrium moisture content [24]. The second falling rate period occurs when the bean surface and outer cotyledon area within the bean are dry (moisture content of about 25-30 % dry basis). The movement of moisture from the inner core of cotyledons to the surface of bean requires higher driving force and longer drying time. This is due to the greater resistance in moisture movement inside cotyledons [36].

2.3.1. Effective diffusivity

Effective diffusivity can be defined as the transport of moisture within solid which occurs through one or more of the following mechanisms: liquid diffusion, vapour diffusion, surface diffusion, Knudsen diffusion or diffusion due to hydrostatic pressure differences. These mechanisms are described below;

- Liquid diffusion occurs when wet solid is dried at a temperature below the boiling point of water.
- Vapour and Knudsen diffusion occurs when liquid vaporizes within the material
- Surface diffusion occurs during drying
- Diffusion due to hydrostatic pressure differences occurs when internal vaporization rates exceeds the rate of vapour transport through the solid to the surroundings and due to combinations of a few or all the mechanisms mentioned above [56,57].

Effective diffusivity is determined by using the general solution of the Fick's second law (e.g: spherical object) as shown in Equation 1 [58].

$$MR = \frac{6}{\pi^2}\sum_{n=1}^{n=\infty}\frac{1}{n^2}\exp[\frac{-n^2\pi^2 D_{eff}t}{r^2}] \quad (1)$$

The estimated effective diffusivity if assumed to follow an Arrhenius relation with respect to the bean temperature can be represented by the Arrhenius equation as shown in Equation 2.

$$D_{eff} = D_0 \exp[-\frac{E_a}{R(T+273)}] \quad (2)$$

Where; D_{eff} is effective diffusivity (m^2s^{-1}), D_0 is Arrhenius constant(m^2s^{-1}), E_a is activation energy ($kJmol^{-1}$) and R is Gas constant (8.314 J $mol^{-1}K^{-1}$) [8]. This equation can be linearized by applying natural logarithm on both sides of ln D_{eff} versus 1/T which will produce a straight line. The activation energy and Arrhenius constant can be determined from the slope and y-intercept, respectively. Various literatures for cocoa drying use this model as shown in Table 2.1.

Table 2.1: Evaluation of drying kinetics for cocoa beans as reported in literature

Type of drying	Activation energy, E_a (kJ K^{-1} mol^{-1})	Effective diffusivity, D_{eff} (m^2 s^{-1})	Ref.
Hot air (60°,70°,80°C)	11.8	$3.73x10^{-10}$ to $4.74x10^{-10}$	[24]
Sun	-	$8.01x10^{-10}$ to 4.84 $x10^{-10}$	
Hot air (55°, 70°,81°C)	39.94	$3.62x10^{-10}$ to $9.98x10^{-10}$	[25]
Hot air (60°,70°,80°C)	28.11	$1.61x10^{-10}$ to $3.23x10^{-10}$	[8]
Sun	-	$8.01x10^{-10}$ to $4.84x10^{-10}$	
Hot air (60°,70°,80°C)	44.92	7.46×10^{-11} to 1.87×10^{-10}	[59]

The effective diffusivity values for hot air and sun drying of cocoa from various literatures are shown to be in the orders of magnitude ranging from 10^{-10} to 10^{-11} m^2 s^{-1}. It is also observed that similar drying methods used in different literatures show differences in the values of activation energy and effective diffusivity. This could be due to the differences in the variety of cocoa beans samples and model of the dryers that were used for experimentation. The sun drying methods show a lower reading of

effective diffusivity values when compared to hot air drying because of the variations in weather patterns experienced using the drying process [24].

2.4. Blanching pre-treatment

In food products, blanching is a pre-treatment method with the aim of inactivating enzymes, modifying texture, preserving colour, flavour and nutritional value and removing trapped air [60]. Hot water or steam are commonly used as the heating medium for blanching food materials. In most blanching methods, a number of studies on the effects of blanching pre-treatments on food products have high polyphenol and antioxidant activities [61,62]. Tomas-Barberan *et al.* [15] reported hot water blanching on fresh cocoa beans before drying. The results for cocoa samples blanched at 95°C for 5 min were optimum where the browning degree was visually found to be the least.

2.5. Polyphenols and antioxidants in cocoa

Polyphenols are compounds produced in secondary metabolism of many plants and play an important role in the defense against micro-organism. The presence of polyphenols in cocoa is dependent on several factors including degree of ripeness, geographical origin, variety, stress reactions, processing and storage; the polyphenol content in cocoa is about 12-18 % of the dry weight of whole bean [63]. Three groups of polyphenols can be identified in cocoa beans: catechins, which constitute about 37% of the polyphenol content in the beans, anthocyanidins (about 4%), and proanthocyanidins (about 58%). Of the catechins, (−)-epicatechin is the most abundant (up to 35%), while (+)-catechin, (+)-gallocatechin, and (−)-epigallocatechin are present in smaller quantities [64].

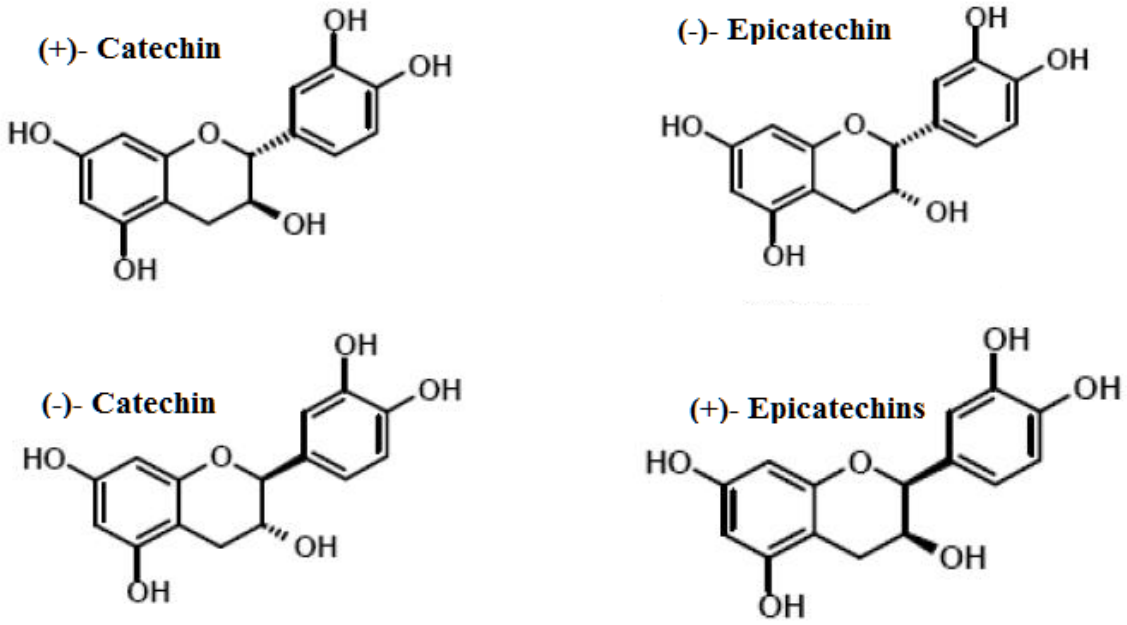


Figure 2.9: Chemical structures of major polyphenolic compounds found in cocoa beans

In recent years, cocoa has gained more attention and have become an important focus of research interest owing to their antioxidant activities. Various beneficial effects on human health have been reported, such as in treatment and prevention of cancer, cardiovascular diseases, antimicrobial and other pathologies [65]. Various literatures have reported the amounts of polyphenols available in cocoa beans based on their geographical origins and planted varieties. Table 2.2 summarizes the total polyphenol content in different regions as reported in literature. The total polyphenolic contents of fermented cocoa ranges from 40-84.2 mg GAE g^{-1} and varies among geographical origins and the planted varieties. Among these, the criollo cocoa variety shows a lower total polyphenol content since it lacks in anthocyanins; which is a type of polyphenol [15].

Table 2.2: Total polyphenol content in cocoa beans in different geographical origins and planted varieties

Geographical origin	Variety	Total polyphenol content	Ref.
Ivory Coast	Forastero	81.5 (mg GAE g^{-1})	
Columbia	Amazon	81.4 (mg GAE g^{-1})	
Guinea Equatorial	Amazon	72.4 (mg GAE g^{-1})	
	Forastero		
Ecuador	Amazon hybrid	84.2 (mg GAE g^{-1})	[15]
Venezuela	Trinitario	64.3 (mg GAE g^{-1})	
Peru	Criollo	50.0 (mg GAE g^{-1})	
Dominican Republic	Criollo	40.0 (mg GAE g^{-1})	
Malaysia	Unknown	71.42-82.68 (mg GAE g^{-1})	[8]
Cameroon	Unknown	86.6-143.6 (mg EC equivalent g^{-1})	[66]

Processing such as fermentation and drying of cocoa beans are essential to develop suitable flavours. However, fermentation and drying degrade the total polyphenols content in cocoa. The various processing methods that affect the polyphenol and antioxidant activity are shown in Figure 2.10. Tables 2.3 summarizes the effects of various primary processing on total polyphenols content.

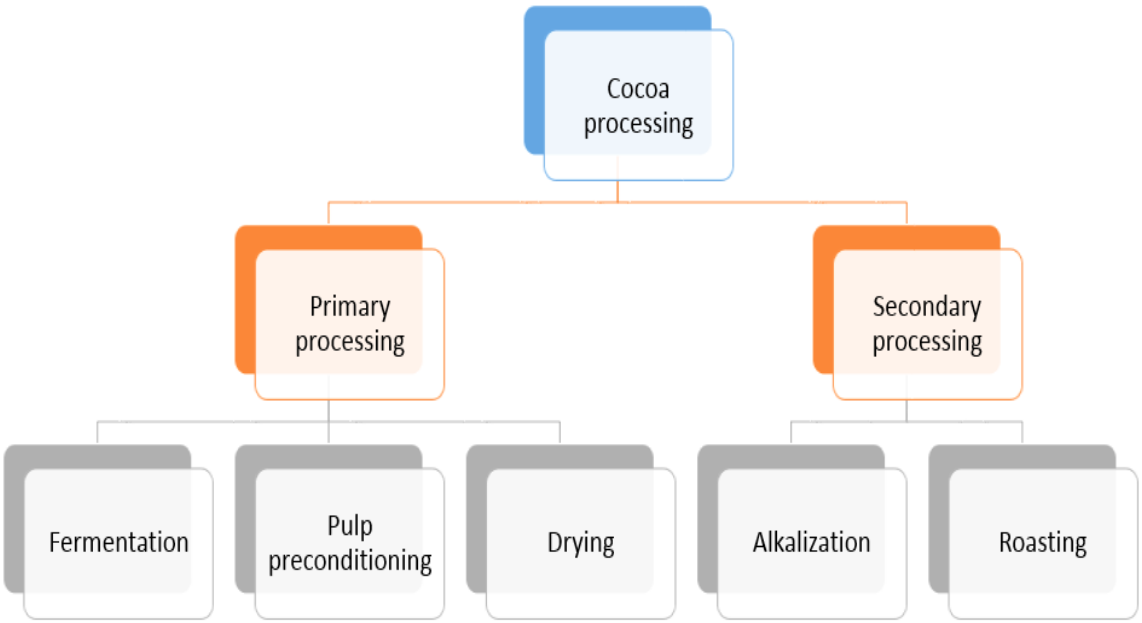


Figure 2.10 Processing methods which affect cocoa polyphenols content

Table 2.3: The effect of primary processing on cocoa polyphenols

Processing step (Primary)	Key finding	Ref.
Fermentation	• Decrease in total polyphenolic contents of cocoa beans as the duration of fermentation increased (15.5 wt. % to 6.01 wt. % in 6 days). • Decrease in the epicatechin content of the cocoa beans as the fermentation duration increased (10 to 20 % of epicatechin and other soluble polyphenols were reduced during fermentation).	[67]
Fermentation	• Fermentation contributes to the elimination of the astringency and the bitter taste characteristics of fresh unfermented cocoa seeds. • Most of the polyphenols (80–90 %) were lost during the first 48 hours of fermentation. • At the end of fermentation and drying, epicatechin concentration was reduced by approximately 75%.	[34]
	• After 144 h of fermentation, the concentration of	

Fermentation	polyphenolic compounds reduced from 13.07 to 16.11 % (wt/wt). 6.57 to 10.11 % (wt/wt). • Concentrations of catechin (±0.16 mg g^{-1}) did not change during fermentation, After 144 h of fermentation more than 70 % of the initial concentration of epicatechin was lost.	[31]
Heat pump Drying	• Total polyphenolic contents reduced as drying temperature were increased in heat pump. Percent retention of polyphenol was found ranging from 44–73 % as compared to the freeze dried sample. • The lower temperature generated from the heat pump dryer could preserve greater amount of cocoa polyphenols during drying.	[37]
Hot air Drying	• After drying the level of phenolic compounds decreased by 32 % compared to the fermented sample (not dried).	[68]
Freeze, sun and hot air drying	• Freeze-dried samples contained significantly more (88.45 mg g^{-1}) polyphenols compared to the sun (61.81 mg g^{-1}) and 80 °C (71.42 mg g^{-1}) oven dried samples. • The freeze-dried samples contained the highest polyphenol content due lack of enzymatic activity and hence no browning reaction. • The sun dried samples showed the lowest total polyphenol content due long drying process.	[8]
Hot air drying	• The antiradical properties decreased significantly (45%) after drying	[69]
Hot air drying	• Polyphenol degradation rate increases with increasing temperature and relative humidity.	[14]

2.4. Polyphenol degradation kinetics

Browning reaction is an important reaction which occurs during drying and browning begins in the final stages of fermentation after the bean is killed due to acidic environment. The browning process occurs due to oxidation of polyphenols by enzymes inside the bean cotyledons. The chemical structure of tannin compounds which give brown colouration to the cocoa beans are shown in Figure 2.11, where letter R represents the number of polymerized units present in the compound [15]. During the final drying stages, void spaces are created between the cotyledons due to moisture loss. The oxygen present in the void spaces further oxidizes the polyphenols into brown pigments, and the enzymatic activity slowly recedes once the beans are dry due to the lack of moisture availability [14]. The reaction kinetics (reaction rate constant k) of the browning reaction are shown in Equation 3 [70].

$$\text{Polyphenol} + \text{Oxygen} \xrightarrow{k} o - \text{Quinone} \quad (3)$$

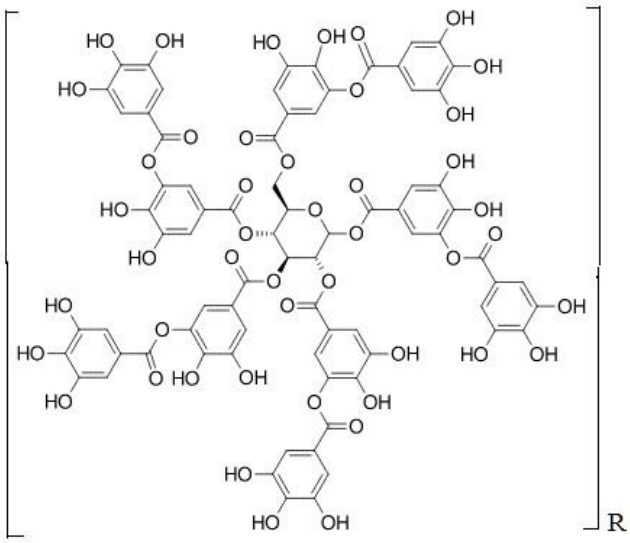


Figure 2.11: The chemical structure of polymerized tannin compound

Kyi *et al.* [14], reported that the degradation kinetics of cocoa polyphenols during hot air drying are usually represented by pseudo-first order reaction mechanism. The authors stated that 'pseudo' was prescribed since the actual reaction mechanism and its kinetics are far more complex in the degradation model. Generally, the action of non-enzymatic polyphenol degradation is lesser compared to enzymatic degradation during drying process. The non-enzymatic reactions usually occur at higher temperature and low oxygen conditions. Roasting at high temperatures (>100°C) has been reported to cause non-enzymatic browning in cocoa beans mainly due to Maillard reactions [71]. A study on cocoa polyphenols degradation kinetics by Teh *et al.* [24], reported that the reaction rate constants were in the range of 0.044 to 0.052 (min^{-1}) for hot air dried cocoa at temperatures ranging from 60°C to 80°C.

$$C = C_0 \exp^{-kt} \quad (4)$$

Where, C is the concentration of polyphenolic contents (mg GAE g^{-1}) measured at drying time; subscript 0 indicates the value for reference sample (before drying), t the

drying time (h) and *k* is the rate constant (h^{-1}) at temperature *T* (K) [62]. The temperature dependence of the rate constant (k) for polyphenol degradation during both drying can be shown by Arrhenius equation (5);

$$k = k_0 \exp^{-E_a/RT} \quad (5)$$

Where, *k* is the rate constant, k_0 is the pre-exponential factor, E_a is the activation energy, *R* is the gas constant and *T* is the temperature at which drying occurred at [14,24]. Examples of polyphenol degradation kinetics reported in various literatures for cocoa and other agricultural products are shown in Table 2.4.

Table 2.4: Evaluation of polyphenol degradation kinetics of cocoa during drying

Treatment type	Activation energy, E_a (kJ K^{-1} mol^{-1})	Rate constant *(k)*	Ref.
Hot air drying (40°, 50° and 60°)	27.8 to 30.3	0.055 to 0.199 h^{-1}	[14]
Hot air drying (60°, 70° and 80°)	9.0	0.044 to 0.052 h^{-1}	[24]

2.7. Sensory evaluation of cocoa

Flavour is one of the most important constituents in cocoa products and the flavour precursors are developed during fermentation and drying of cocoa beans [10]. Studies reported on the effects of artificial drying methods on the sensory attributes of cocoa have been discussed in section 2.2 of this Chapter. Sourness flavour attributes are highly dependent on drying method used where fast drying at high temperature usually leads to retention of acids in cocoa bean and expressed in sourness scores during sensory

evaluation [17]. Advanced drying method such as heat pump drying are reported to produce cocoa with high flavour quality which are comparable to Ghanaian cocoa [18]. Cocoa beans used in chocolate manufacturing have to undergo roasting process by means of dry heat treatment for the development of chocolate flavour. Flavour precursors developed during fermentation and drying interact in the roasting process to produce the desired chocolate flavour. Roasting leads to development of characteristic brown colour, mild aroma and texture of roasted beans. The flavour produced is a result of combinations of 400–500 compounds including pyrazines, aldehydes, ethers, thiazoles, phenols, ketones, alcohols, furans and esters [72]. Aldehydes and pyrazines are among the major compounds formed during roasting. Figure 2.12 shows the cocoa flavour attributes and the various factors contributing to its development.

Ramli *et al.* [73], studied the effect of roasting conditions on the sensory evaluation of dark chocolates. The authors reported that higher temperature and longer roasting time leads to lower astringent taste. This is because polyphenol compounds (epicatechin, catechin and procyanidin) which are responsible for the astringent taste in cocoa [74] degrades at high temperature and long roasting time. When temperature was increased from 120°C to 170°C, there was a significant increase in the bitter taste. Xanthine alkaloids (caffeine) and theobromine are responsible for the bitter taste of cocoa beans.

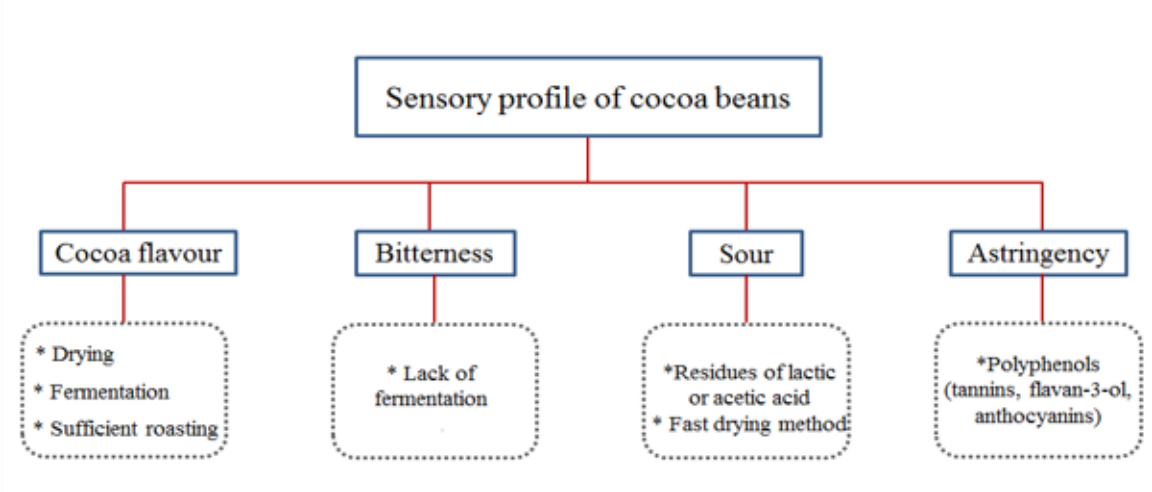


Figure 2.12: Major cocoa bean flavour attributes and the flavour contributing factors

The cocoa flavour increased with increasing temperature and time of roasting. This is due to evaporation of volatile compounds which mask the cocoa flavour attribute [38]. Misnawi *et al*. [41] studied the effects of polyphenol concentration and roasting duration on sensory properties of cocoa liquor. Similar to Ramli *et al*., the authors reported that cocoa flavour increased with longer duration of roasting. However, at higher concentrations of polyphenol (170 g kg^{-1}) the opposite effects of strong astringent and bitter sensations were observed. This was due to strong reduction effects of polyphenol against cocoa flavour formation and leads to interference. However, roasting duration did not significantly (p > 0.05) influence astringency and bitterness properties. This may be due to the lower levels of polyphenol concentration after fermentation and drying process which are not affected by roasting.

Misnawi *et al.* [75] studied the ability of polyphenols to produce astringency during cocoa roasting through an evaluation of the polyphenol-protein interaction in cocoa cake/liquor roasted at 120°C for 45 min, with and without enrichment with polyphenol

extract. It was reported that roasting decreased capacity of polyphenols to interact with protein, causing a decrease in astringency. Table 2.5 shows the flavour scores of cocoa beans after various processing methods that are reported in literature.

Table 2.5: Flavour scores of cocoa beans after various processing methods

Criteria	Cocoa	Sour	Bitterness	Astringency	Ref.
Cocoa beans roasted at 120°C for 15 minutes	6.3	-	3.1	3.3	[41]
Cocoa beans roasted at 120°C for 25 minutes	6.3	-	3.7	4.0	
Cocoa beans roasted at 120°C for 35 minutes	6.5	-	2.9	3.6	
Cocoa beans roasted at 120°C for 45 minutes	6.5	-	2.9	3.4	
Cocoa liquor obtained from a hybrid variety of cocoa beans	6.5	2.1	6.3	1.5	[76]
Cocoa liquor (beans roasted at 120°C)	-	-	-	2.8	[75]
Cocoa liquor of heat pump (step up) dried cocoa	5.4	2.6	3.6	3.4	[18]
Cocoa liquor of sun dried dried cocoa	4.9	2.8	3.0	3.8	
Cocoa liquor of direct solar dried cocoa (20 kg)	4.8	2.2	3.2	3.5	[40]

CHAPTER 3

GENERAL MATERIALS AND METHODS

3.1. Preparation of samples

Mixed clonal varieties of fermented and fresh beans were obtained from Malaysia Cocoa Board, Jengka, Pahang. The cocoa beans were obtained from matured ripe fruits (yellow or yellowish-red in colour) which contained about 30-40 beans. Around 1 kg of fresh cocoa beans could be extracted from around 10 matured cocoa fruits. The average sizes of the beans were of ca. 2.7 X 1.5 X 1.0 cm (l X w X h). Diseased beans were discarded and only healthy beans were used in experiments.

3.1.1. Fresh cocoa beans

Freshly harvested cocoa pods after procurement, were cut open. The beans along with the pulp were stored in air-tight plastic containers (ca. 100 g) at -18°C to prevent fermentation and preserve the beans from losing its freshness. Prior to experiments, the frozen bean samples were allowed to defrost sufficiently at ambient condition and made suitable for drying. Figure 3.1 shows an image of fresh cocoa beans.

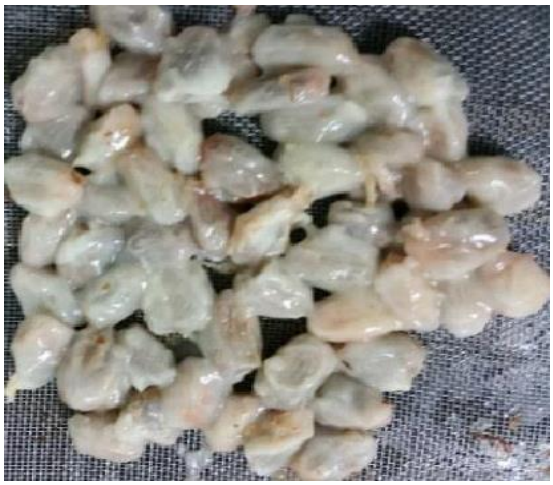


Figure 3.1: Fresh cocoa beans used for experiments

3.1.2. Fermented cocoa beans

The cocoa beans after harvesting from the farms were fermented at Malaysian Cocoa Board (Jengka, Malaysia), using wooden boxes of dimensions and cocoa mass capacity of 60 x 91 x 30 cm (l x w x h) and 150 kg, respectively. The beans were fermented according to the standard protocol for Grade SMC 1 quality developed by Malaysian Cocoa Board for 5 to 7 days until the beans have turned dark reddish brown [43]. The beans were turned every 48 hours using a wooden shovel for aeration and consistent fermentation. After fermentation, the beans were divided and stored in air-tight plastic containers (ca. 100 g) at -18°C in deep freezer (FZ301, Khind, Malaysia). Figure 3.2 shows typical image of fermented cocoa beans used in experiments.

Figure 3.2: Fermented cocoa beans used for experiments

3.2. Drying methods

3.2.1. Oven drying

Drying experiments were carried out using an oven (Memmert, humidity controlled drying chamber HCP 108, Germany) with overall dimensions of 48 × 56 × 40 cm (l X w X h). The beans were spread thinly on a meshed tray with square openings measuring 0.1 × 0.1 cm. Heat was generated by heaters integrated into the walls of the chamber. Figure 3.3 shows the image of the humidity controlled drying chamber. The relative humidity level inside the oven was maintained at 50 % as recommended by Kyi *et al*., 2005 [14] where at 50% relative humidity, the highest polyphenolic contents in cocoa beans were recovered. The air velocity was ca. 0.01 m s^{-1}.

Figure 3.3: Pictures of oven from front in; (a) closed door; (b) open door conditions

3.2.2. Adsorption drying

The adsorption dryer used in the study was fabricated by Dawnyx Technologies SDN BHD, Malaysia. The schematic representation of the dryer is shown in Figure 3.4. The adsorption dehumifidification was performed using zeolite adsorbent (diameter of 0.2 cm). Two cylindrical adsorption columns (100 cm in length and 25.4 cm in diameter) were used to hold the adsorbent by wire meshes with square openings (1 X 1 mm mesh size). The mass of zeolite beads used in each adsorption cylinders were approximately 4 kg each. This approximately filled 60% of the volume of adsorption columns and were ideal for operation. The zeolite adsorbents used in the experiment were de-saturated (regenerated) prior to the drying experiment at 100°C for 1 h in an oven [49].

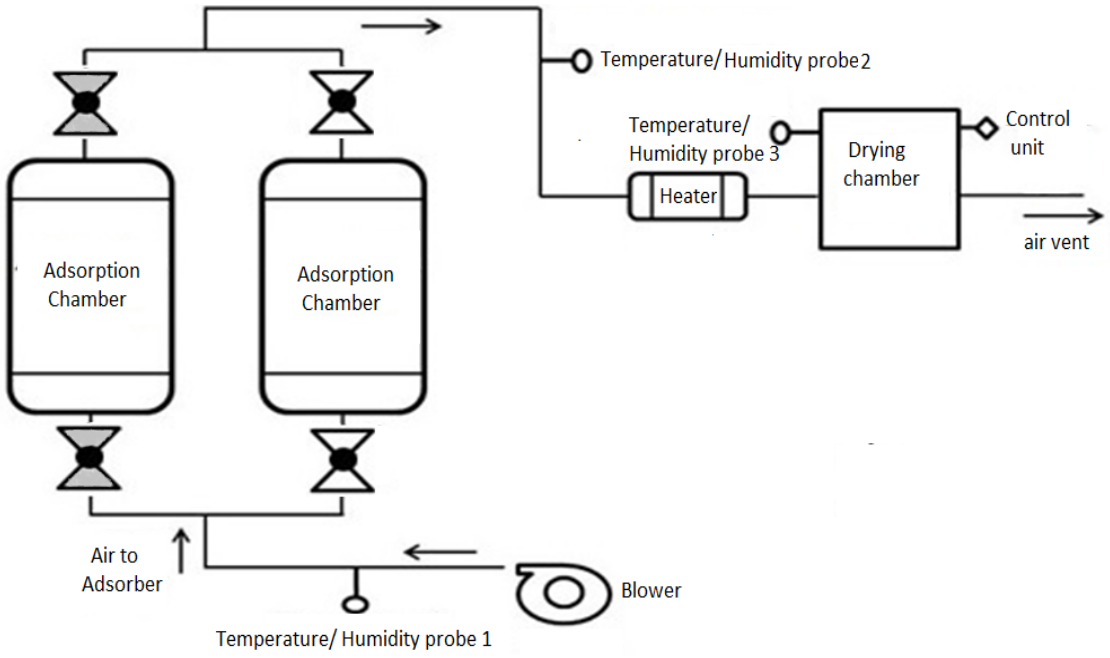


Figure 3.4: Schematic representation of the adsorption dryer

The adsorption dryer setup is shown in Figure 3.5, the various components marked in the figure were the following; (A) the drying chamber, (B) the digital control unit, (C) the blower unit, (D) the valves used to channel the air into chamber and control the air flow rate, (E) the adsorption chambers, (F) the humidiprobe sensors (G) the flowmeter (Dwyer, USA) to record the airflow rate in l min^{-1} and (H) the heating coil.

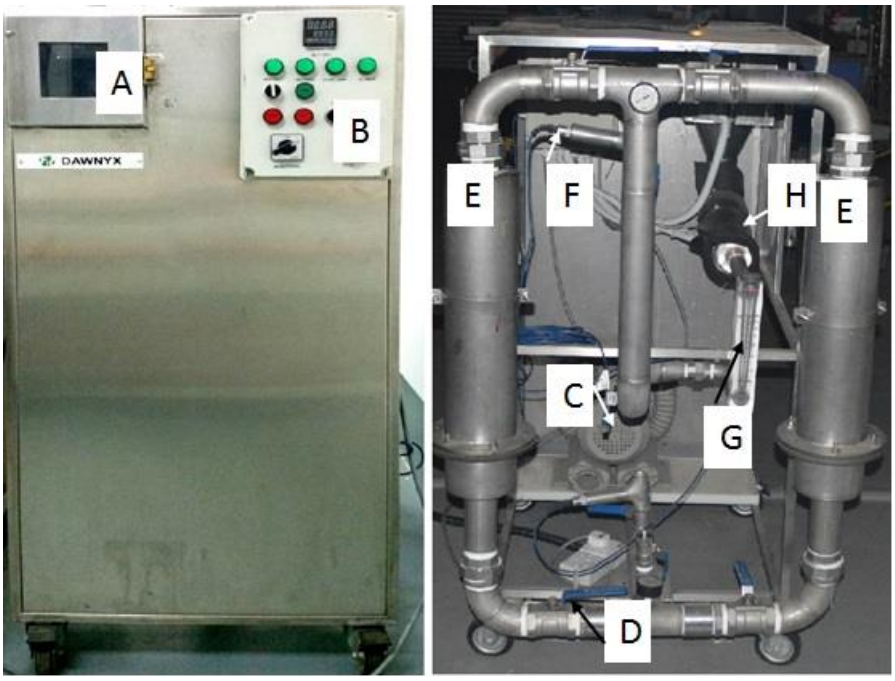


Figure 3.5: Adsorption dryer setup; front and rear view

Atmospheric air from the surrounding was channeled into the adsorption chamber by a blower (HB-429, Apex Dargang, Taiwan). The inlet air were specifically channeled into one adsorption column during drying and were alternated every 6 h (Figure 3.4). The switch of air channel into either one of the adsorption columns every 6 h were for the purpose of using fresh dehumidified zeolite for efficient drying purpose. An embedded heating coil was installed before the drying chamber in dryer setup to dry samples at high temperature (above ambient air conditions). However, the heating parameter was not used for the present drying operation, and ambient air were channeled into the adsorption chamber and subsequent drying chamber. The drying chamber dimensions are 18 x 21 x 27 cm (l X w X h). The temperature and relative humidity values at various points of the adsorption dryer; before adsorption, after adsorption and drying chamber were measured using a sensor (humidiprobe AQ 518, Pico technology, USA). After

drying, the air was channeled out from the drying chamber through a small opening valve on top. The average temperature of the drying chamber was recorded to ca. 60°C. The relative humidity profile of the adsorption dryer is shown in Figure A1 in section Appendix A. The average relative humidity of the drying chamber after adsorption process was 9-10 %. The air velocity during the experiments were noted to be 4.1 ± 0.6 m s^{-1}.

3.2.3. Freeze drying

Freeze drying experiments were carried out to establish a benchmark for quality assessment of cocoa beans. The cocoa beans were frozen (-18°C) before the freeze drying process. Drying was carried out using a freeze dryer (Alpha 1-2 LDplus Martin Christ Gefriertrocknungsanlagen GmbHChrist, Germany) as shown in Figure 3.6. The vacuum chamber or lyophilisation unit was a transparent acrylic cylinder (diameter= 20 cm, height= 25 cm). There was a two-tier sample stand which held the sample during drying. A vacuum pump (Vacuubrand, RZ 2.5, Germany) sucked in air from the condenser unit and drying chamber and maintained the pre-set pressure conditions throughout the drying process. The vacuum pump and condenser unit were switched on and warmed up for 30 min, before drying process. This was for the warming up of the vacuum pump and also for cooling the condenser unit to the desired temperature. The drying process followed Hii *et al*. [8] with slight modifications. The drying process consisted of two stages namely; main drying (time= 24 h, condenser temperature= -30°C and pressure ca. 0.040 mbar) and final drying (time= 4 h, condenser temperature= -50°C and pressure ca. 0.015 mbar). According to Hii *et al.* [8], cocoa beans dried in freeze

dryer following these parameters were reported to achieve a final moisture content of less than 7.5%.

Figure 3.6: Image of freeze dryer setup

3.2.4. Vacuum drying

The vacuum dryer (Memmert VO 200, Germany) consisted of two portions namely; drying chamber (oven) and a vacuum pump. The drying unit had overall dimensions of 55 x 55 x 71 cm (l x b x h). The dryer was equipped with two thermo-shelves which generates the required heat to be cocoa sample. A digital controller present in the drying unit controlled the thermo-shelf temperature and vacuum pressure in chamber. The vacuum level was set to the lowest at 150 mbar to reduce the boiling point of water

as low as possible to facilitate drying. The cocoa samples were placed thinly spread evenly in a meshed tray of square opening (0.1 X 0.1 cm) on the thermo-shelf. Both the drying chamber and vacuum pump were allowed to operate for 30 min prior to every experiment for pre-conditioning the thermos-shelves (raising the temperature to desired temperature) and warming up the vacuum pump. The vacuum drying setup is shown in Figure 3.7. The operating temperature for vacuum drying was set at 60°C, published literatures have reported that any temperature above 60°C is detrimental for cocoa quality [8].

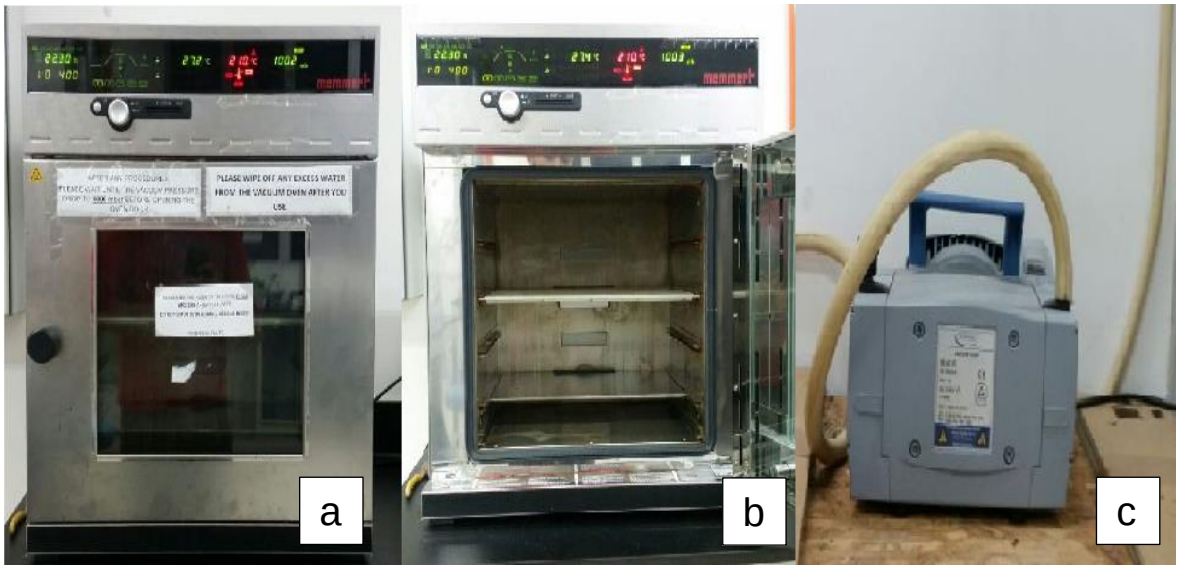


Figure 3.7: Pictures of the vacuum oven in; (a) closed door; (b) open door; (c) vacuum pump

3.2.5. Sun drying

Sun drying of cocoa was performed from 7 am to 7 pm (from August 2015 to November 2015) at a concrete floor besides the parking area of Engineering Research Building, The University of Nottingham Malaysia Campus (N02°56.622' and E101°52.405'). The cocoa beans were evenly spread and dried on a rectangular tray of dimensions 30 X 28 cm (l X w) under direct sunlight. The beans were manually mixed every hour to ensure uniform drying. The tray containing beans was placed 50 cm above ground level as shown in Figure 3.8. The ambient temperatures recorded during experiments were between 26 - 33°C. The air velocity was measured to be ca. 1.3 m s^{-1} and relative humidity was about 65-75-%. The beans were left at a covered area at ambient temperature during night time (7 pm to 7 am). This process is helpful in re-distributing the internal moisture of the cocoa beans to the bean surface [44].

Figure 3.8: Sun drying experimental setup

3.3. Drying Kinetics

3.3.1. Moisture content

Moisture content (X_i) in dry basis was determined hourly based on the weight of the whole beans (M_i) using equation (6). However, for freeze drying this could not be performed as this process would disrupt the equilibrium condition established inside the chamber during operation.

$$X_i = \frac{M_i - M_{ds}}{M_{ds}} \times 100\% \quad (6)$$

Dry solid weight of the beans (M_{ds}) was determined by drying the cocoa beans in the oven at 105°C for 24 h following a method used by Hii *et al.* [37].

3.3.2. Drying rates

The drying rates was calculated by approximation of the derivatives of finite differences [78] based on the following equations (7-9),

At $t = t_0$ (initial time),

$$\frac{dX}{dt} = \frac{X_1 - X_0}{t_1 - t_0} \quad (7)$$

At $t = t_i$ (i= 1, … , N-1), where X denotes the moisture content (g H_2O g^{-1}).

$$\frac{dX}{dt} = \frac{X_{i+1} - X_{i-1}}{t_{i+1} - t_{i-1}} \quad (8)$$

At, $t = t_N$ (final time, h),

$$\frac{dX}{dt} = \frac{X_N - X_{N-1}}{t_N - t_{N-1}} \tag{9}$$

3.3.3. Effective diffusivities

The general solution of Fick's second law diffusion model was used to determine the effective diffusivity during the moisture removal process [58]. The cocoa beans were assumed spherical in shape in this model with an equivalent radius (r) of 0.67 cm. The general solution of the Fick's law for spherical object as shown in equation (10) below;

$$MR = \frac{6}{\pi^2}\sum_{n=1}^{n=\infty}\frac{1}{n^2}\exp[\frac{-n^2\pi^2 D_{eff}t}{r^2}] \tag{10}$$

Where, MR is the moisture ratio, which can be calculated from the equation given below,

$$MR = \frac{X_i - X_e}{X_o - X_e} \tag{11}$$

Where, subscripts i, e and o denote at time t_i, equilibrium and initial, respectively.

Only the first term of the equation (10) was used and upon linearization by applying natural logarithm at both sides, a straight line graph can be plotted (ln MR vs t) using equation (12). The slope of the graph was used to obtain the values of D_{eff} [59].

$$lnMR = ln(\frac{6}{\pi^2}) - (\frac{\pi^2 D_{eff}t}{r^2}) \tag{12}$$

Where, *r* represents the radius of cocoa beans.

The dependence of effective diffusivity on temperature can be further described using the Arrhenius equation as shown in equation (13):

$$D_{eff} = D_o \exp\left[-\frac{E_a}{R_g(T+273.15)}\right] \quad (13)$$

Where, D_0 is Arrhenius constant, E_a is Activation energy and R_g is gas constant (8.314 J $mol^{-1}K^{-1}$). This equation can be linearized by applying natural logarithm on both sides and ln D_{eff} versus $1/T$ will produce a straight line. The activation energy and Arrhenius constant can be determined from the slope and y-intercept respectively.

3.4. Quality Analysis

3.4.1. Total polyphenols content

Polyphenols analyses were carried out according to the method proposed by Kim and Keeney [74]. Dried cocoa beans were peeled to separate the nibs from the shells for analyses. The samples were ground in a dry mill and then sieved through a 600 µm screen to obtain the fine powders. Five gram of ground samples were defatted for 2 h using petroleum ether (Spectrum chemicals, USA) in Soxtherm (Gerhadt, Germany). The samples were filtered through Whatman No. 1 filter papers (110 mm, Sigma-Aldrich, USA) and the residues were oven dried at 60°C for 6 h to allow the traces of petroleum ether to evaporate. For extraction of polyphenols, 10 ml of 70 % acetone (Sigma-Aldrich, USA) solution was added to 0.1 g of dried defatted samples and sonicated (Elmasonic

Palmer, Germany) for 30 min in ice water. The samples were then centrifuged (Eppendorf, Germany) at 5000 rpm for 10 min.

Subsequent steps were carried out by diluting 100 µl of the supernatant liquid with 7.9 ml of distilled water. Then, 500 µl of Folin-Ciocalteau (Sigma-Aldrich, USA) reagent was pipetted into the test tube, shaken to allow it to mix well and left for 8 min. Next, 1.5 ml of 20 % sodium carbonate solution was pipetted into the mixture and were kept for 2 h for colour development. These steps were repeated with standard Gallic acid solutions. The calibration curves are reported in Figure A2, in section Appendix A. The absorbances of the standards and polyphenols extracts were as measured with a UV-Vis spectrophotometer (PerkinElmer, USA) at 765 nm. The results were reported as Gallic acid equivalents (GAE) per gram dry weight of cocoa.

3.5. Statistical Analyses

The experiments were carried out as completely randomized experiments. Each experiment is carried out in three replicates and the data were analyzed using one-way ANOVA and mean comparison using Duncan's Multiple Range Test at 95 % confidence level (SPSS version 20, IBM, USA).

CHAPTER 4

DEGRADATION KINETICS OF POLYPHENOLS DURING CONVENTIONAL COCOA DRYING

4.1. Introduction

Cocoa (*Theobroma cacao* L.) is a widely consumed commodity and its application can be found in manufacturing of chocolates, beverages, cosmetics, pharmaceuticals and toiletries products. Recent studies have revealed several positive health implications of cocoa polyphenols ranging from preventing cardiovascular disease, lowering blood pressure, improving endothelial function, inhibiting platelet aggregation and reducing inflammatory responses [38,79]. Typically, the total phenolic compounds in cocoa are about 6 to 8 % by weight of a dried fermented cocoa bean [80]. The composition and amounts of polyphenols in cocoa beans vary significantly with bean type, origin and methods of processing. It has been reported that fermentation, drying and alkalization could lead to substantial decrease in polyphenols amounted to nearly 60 % of total flavonoids [59,81,82].

Drying after fermentation is an important step as it has a huge role in governing the final quality of dried cocoa beans. Conventionally, cocoa farmers use sun and hot air to dry cocoa beans to achieve desired moisture content conducive for safe storage. The drying process ensures various chemical and bio-chemical changes necessary to form the flavour and aroma precursors are produced for subsequent roasting process in secondary processing [83,84]. However, drying degrades polyphenols in cocoa beans via complex reaction known as browning and also due to thermal degradation due to heat.

Although polyphenols in bean degrade considerably during drying, the remaining amount will impart an astringent taste to the chocolate products after processing [14]. The degradation reaction of polyphenols can be explained as browning process where phenolic compounds are degraded due to enzymatic and non-enzymatic reactions. The precursor for enzymatic reaction (polyphenol-oxidases enzymes) is usually activated towards the end of fermentation and its activity continues during drying. At drying temperatures higher than 60°C, non-enzymatic reaction plays a more dominant role in degrading polyphenols in cocoa beans. Under the action of heat, the non-enzymatic browning (Maillard reactions) process involves the carbonyl groups of reducing sugars and amino groups of proteins to undergo chain reactions to form coloured polymeric compounds [14].

The degradation of cocoa polyphenols under the action of heat during drying are scarcely reported in literatures. Kyi *et al*. [14] reported kinetics of polyphenols degradation at drying temperatures ranging from 40°C to 60°C for the first 5 h of drying inside an hot air oven. However, in commercial cocoa drying the operating temperature is above 60°C for a longer drying duration (typically more than 10 h). Both enzymatic and non-enzymatic reactions play significant role and can be affected under the action of heat. Therefore, this chapter aims to investigate polyphenols degradation based on conditions used in commercial practice to get a better understanding on the polyphenols degradation kinetics and its impacts on product quality.

4.2. Materials and Methods

4.2.1. Sample preparation

Fermented cocoa beans were obtained from Malaysian Cocoa Board, Jengka, Pahang. The beans were kept in deep freezer (FZ301, Khind, Malaysia) at -18°C to prevent further fermentation process to occur. Prior to drying, the beans were allowed to defrost overnight at room temperature. Approximately 20-30 g of samples were used in each drying experiment.

4.2.2. Drying procedure

Drying experiments for fermented cocoa beans were carried out using convective air oven (Humidity controlled drying chamber, Memmert HCP 108, Germany). The equipment was allowed to pre-heat for more than 10 h prior to experiment to achieve stable drying temperature and relative humidity. The relative humidity was set constant at 50 % throughout drying.

4.2.3. Drying kinetics

The drying kinetics analysis was carried out as described in section 3.3.2 under General Materials and Methods (Chapter 3). The moisture content was determined based on an hourly recording of the weight of cocoa beans for a period of 26 h in the oven. The exposure time (26 h) was chosen to match the typical drying time used in industry.

4.2.4. Effective diffusivities

The effective diffusivities were determined following the steps as mentioned in section 3.3.3, General Materials and Methods (Chapter 3).

4.2.5. Total polyphenolic content analysis

Total polyphenolic analysis was carried out as described in section 3.4.1 under General Materials and Methods (Chapter 3) for all the experimental parameters shown in Table 4.1. The exposure time used were 12 h, 24 h, 32 h and 40 h and these were selected to analyze effects of prolonged drying (0 – 40 h) on the polyphenolic contents of cocoa beans.

Table 4.1: Parameters of oven drying

Label	Temperature (°C)	Exposure time (h)	Relative humidity (%)
H60	60	12, 24, 32, 40	50
H70	70		
H80	80		

4.2.6. Polyphenol degradation kinetics

The following are the main focus of polyphenols degradation model:

$$\text{Polyphenols} + \text{Oxygen} \xrightarrow{k} \text{o} - \text{Quinone} \quad (14)$$

For the determination of the reaction rate constant (*k*), a first-order reaction model was assumed to represent the above reaction:

$$\frac{dC_p}{dt} = -kt \quad (15)$$

$$C_p = C_0 \exp^{-kt} \quad (16)$$

Where, C_p is the concentration of polyphenols measured at a drying time *t.* C_0 is the concentration of polyphenols of the reference sample or at time *t*=0.

Equation (16) can be linearized and plotting a straight line graph (ln C_p versus *t*), the rate constant (h^{-1}) is determined from the gradient of the straight line graph;

$$\ln C_p = lnC_o - kt \quad (17)$$

The activation energy (E_a) can be determined by modifying Arrhenius equation shown as following, where k_0 is the pre-exponential factor ($m^2\ s^{-1}$)

$$k = k_0 \exp^{-E_a/RT} \quad (18)$$

4.2.7. Statistical analysis

The statistical analyses were carried out as described in section 3.5 under General Materials and Methods (Chapter 3).

4.3. Results and discussion

4.3.1. Drying kinetics

Figure 4.1 shows that moisture ratios of the cocoa beans decreased exponentially with time as reported for most agricultural products in published literatures [25,85,86] and the moisture ratio for samples dried from H80 is lower than samples dried at H70 and H60. Figure 4.2 shows the drying rates for H60, H70 and H80, respectively. The initial

drying rates were estimated at 0.15 g H_2O g^{-1} dry solid h^{-1}, 0.16 g H_2O g^{-1} dry solid h^{-1} and 0.21 g H_2O g^{-1} dry solid h^{-1} for H60, H70 and H80, respectively. The higher drying rate for H80 was due to the higher temperature which initiates greater driving force for mass transfer to occur. Only falling rate period was observed in H80 while for H70 and H60 treatments. The initial phase showed a rather short existence of constant rate period. This was due to the lower drying temperature requiring more heat to evaporate the surface moisture.

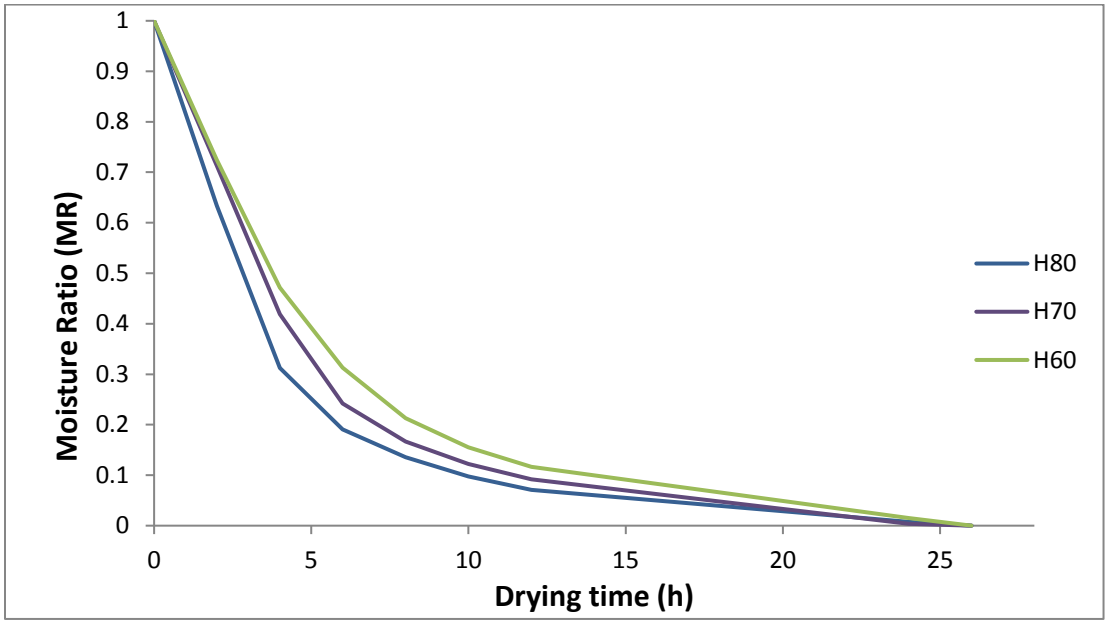


Figure 4.1: Moisture ratio curves for cocoa beans dried for trials H60, H70 and H80

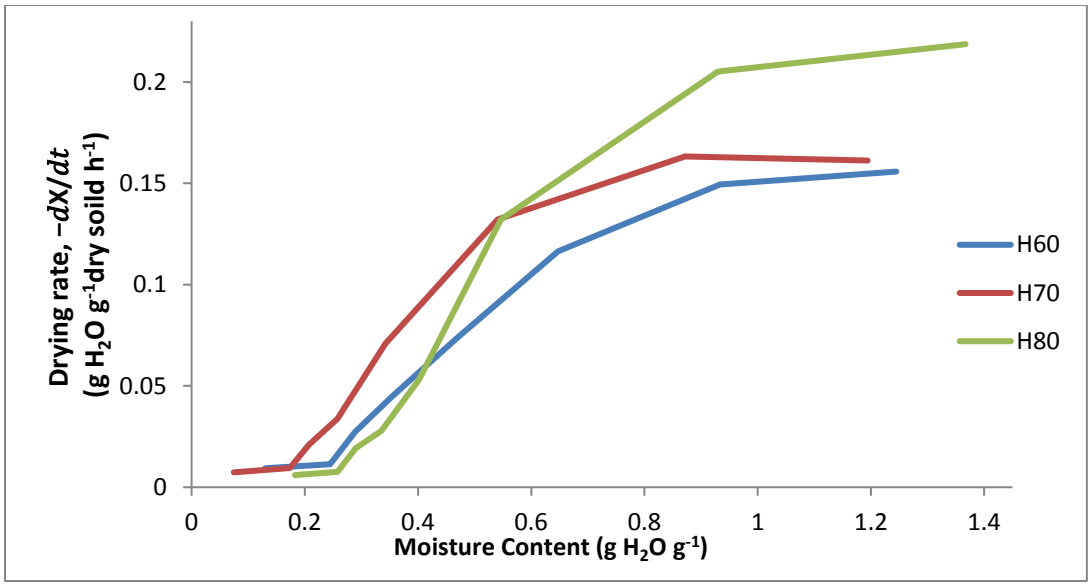


Figure 4.2: Drying Rate curves of cocoa for trials H60, H70 and H80

Generally, for cocoa dried using hot air drying methods only falling rate periods are observed, and this is due to the higher rates of drying initially, in hot air method as opposed to sun drying which takes longer time durations for the cocoa beans to mediate moisture evaporation from the surface due to the mild drying temperature present [24,25,59]. The drying rates for all the experiments reduced significantly as the moisture content approaches 0.2 g H_2O g^{-1} dry solid h^{-1}. For hot air drying, the drying kinetics of fermented cocoa beans were reported by Hii *et al*. [8] at 60°, 70° and 80°C, respectively. It was reported that the drying rates diminished as the drying temperature reduced; 0.2652 g H_2O g^{-1} dry solid h^{-1}, 0.1556 g H_2O g^{-1} dry solid h^{-1} and 0.1436 g H_2O g^{-1} dry solid h^{-1} for treatments 80°C, 70°C and 60°C drying temperatures, respectively. The high rates of drying are mostly due to low internal resistance of moisture at the initial phases of drying. Gradually, when the external moisture is evaporated, the heat mediates the movement of moisture towards the outer regions of the beans. For H80

after ca. 0.55 g H_2O g^{-1} moisture content there is a sudden drop in drying rates (2^{nd} falling rate period). This could be due to case hardening of cocoa beans due to fast drying occurring due to high temperature (80°C) in initial periods of drying. The hardened testa could prevent the movement of free moisture from inside to the outside [44].

4.3.2. Effective diffusivities

The effective diffusivities determined were in the range of 2.36 x 10^{-10} to 2.86 x 10^{-10} m^2s^{-1} (Table 4.2) which are within the order of magnitudes reported for drying of food materials (10^{-8} - 10^{-12} m^2s^{-1}) [85,87]. Although the effective diffusivity values were lower than those reported in literatures [8,59,24] but the order of magnitude determined were quite comparable in the range of 10^{-10} to 10^{-11} m^2s^{-1}.

Table 4.2: Effective diffusivities of oven dried cocoa samples

Drying Method	Effective Diffusivity (m^2s^{-1})	R^2
H60	2.36 X 10^{-10}	0.9963
H70	2.66 X 10^{-10}	0.9876
H80	2.86 X 10^{-10}	0.9792

The plot as shown in Figure 4.3 explains the relationship between effective diffusivity and temperature. The Arrhenius constant is a diffusivity constant equivalent to the diffusivity at infinitely high temperatures [8,59]. The values of D_0 and E_a were calculated based on the Arrhenius relationship at 7.16 x 10^{-9} m^2s^{-1} and 9.43 kJ mol^{-1}, respectively,

as shown in Equation (19). The activation energy calculated was much lower than the published values [8,24,59] which could be due to the different bean samples used and different types of dryers. The activation energy indicates the minimum barrier that needs to be overcome to initiate the moisture diffusion process during drying [8].

$$D_{eff} = 7.16X10^{-9} \exp\left[-\frac{9.43}{R_g(T+273.15)}\right] \quad (19)$$

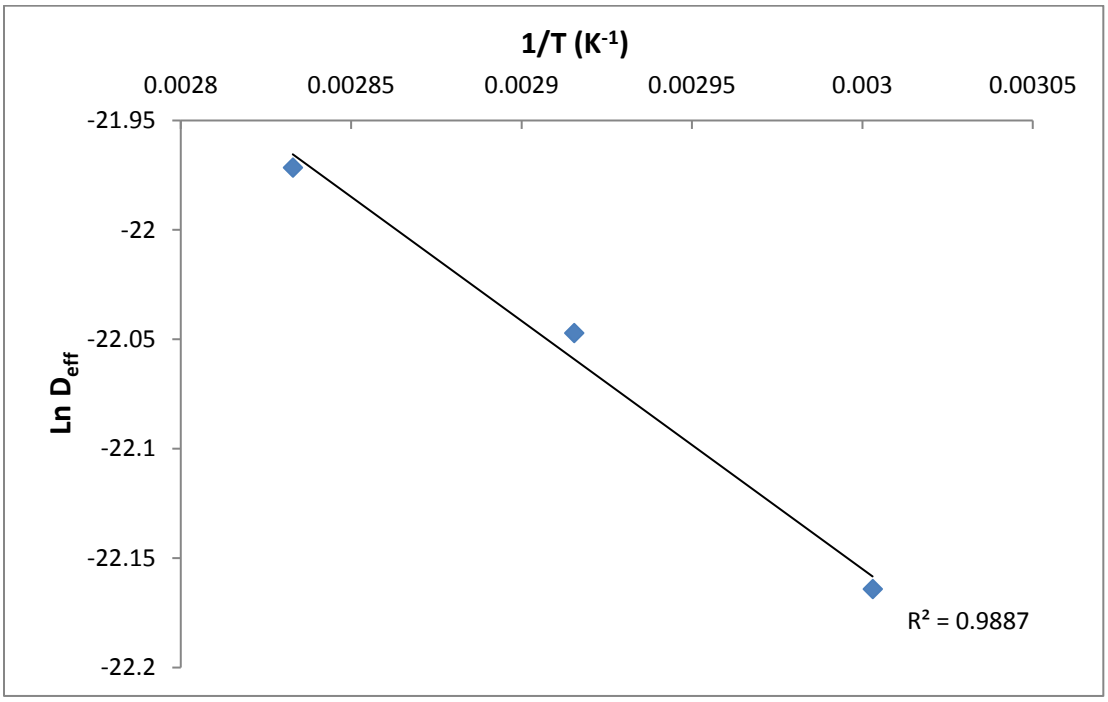
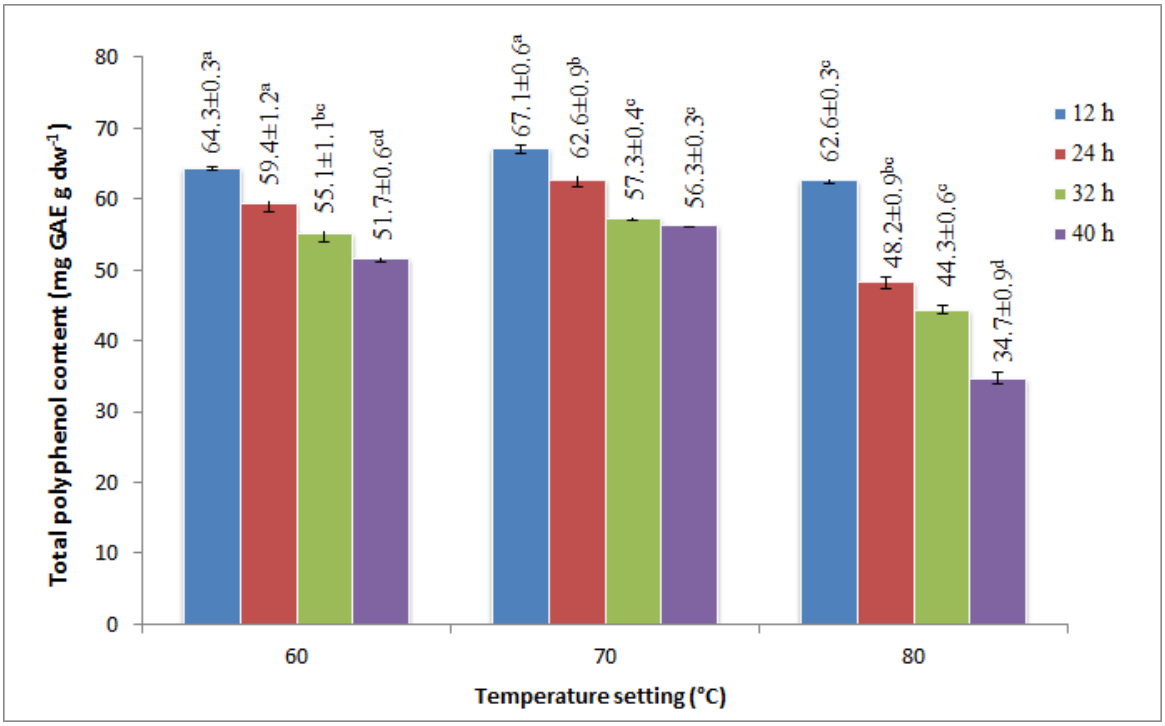


Figure 4.3: The Arrhenius relationship between effective diffusivities and temperature

4.3.3. Total polyphenolic content

Figure 4.4 shows the total polyphenolic content of cocoa beans from H60, H70 and H80 trials at 12 h, 24 h, 32 h and 40 h of drying, respectively. General trend shows reduction of total polyphenol content with increasing duration of drying. Significant differences ($p < 0.05$) were also noted for the polyphenol content dried at 12 h and 40 h in all

temperature treatments. Generally, in cocoa bean drying, thermal decomposition plays a more critical role in polyphenols degradation at higher drying temperature range (60°C and above). At lower temperature range (60°C and below), the mechanism attributed is mostly enzymatic degradation during drying [70]. For example, Hii *et al.* [37] reported that cocoa beans dried using heat pump dryer at 28.2°C recovered high amount of polyphenols (73.9 mg GAE g^{-1}) after drying. Several literatures have published total polyphenols content of fermented cocoa beans dried using hot air drying methods, the values of reported results vary from 45 to 74 mg GAE g^{-1} dw [24, 52, 81, 93].



*Mean values (± standard deviation) having a common letter among same temperature setting are not significant according to the Duncan's Multiple Range test at 5% Level

Figure 4.4: The total polyphenol contents of dried cocoa samples.

The results show that maximum value of total polyphenols was recorded at treatment H70. Hii *et al*. [8] reported the polyphenols content for cocoa beans dried in hot air dryer (60°C, 70°C and 80°C) at 77.2 mg g^{-1}, 82.68 mg $g^{-1}$and 71.42 mg g^{-1}, respectively. The results of total polyphenols content for cocoa samples dried at 70°C (82.68 mg g^{-1}) and samples dried using freeze drying (88.45 mg g^{-1}) showed no significant difference ($p > 0.05$). A similar trend of results was obtained in the current study where treatment H70 shows high polyphenols recovery (67.1 mg GAE g^{-1} dw, 62.6 mg GAE g^{-1} dw, 57.3 mg GAE g^{-1} dw and 51.7 mg GAE g^{-1} dw) at exposure times ranging from 12 h to 40 h. The high recovery of polyphenols could be due to the optimal drying condition (temperature and exposure time) that not only reduces the enzymatic activity but also minimizes rate of thermal degradation of polyphenols. At 70°C, the temperature is neither too high to induce thermal degradation or Maillard browning of cocoa beans, nor too less to prevent the cocoa beans from drying effectively to produce cocoa beans with higher polyphenolic content.

The results for treatment H80 at 24 h, 32 h and 40 h durations show significantly lower ($p < 0.05$) values when compared with H60 and H70 and similar drying durations. This shows that higher drying temperature (80°C) and prolonged drying lead to greater thermal degradation and substantial oxidation of polyphenols, respectively, as shown in H80. Comparing the results for treatments H60 and H70 no significant differences ($p > 0.05$) were noted for any duration of drying. This could be due to the lower moisture content removed in these treatments as opposed to H80. When the moisture content is higher oxidation of polyphenols occurs at a much reduced rate.

4.3.4. Polyphenols degradation kinetics

Table 4.3 shows the rate constants (*k*) determined at experimental settings H60, H70 and H80 from the reaction kinetics as proposed in Equation (14). The results obtained from Figure 4.4 suggests that first order kinetics is sufficient to describe the polyphenol degradation process during drying with rate constant (*k*) values determined within the range of 0.0066 to 0.0202 h^{-1} and R^2 value ranges from 0.964 to 0.9919 (Table 4.3). Generally, degradation for food compounds can be described by first order exponential model which is similar to moisture diffusion model [14]. This could be due to the dependency of polyphenol degradation to the moisture content present within the beans [24]. The activation energy was determined to be 38.2 kJ mol^{-1} and in line with results obtained from several literatures related to cocoa drying (9 - 49 kJ mol^{-1}) [14,24]. The Arrhenius model is as shown in equation (20). This is in agreement to the results obtained in published literatures; Kyi *et al*. [14] reported activation energy values for cocoa beans (dried between 50°C to 80°C for duration 1 h to 6 h) in the range of 27.8 - 30.3 kJ mol^{-1}. The focus of the current study is on drying parameters that are similar to conventional practices where cocoa beans are dried at higher temperatures for longer durations. Teh *et al.* [24], reported the polyphenol degradation kinetics for cocoa beans dried at 60°C, 70°C and 80°C, respectively and reported activation energy for polyphenol degradation to be 9 kJ mol^{-1}.

$$k = k_0 7238.72 \exp\left[-\frac{38.2}{R(T+273.15)}\right] \quad (20)$$

Table 4.3: Rate constants for polyphenol degradation

Temperature (°C)	Rate constant (h^{-1})	R^2
60	0.0091	0.9919
70	0.0066	0.964
80	0.0202	0.9798

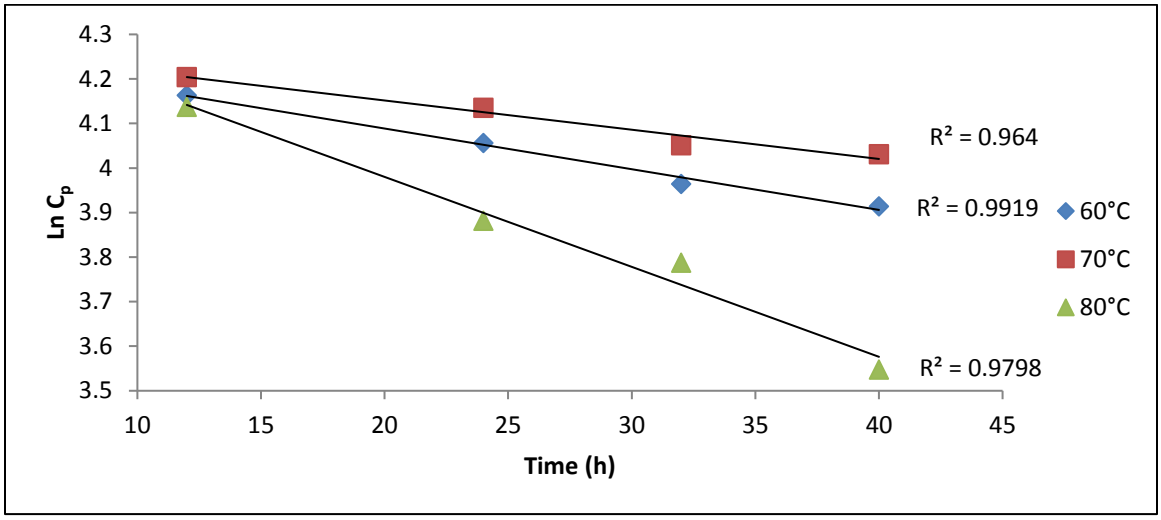


Figure 4.4: Plot of ln C_p against time for rate constant determination.

4.4. Conclusion

Results show that drying rate has a strong relationship with the drying temperature and moisture content of cocoa beans. The drying rates for the samples showed short constant rate periods for 60°C and 70°C drying treatments and falling rate period for 80°C drying are in line with published literatures for hot air drying of cocoa. The activation energy required to initiate moisture diffusion was determined at 9.43 kJ mol^{-1}. Total polyphenols content showed the highest retention in cocoa beans dried at 70°C. Therefore, this treatment was considered optimal and was used for subsequent drying experiments involving hot air oven. The polyphenol degradation kinetics were analyzed

and activation energy was determined to be 38.3 kJ mol^{-1} using the first-order reaction kinetics model. The results from this chapter provide better understanding the polyphenols degradation kinetics under the action of heat with drying conditions similar to conventional drying practices used in industries.

CHAPTER 5

COMPARSION OF DRYING KINETICS OF FRESH AND FERMENTED COCOA BEANS USING ARTIFICIAL AND SUN DRYING METHODS

5.1. INTRODUCTION

The application of fresh cocoa beans for producing cocoa products with high polyphenols is an area of research, which has not been widely explored. Processing methods such as fermentation and drying are responsible for degradation of polyphenols from cocoa beans. Fermentation is able to reduce about 60 % of polyphenols [67] and the remaining polyphenols constituents are further reduced by about 32 % by drying [68]. Thus, the application of fresh cocoa beans for the production of high polyphenols chocolate and other health beneficial products is an important area of research that needs to be envisaged. Unfermented cocoa beans have been reported to contain high polyphenols content which can be compared with that of red wine and green tea [12].

Drying technology in recent years have had rapid advances with the discovery of freeze drying, vacuum drying, microwave drying, superheated steam drying, heat pump drying, etc. However, cocoa beans are still dried using very crude drying methods such as hot air and sun drying methods. Hii *et al*. [18] has reported the use of heat pump drying for cocoa beans. Heat pump dried cocoa samples at 28.2°C were found to recover cocoa polyphenols as high as 73.9 mg GAE g^{-1}, this accounted to 73 % recovery when benchmarked with freeze dried cocoa beans [37]. With high polyphenols recovery

achieved using advanced drying methods; there is a greater need for application of new drying technologies for cocoa beans.

The study on the drying kinetics of cocoa beans using various artificial drying methods in this research will be highly beneficial in transfer of technology for large scale production of polyphenols rich cocoa beans. The studies of cocoa drying by Bravo and McGaw [36] form the basis of most subsequent research on drying kinetics studies [3–6].

Applications of vacuum and adsorption drying for cocoa beans have not been reported in any scientific literatures. The drying process in vacuum drying methods is due to the lowering of boiling point of water at sub-atmospheric conditions which is different from other heat based systems. Adsorption drying process involves a drying mechanism that adsorbs moisture from air to facilitate drying. This study is highly beneficial as drying kinetics for these drying methods can be compared with that of commercial drying technology mainly involving hot air and sun drying. A comparison between drying kinetics of fresh and fermented beans dried using oven drying method are also studied.

The current study emphasized on the analysis of drying kinetics of artificial drying (adsorption, oven hot air and vacuum drying) and sun drying between fresh and fermented cocoa beans. The presence of fresh pulps could have an implication on the exposure time and drying rate due to the much higher initial moisture content. Ultimately, fresh beans are proposed for the production of dried beans with high polyphenolic content.

5.2. Materials and methods

5.2.1. Sample preparation

The fresh and fermented cocoa beans were prepared as mentioned in sections 3.1.1 and 3.1.2, respectively in General Materials and Methods (Chapter 3).

5.2.2. Drying procedure

5.2.2.1. Oven drying

The experimental setup is as mentioned in section 3.2.1, General Materials and Methods (Chapter 3). The drying parameters are as shown in Table 5.1 and denoted as H70.

5.2.2.2 Adsorption drying

The experimental setup is as mentioned in section 3.2.2, General Materials and Methods (Chapter 3). The drying parameters are as shown in Table 5.1 denoted as A60.

5.2.2.3. Vacuum drying

The experimental setup is as mentioned in section 3.2.4, General Materials and Methods (Chapter 3). The drying parameters are as shown in Table 5.1 denoted as V60.

5.2.2.4. Sun drying

The experimental setup is as mentioned in section 3.2.5, General Materials and Methods (Chapter 3). The drying parameters are as shown in Table 5.1 denoted as SUN.

Table 5.1: Experimental drying parameters

Drying Method	Drying time (h)	Drying Parameters
H70	30	T=70°C, RH=50 %, 0.01 m s^{-1}
A60	24	Zeolite adsorbent, (T=ca. 60°C), RH= 9-10%, air flow rate = 4.1 m s^{-1}
V60	24	T= 60°C, P=150 mbar
SUN	36	Direct sun light exposure (7 am to 7pm), T=26°C to 36°C, RH= 65-75 %, air flow rate 1.3 m s^{-1}

5.2.3. Temperature profiles

Bean surface temperatures were recorded using an infrared thermometer (Oakton, SP2224A-SP, USA). The air temperature during drying for adsorption, oven and vacuum oven were recorded using temperature sensors installed in the dryer. During sun drying, the air temperatures were recorded using a digital thermometer (UWI, PDT 550, China). The drying air temperature and cocoa bean surface temperature were recorded every 3 h interval.

5.2.4. Moisture content

The calculation steps are as mentioned in section 3.3.1, General Materials and Methods (Chapter 3). The drying treatments were terminated when the cocoa beans attained a moisture content of less than 7 % dry basis.

5.2.5. Drying rates

The calculation procedures are as mentioned in section 3.3.2, General Materials and Methods (Chapter 3).

5.2.6. Effective diffusivities

The effective diffusivities were determined following the steps as mentioned in section 3.3.3, General Materials and Methods (Chapter 3).

5.3. Results and discussion

5.3.1. Temperature profiles

Figure 5.1 shows the mean air and bean surface temperature (fresh cocoa beans) profiles recorded for various drying methods as mentioned in Table 5.1. All the temperature profiles were below 65°C except for H70. According to literature, drying temperature more than 65°C is detrimental to quality especially in terms of flavour [89]. The highest temperature range (67.4°C to 70.4°C) were recorded for treatment H70 while treatment SUN recorded the lowest temperature range (27.5°C to 35.4°C). The initial air temperature for A60 was noted to be lower (36.4°C) because no pre-heating of the dryer was performed to prevent the saturation of zeolite adsorbent prior to drying process. There is a considerable fluctuation in the temperature range of treatment SUN on comparison with the values generated for other methods. This is due to the considerable temperature fluctuation that occurs during the course of sun drying in a day. The beans temperatures for artificial drying methods namely H70, A60, V60 in the initial period showed a slow rise in surface temperature. Approximately after 6 h of

drying time, the bean temperatures hold up at a constant temperature, which was determined by the maximum temperature pre-set in all drying methods.

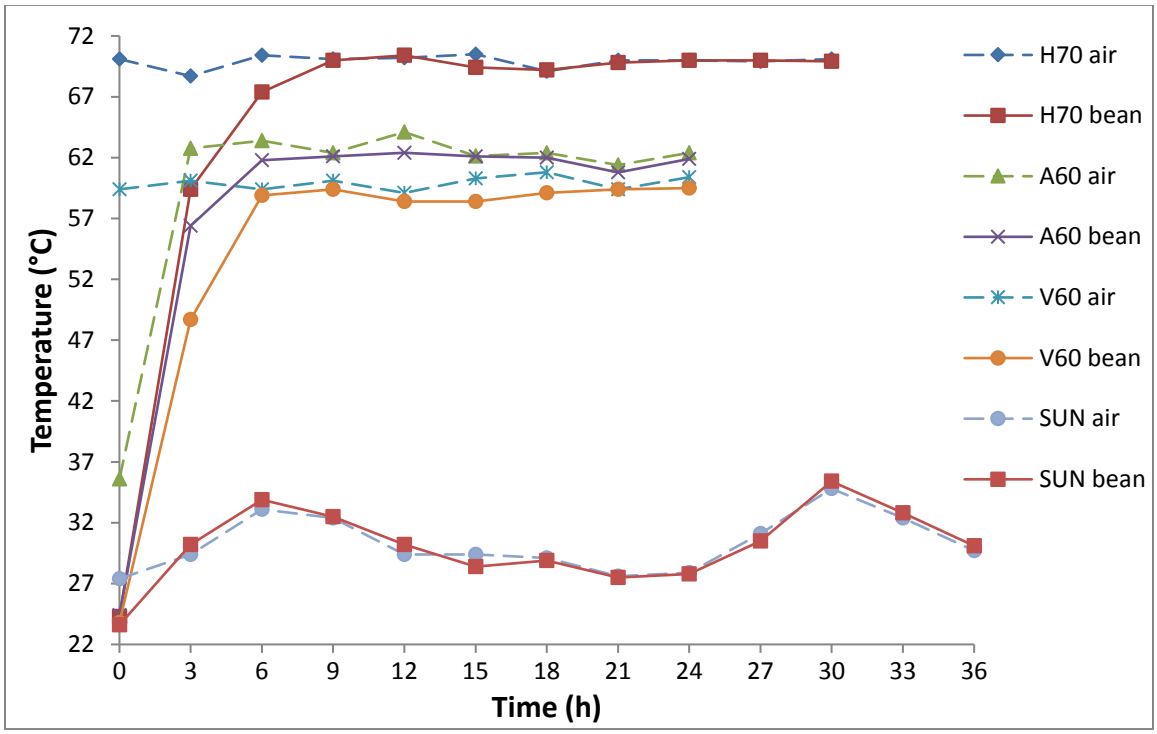


Figure 5.1: Measured air and bean temperature profile during drying of fresh beans

5.3.2. Drying Kinetics

5.3.2.1. Drying kinetics analysis of fresh cocoa beans

Figure 5.2, shows the moisture ratio profiles of the cocoa samples dried using the various drying techniques. In all cases it can be observed that moisture ratios fell exponentially with time as reported for most agricultural products [24]. A60 shows a lower moisture ratio profile on comparison with H70, V60 and SUN. The SUN drying profiles are lower due to the significantly mild drying temperatures the beans were exposed with. For the preservation of bioactive compounds within the beans it is recommended that fast drying (T >80°C) with short durations (< 6 h) are not

appropriate, however for sun drying methods, considerable polyphenols in cocoa are lost owing to the long drying durations and thus higher loss of polyphenols [8]. It is worth noting the trends observed by treatments V60 and A60 dried faster than H70 and SUN. This trend was observed inspite of treatment H70 having higher drying temperature. This could be attributed towards the higher loss of moisture achieved by evaporation through lowering of boiling point in V60 and faster drying occurs at low relative humidity conditions and high volumetric flow rate of dry air (RH= 9-10 %) experienced by cocoa beans in treatment A60, respectively.

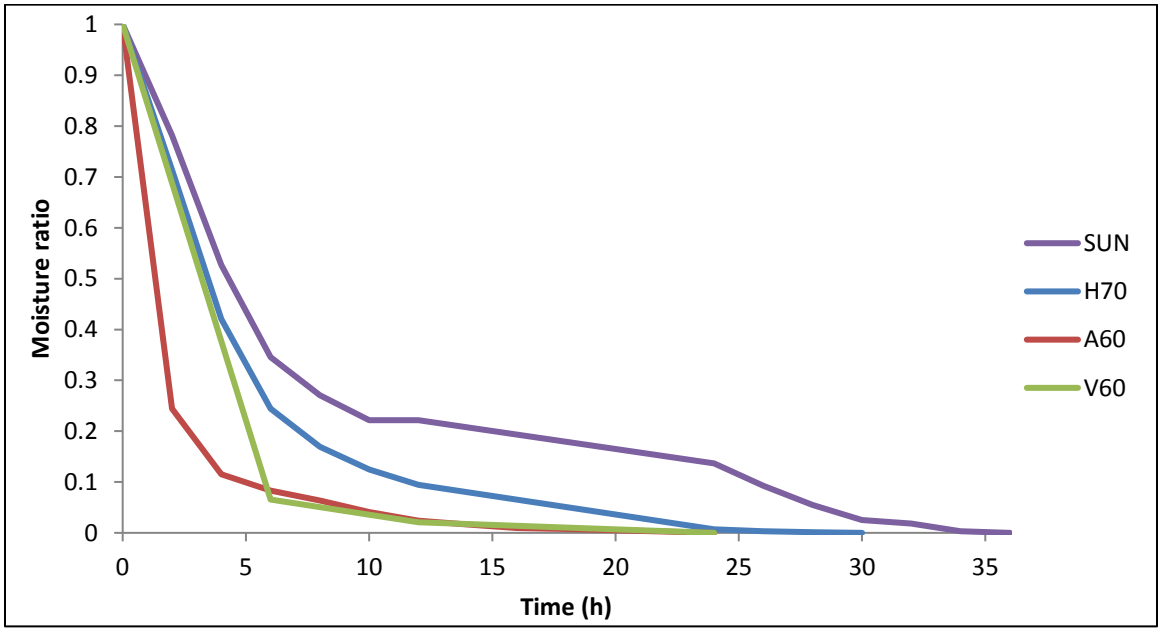


Figure 5.2: Moisture ratio profiles for cocoa beans dried using different drying methods

Figure 5.3 shows the drying rates of the cocoa bean samples, the initial drying rates were estimated at 0.177 g^{-1} dry solid h^{-1}, 0.505 g^{-1} dry solid h^{-1}, 0.249 g^{-1} dry solid h^{-1} and 0.164 g^{-1} dry solid h^{-1} for H70, A60, V60 and SUN treatments, respectively. At the lower moisture content region, temperature plays an important role on drying rate due to the

lower drying force between the interior moisture content and the surface. It is observed that there is an existence of constant rate periods for H70 and SUN treatments, respectively. No constant rate periods are observed for A60 and V60 due to the high airflow (adsorption dryer) and low boiling point of water (vacuum drying), respectively, that causes higher rate of surface moisture evaporation to the surrounding.

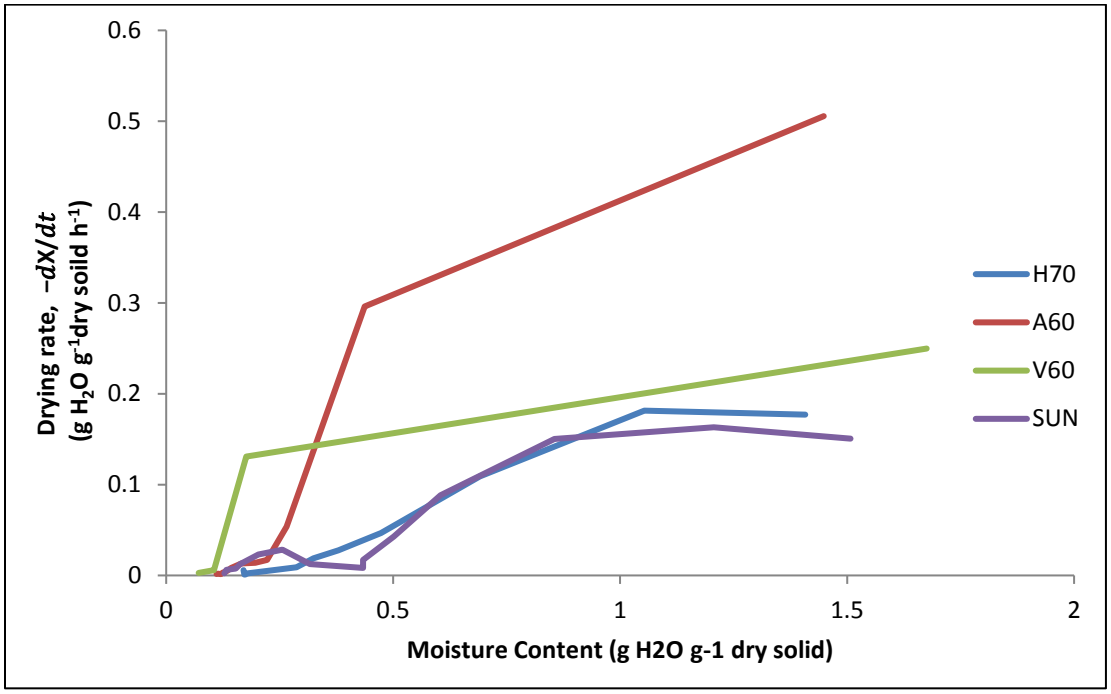


Figure 5.3: Drying rate curves of cocoa dried using different drying methods

Constant rate periods in food products are observed when there is the presence of a consistent layer of saturated moisture which evaporates using heat energy. There is neither an increase nor decrease in the drying rates in constant rate periods [56]. Falling rate periods are observed after evaporation of the free moisture from the mucilaginous pulp of fresh cocoa beans and diffusion of moisture from inner cotyledon area to the outer testa sets in [77]. The drying rate curves for adsorption drying and vacuum drying

experienced falling rate period only. The bean surfaces are dried but the inner core contains remaining moisture which requires a greater driving force for diffusion to surface to occur [36]. Subsequently, drying rate diminishes as the moisture content reduces to less than 0.17 $gH_2O\ g^{-1}$ dry solid towards the end of drying. Two distinctive falling rates are observed in V60 and A60, respectively, namely the first and second falling rate periods. The occurrence of the second falling rate period could be due to the case hardening phenomenon that further restrict the movement of moisture from the inner core to the bean surface.

5.3.2.2. Comparison of the drying kinetics of fresh and fermented cocoa beans

The comparisons between drying kinetics of fresh and fermented cocoa beans after oven drying are shown in Figures 5.4 and 5.5, respectively. The fresh and fermented cocoa beans were dried for 30 h and 26 h, respectively. The moisture ratios of fresh and fermented shows that the fermented cocoa beans dried at a faster rate than fresh cocoa beans. This is because in fresh beans, there is an existence of thick mucilaginous pulp which contributes an additional resistance to drying. It is reported that cocoa beans loses as much as 30-35 % (wet basis) of moisture after fermentation process [90].

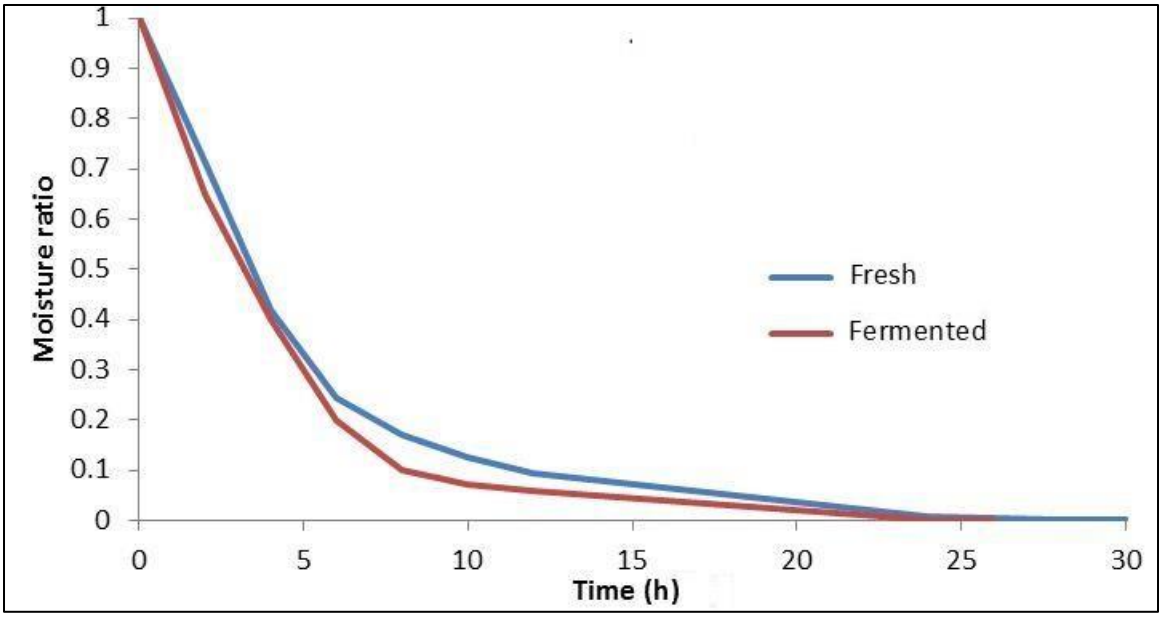


Figure 5.4: Moisture ratios of fresh and fermented cocoa beans after oven drying

The initial drying rates for fresh and fermented cocoa beans were determined at 0.177 g H_2O g^{-1} dry solid h^{-1} and 0.181 g H_2O g^{-1} dry solid h^{-1}, respectively. This is a small difference and could be due to the similar amount of moisture content present in the outer layers of cocoa beans initially. There is a trend of lower drying rates for fresh cocoa beans after drying on comparison with fermented cocoa beans. In fermented cocoa beans, there is availability of free moisture (moisture retrieved from storage cells after bean death) and acids in beans after fermentation [28]. In fermented beans, diffusion of moisture occurs freely through the non-existence pulp (which is degraded after fermentation). This is evident from the rates of drying for fresh cocoa beans showing higher values in the initial period, which predominantly is the loss of moisture from the outer pulp of beans. Fresh beans owing to the higher moisture contents were also dried until 30 h on comparison to 26 h for fermented cocoa beans to compensate the drying of extra moisture content.

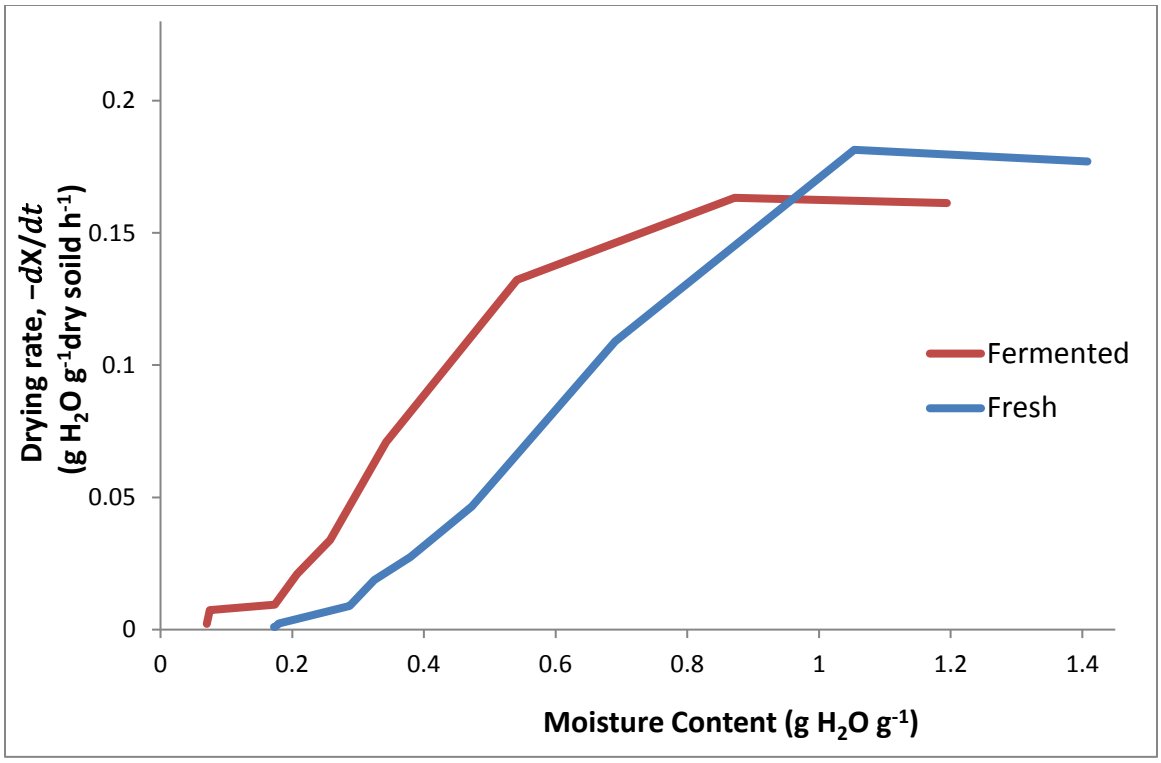


Figure 5.5: Drying rates of fresh and fermented cocoa beans after oven drying

5.3.3. Effective diffusivities

Table 5.2 shows the effective diffusivity values determined for each drying method. Regression analysis shows that the coefficients of determination are in the range of 0.7553 to 0.9998 for artificial drying treatments namely H70, A60, V60 and SUN. Determination of effective diffusivities during drying is in the range of 1.58 X 10^{-10} to 9.04 X 10^{-10} m^2s^{-1}. These values are typically reported in various agricultural products, particularly for cocoa drying which are in ranges of (10^{-10} to 10^{-12} m^2s^{-1}) [20, 24, 45, 46]. From Figure 5.3, it is shown that treatments V60 and A60 experience two distinct falling rate periods, hence the effective diffusivities of these treatments were obtained for each falling rate period distinctively. The highest values of effective diffusivities (9.04 X 10^{-10} m^2s^{-1}) were observed in treatment A60 for the first falling rate period, this could be

due to the high losses of saturated moisture present on the surface of the beans due to evaporation by dry air (RH= 9-10 %). The effective diffusivity values for second falling rate period of A60 were observed to be 3.67 X 10^{-10} m^2s^{-1}, which is lower than the first falling rate period. This is mainly due to the lesser of moisture diffusion occurring and higher driving force required for inner moisture particles to approach the testa of cocoa beans. The values of effective diffusivities for V60 are 5.66 X 10^{-10} m^2s^{-1} and 4.45 X 10^{-10} m^2s^{-1} for first and second falling rates periods, respectively. The high rate of moisture diffusion is achieved in V60 due to low-pressure conditions decreasing the boiling point of water inside the beans [23]. It is to be noted that the effective diffusivity of the second falling rate period of treatment V60 (4.45 X 10^{-10} m^2s^{-1}) is higher than treatment A60 (3.67 X 10^{-10} m^2s^{-1}). From Figure 5.2, it is shown that A60 undergoes faster drying in the initial stages of drying. The moisture is encapsulated in the inner core of the beans will therefore take a much higher driving force to be removed through diffusion. This could be correlated with the higher effective diffusivity values of second falling rate periods in V60. SUN treatment recorded the lowest value (1.58 X 10^{-10} m^2s^{-1}) as expected mainly due to the lower driving force for drying from the ambient temperature condition (27-33°C). Similar literatures for sun drying process have been reported where the effective diffusivity values were considerably lower than that of artificial drying methods [8,24,37]. Treatment H70 observed effective diffusivity values of 2.79 X 10^{-10} m^2s^{-1} and 2.66 X 10^{-10} m^2s^{-1} for fresh and fermented cocoa beans, respectively. The values recorded identical values, which is similar to the drying kinetics analysis comparing fresh and fermented coco beans in treatment H70. The differences

in the moisture ratios and rates of drying were negligible. The effective diffusivity values of H70 are the lowest among the artificial drying methods (V60 and A60). Similar patterns of results were observed in section 5.3.2, where treatments A60 and V60 dried faster than H70. The study on effective diffusivity of fresh cocoa beans has still not been reported in any literatures. More analysis needs to be carried out in order to verify the moisture diffusion process of various drying methods.

Table 5.2: Effective diffusivity of cocoa dried using different drying methods

Bean type	Drying treatments	Effective diffusivity, D_{eff} (m^2s^{-1})	R^2
Fresh	SUN	1.58×10^{-10}	0.8617
Fresh	A60	9.04×10^{-10} *	0.9998
		3.67×10^{-10} **	0.8343
Fresh	V60	5.66×10^{-10} *	0.9997
		4.45×10^{-10} **	0.7553
Fresh	H70	2.79×10^{-10}	0.9941
Fermented	H70	2.66×10^{-10}	0.9876

* Denotes the effective diffusivity for 1st falling rate period, ** the effective diffusivity for 2nd falling rate period

5.4. Conclusion

This chapter highlights the drying kinetics of vacuum and adsorption drying methods and compares it with that of traditional drying methods such as oven drying and sun drying. The drying kinetics analysis in the current research helped in establishing that the rate of drying varied depending upon the drying parameters such as temperature and moisture content, this is in accordance with reported literatures. It is shown that adsorption and vacuum drying methods take shorter drying time and removing moisture

at higher rates when compared to oven and sun drying methods. The comparison between drying kinetics of fresh and fermented cocoa beans using oven drying method were analyzed. It was observed that fermented cocoa beans dried faster than fresh cocoa beans. The findings in this chapter also demonstrated that cocoa beans dried using vacuum and adsorption drying methods shows promising results on comparison with conventionally used, oven and sun drying techniques.

CHAPTER 6

EFFECTS OF HOT WATER BLANCHING ON THE TOTAL POLYPHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF COCOA BEANS

6.1. Introduction

Cocoa has the highest flavanol content of all foods on a per-weight basis and is a significant contributor to the total dietary intake of flavonoids [12,16]. Cocoa antioxidants such as flavonoids are intimately involved in the prevention of free radical damage, but it also has a central role in boosting collagen protection. This means that antioxidant-rich foods like cocoa are not only good cancer prevention and other degenerative diseases, but also provide other benefits such as anti-aging properties. The polyphenol degradation of cocoa beans in standard processing methods is primarily due to fermentation, drying and roasting. The main reason for this phenomenon is due to the activation of polyphenol oxidases enzyme which is a pre-cursor for polyphenol degradation (browning reaction).

By using blanching as a pre-treatment on cocoa beans, it can degrade the polyphenol oxidases enzyme, which in turn would help in substantial recovery of polyphenol contents in cocoa after drying. Water is non-reactive to cocoa beans (inert) during short period blanching process and able to inactivate the polyphenol oxidases enzyme [15]. Freshly harvested cocoa which are not subjected to any fermentation or other processing methods contain high levels of low molecular weight polyphenols. These polyphenols are polymerized by oxidation process once the bean is opened or subjected

to processing methods such as hot air drying and roasting. Hence, these polymeric compounds are no longer available for bio-absorption in human diet [80].

Since there is no economically feasible method to avoid such polymerization process from occurring, there is a major requirement to look into blanching unfermented cocoa beans. Conventionally, cocoa farmers use sun and hot air to dry cocoa beans. The drying process ensures various chemical and bio-chemical changes that are necessary to form the flavour and aroma precursors are produced [8]. The conventional drying methods for cocoa is reported to reduce the polyphenols by about 24 % [34]. The application of artificial drying techniques for cocoa beans have been studied by various researchers, however study on effects of hot water blanching pre-treatment prior to adsorption, vacuum, freeze, oven and sun drying methods have not been reported.

6.2. Materials and methods

6.2.1. Sample preparation

The cocoa samples were prepared following the methods mentioned in section 3.1, in General Materials and Methods (Chapter 3). For the half cut cocoa bean experiment, the beans were cut longitudinally using a knife, along the bean length before subjecting it to blanching process.

6.2.2. Blanching pre-treatment

The blanching experiment setup is shown in Figure 6.1 following the parameters as mentioned in Table 6.1. Blanching was performed using a 1000 ml beaker filled with 700 ml of water. A metal sieve (1 X 1 mm, mesh size) was used to hold the samples during

blanching. Heating was carried out using a hot plate (Cole Palmer 4568-456800, USA). The operating temperature was set according to the parameters shown in Table 6.1.

Table 6.1: Experimental protocol for blanching experiment for fresh, fresh cut, fermented and fermented cut cocoa bean samples

All Samples	
Blanching temperatures (°C)	**Blanching duration (min)**
70	5,10 and 15
80	
90	

Figure 6.1: Blanching experiment setup

The experiments commenced when the water medium attained the desired temperature. The sieve was gently immersed into the beaker and approximately 10-15 cocoa beans were placed into the blanching medium (within the sieve). A thermometer was used to record the temperature during blanching along with a stopwatch to record the exposure time. After blanching, the cocoa beans were dipped in an ice bath (ca. 4°C)

for 15 seconds to reduce the temperature of the beans as soon as possible. This step was very important to prevent the bean from remaining in a state of high temperature which could lead to degradation of phenolic compounds. The beans were then pat dried using tissue paper to remove any extra moisture present in the surface of the beans. The highest blanching temperature was set at 90°C as any temperature above it would thermally degrade the bioactive compounds in cocoa beans [15]. The water used for blanching process was replaced after each experiment for uniformity.

6.2.3. Drying procedure

For the analysis of total polyphenolic contents of fresh and fermented (full and half cut) cocoa beans after blanching pretreatments, oven drying at 70°C at 50 % RH were used. The drying procedures for experiments of fresh cocoa beans are as shown in Table 6.2.

Table 6.2: Experimental drying procedure for fresh cocoa beans after blanching

Drying method	Drying time (h)	Drying Parameters
H70	30	T= 70°C, RH= 50%, air flow rate 0.01 m s^{-1}
A60	24	Zeolite adsorbent, (T=ca. 60°C), RH= ca. 9-10%, air flow rate= 4.1 m s^{-1}
V60	24	T= 60°C, P= 150mbar
FD	24	Main drying: T= -30°C , 24 h; Final drying T= -50°C, 4 h P= ca. 0.015mbar
SUN	36	Direct sun light exposure (7 am to 7pm), T= 26°C to 36°C, RH= 65- 75%, air flow rate 1.3 m s^{-1}

6.2.3.1. Oven drying

The experimental setup is as mentioned in section 3.2.1, in General Materials and Methods (Chapter 3). The drying parameters are shown in Table 6.2.

6.2.3.2. Adsorption drying

The experimental setup is as mentioned in section 3.2.2, in General Materials and Methods (Chapter 3). The drying parameters are shown in Table 6.2.

6.2.3.3. Freeze drying

The experimental setup is as mentioned in section 3.2.3, in General Materials and Methods (Chapter 3). The drying parameters are shown in Table 6.2. The quality analysis (total polyphenolic content and antioxidant assays) of samples after freeze drying were used for benchmarking purposes. Freeze dried cocoa has been reported to recover the highest bioactive compounds in cocoa beans [24].

6.2.3.4. Vacuum drying

The experimental setup is as mentioned in section 3.2.4, in General Materials and Methods (Chapter 3). The drying parameters are shown in Table 6.2.

6.2.3.5. Sun drying

The experimental setup is as mentioned in section 3.2.5, in General Materials and Methods (Chapter 3). The drying parameters are shown in Table 6.2 and duration of drying were from 7 am to 7 pm daily.

6.2.4. Total polyphenolic contents

The experimental steps are as mentioned in section 3.5.1, in General Materials and Methods (Chapter 3).

6.2.5. Antioxidant assay

6.2.5.1 DPPH radical scavenging assay

DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was executed by following the method as described by Blois [92]. The DPPH (Sigma-Aldrich, USA) solution was freshly prepared by dissolving 0.1 mM of DPPH in methanol (Sigma-Aldrich, USA) and kept aside for 30 min. To 50 µl each of cocoa extracts which were prepared by grinding dried cocoa sample with 70 % methanol, an aliquot of 150 µl of DPPH solution was added and the mixture was incubated in the dark for 30 min at room temperature. The absorbance was measured at 517 nm using a microplate reader (Thermo ScientificTM MultiskanTM GO, Finland). A standard calibration curve was obtained (R^2 = 0.9985) by using 1-40 µg ml^{-1} of Trolox (Sigma-Aldrich, USA) in methanol and the obtained results have been expressed as mg of Trolox equivalent per gram dry weight of the extract, the calibration curves are reported in Figure A3 in section Appendix A.

6.2.5.2. *ABTS radical cation decolourization assay*

ABTS assay was carried out by following the method of Re *et al*. [93] with some modifications. The stock solutions for this assay include 7 mM of ABTS (Sigma-Aldrich, USA) solution and 2.45 mM of potassium persulphate (Sigma-Aldrich, USA). The working solution was prepared by mixing both the stock solutions in equal volume and allowed to react for 12-16 h at room temperature. The working solution was then diluted by mixing with 95% ethanol to get an absorbance value of 0.7±0.02 at 734 nm. Fresh solutions were always prepared for each assay. An aliquot of 20 µl extract was mixed

with 200 µl of working solution and allowed to stand at room temperature for 30 min followed by measuring the absorbance (734 nm) in a microplate reader (Thermo Scientific™ Multiskan™ GO, Finland). A standard curve was obtained (R^2 = 0.9968) with Trolox (1-100 µg ml^{-1}) and the obtained values have been expressed as mg of Trolox equivalent (TE) per g dry weight of the sample, the calibration curves are reported in Figure A4 in section Appendix A.

6.2.6. Percentage differences in total polyphenols content and antioxidants capacity

The percentage differences between the results obtained for the total polyphenols content and antioxidant capacities were calculated based on equation (21) as shown below;

$$\text{Percentage differences} = \left[\frac{value\ (blanched\ sample) - value\ (unblanched\ sample)}{value\ (blanched\ sample)}\right] X100\ (\%) \qquad (21)$$

6.2.7. Statistical analysis

The statistical analysis was performed as mentioned in section 3.5, in General Materials and Methods (Chapter 3)

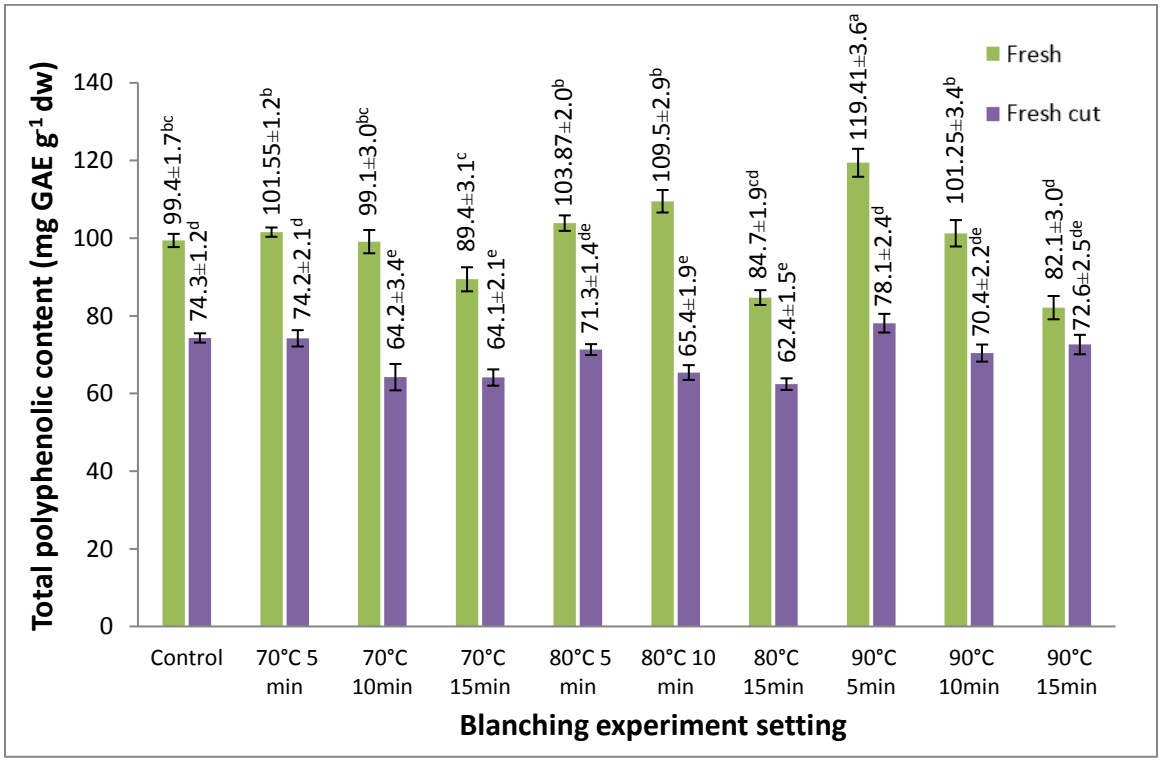
6.3. Results and Discussion

6.3.1. Comparison of total polyphenols contents of fresh and fermented cocoa beans after hot water blanching and oven drying.

6.3.1.1. Total polyphenolic content

Figures 6.2 and 6.3 compare the polyphenolic contents of cocoa beans that were subjected to blanching pre-treatments according to the experiment parameters in Table

6.1. On examining the results for fresh beans initially (Figure 6.2), the maximum polyphenols recovery was found in cocoa beans blanched at 90°C for 5 min (119.41 and 78.1 mg GAE g^{-1} dw for whole and half cut beans, respectively). A similar pattern was noted in research by Tomas-Barberan *et al*. [15]; where cocoa beans blanched at 95°C showed the lowest enzymatic browning for fresh beans. The author explained the importance of inactivating Polyphenol Oxidases (PPO) to promote high recovery of polyphenolic compounds in cocoa beans. During high temperature (90°C and above) blanching, there is occurrence of thermal degradation of PPO activity which would disrupt the polyphenol degradation [15,94].



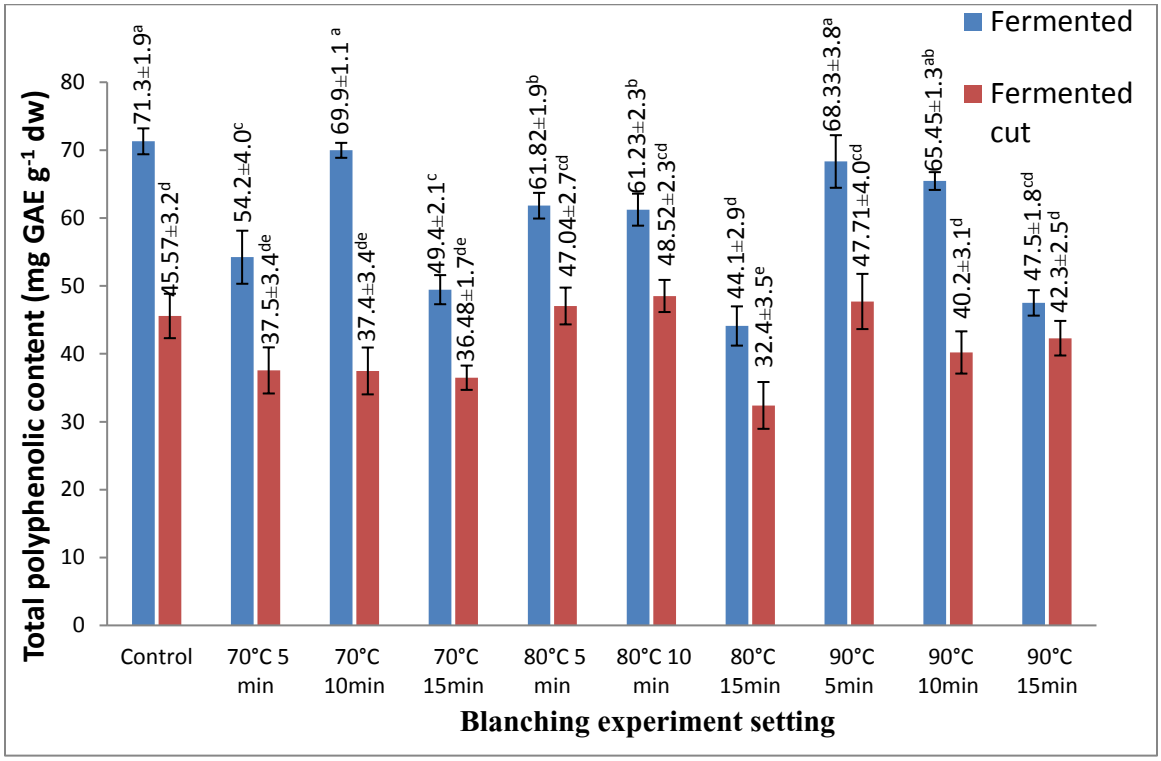
*Mean values (± Standard deviation) having common letters among same blanching treatments are not significant according to the Duncan's Multiple Range test at 5% level

Figure 6.2: The total polyphenolic content of dried fresh and fresh cut cocoa samples after blanching pre-treatments

Blanching temperature and durations vary for food products depending on the quantity of phenolic compounds involved as well as nature of structure of food material analysed. For example, conditions for carrot blanching are at a temperature of 95°C for 1 min for the complete inactivation of polyphenol oxidases and peroxidases [60]; salak blanching temperatures should be below 70°C for blanching durations up to 5 min [95]. Half cut fresh beans also show high contents of polyphenols when blanched at 90°C for 5 min setting (78.1 mg GAE g^{-1} dw). From Figure 6.2, the total polyphenolic contents for fresh whole beans are significantly higher ($p < 0.05$), when compared with half cut fresh beans for all blanching treatments except 90°C for 15min (82.1 mg GAE g^{-1} dw and 72.6 mg GAE g^{-1} dw). The reduced amounts of polyphenols for half cut beans are due to losses through leaching of mostly flavonoids, which are present in the outer parts of plant organs and tissues [60]. Blanching at 90°C for 15 min does not show any significant difference ($p > 0.05$) among whole and cut fresh beans due to the thermal decomposition of polyphenolic compounds due prolonged exposure (15 min) of hot water (90°C) [15].

Fermentation is a deterrent for polyphenols recovery as the browning process occurs. This can be observed in the lower values of polyphenols content recorded for fermented beans (Figure 6.3). The polyphenols content for fresh and fermented beans were recorded to be 102.7 mg GAE g^{-1} dw and 71.3 mg GAE g^{-1} dw, respectively at control setting. The results for the half cut fermented beans are much lower in comparison to whole fermented bean. The lower amount of polyphenols for half cut fermented bean samples could be explained on the basis of chemical constituents within the bean that

leaches into water (during blanching) [84]. No significant differences ($p > 0.05$) are shown among fermented cut samples after blanching. This is because of the significant loses of polyphenols due to fermentation by browning, and losses of flavonoids by leaching from cocoa outer storage cells during blanching and oxidation while exposed to air during cutting of beans [96].



*Mean values (± Standard deviation) having common letters among same blanching treatments are not significant according to the Duncan's Multiple Range test at 5% level

Figure 6.3: The total polyphenols content of dried fermented and fermented cut cocoa samples after blanching pre-treatments

6.3.1.2. Selection of optimal blanching parameter

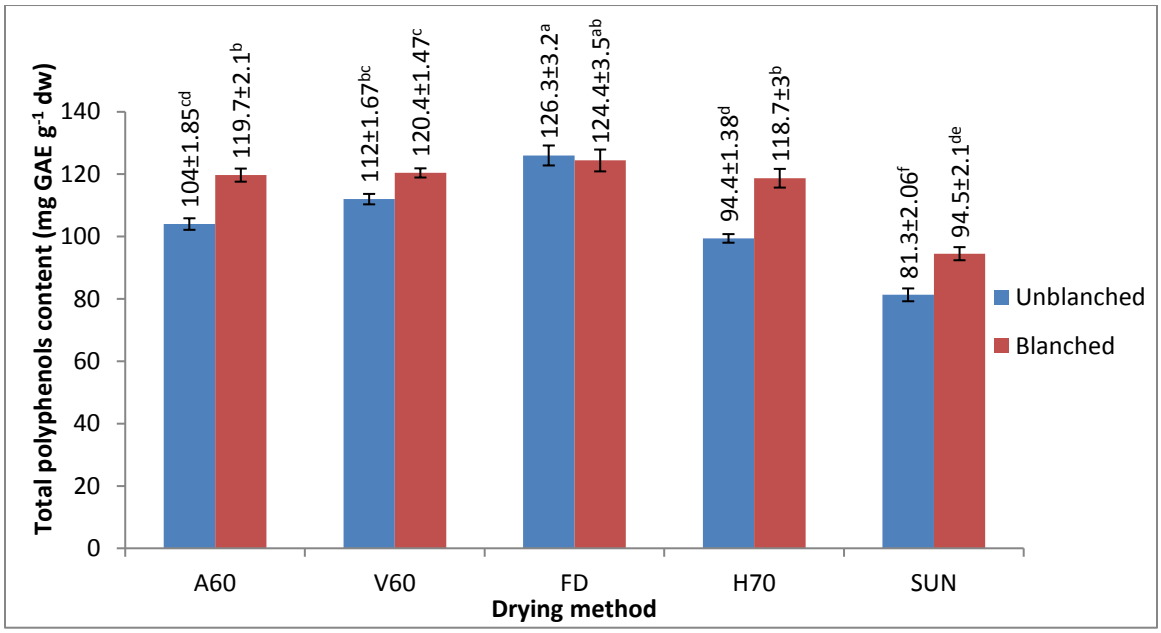
From the results obtained in section 6.3.1.1, blanching parameter of 90°C for 5 min shows the highest recovery of total polyphenolic contents in cocoa beans. Since the objective of the current work is to produce cocoa beans with high polyphenolic contents, fresh cocoa beans blanched at 90°C for 5 min were used for all subsequent

studies. From the results obtained in Figure 6.2 it is noted that for fresh cocoa beans, for a blanching parameter of 5 mins the total polyphenolic content increases from 101.55 to 119.41 mg GAE g^{-1} dw when the blanching temperature is raised from 70°C to 90°C. Further investigation must be performed to better understand the polyphenolic activity at blanching temperatures above 90°C.

6.3.2. Effects of hot water blanching on the total polyphenols and antioxidant activity of fresh cocoa beans from various drying methods

The total polyphenols content of the cocoa samples after blanching at 90°C for 5 min and drying treatments of H70, A60, V60, FD and SUN are as shown in Figure 6.4. The highest amounts of polyphenols recovery was recorded by label FD. This is in accordance with several published literature where polyphenols recovery of freeze dried cocoa sample were significantly high due to the inactive enzymatic activity under vacuum condition leading to impeded browning process [97]. The least amount of polyphenols content was found in cocoa beans dried using label SUN (81.3 mg GAE g^{-1} dw), which could be due to the long drying time and browning process occurring at mild temperature conditions as almost similar values were reported in several literatures [16, 24, 33]. Using the total polyphenols value of FD treatments as a benchmark, the other treatments namely A60, H70, V60 and SUN show significantly lower values ($p < 0.05$). Comparing the results among the drying treatments it is noted that, no significant difference ($p > 0.05$) of polyphenols content were recorded for cocoa beans dried using A60 and H70 as both the drying conditions are at high temperature ($T > 60$°C). High

temperature drying process leads to thermal degradation of polyphenols as well as an increase in oxidation of polyphenols at high temperature which has been reported in literature [71].



*Mean values (± standard deviation) having a common letter within same drying methods are not significant according to the Duncan's Multiple Range test at 5% level

Figure 6.4: The total polyphenols content for blanched and unblanched cocoa beans after drying.

The reason for comparatively high retention of total polyphenols contents for samples dried in treatment V60 (112 mg GAE g^{-1} dw) is due to considerably low oxygen availability in low pressure condition (150 mbar). At low oxygen conditions, the enzymatic polyphenol degradation is minimal accounting to highest total polyphenols values [49, 50]. It is to be noted that, the results for A60 (104 mg GAE g^{-1} dw) show no significant difference ($p > 0.05$) with V60. This phenomenon of reasonably high recovery of polyphenols for A60 could be attributed to the low humidity air conditions present during adsorption drying. At low relative humidity drying conditions, drying rate is

higher and hence the polyphenols degradation due to prolonged drying conditions are minimal [46].

The total polyphenols content of cocoa beans after blanching pre-treatment for treatments A60, H70 and SUN show significantly higher ($p < 0.05$) values in comparison to unblanched beans (Figure 6.4). The percentage differences of total polyphenols content for blanched and unblanched samples are shown in Table 6.3.

Table 6.3: The percentage differences in total polyphenolic contents of blanched and unblanched cocoa beans.

Drying method	Percentage differences (%)
A60	12.46
V60	6.66
FD	-1.26
H70	15.31
SUN	10.47

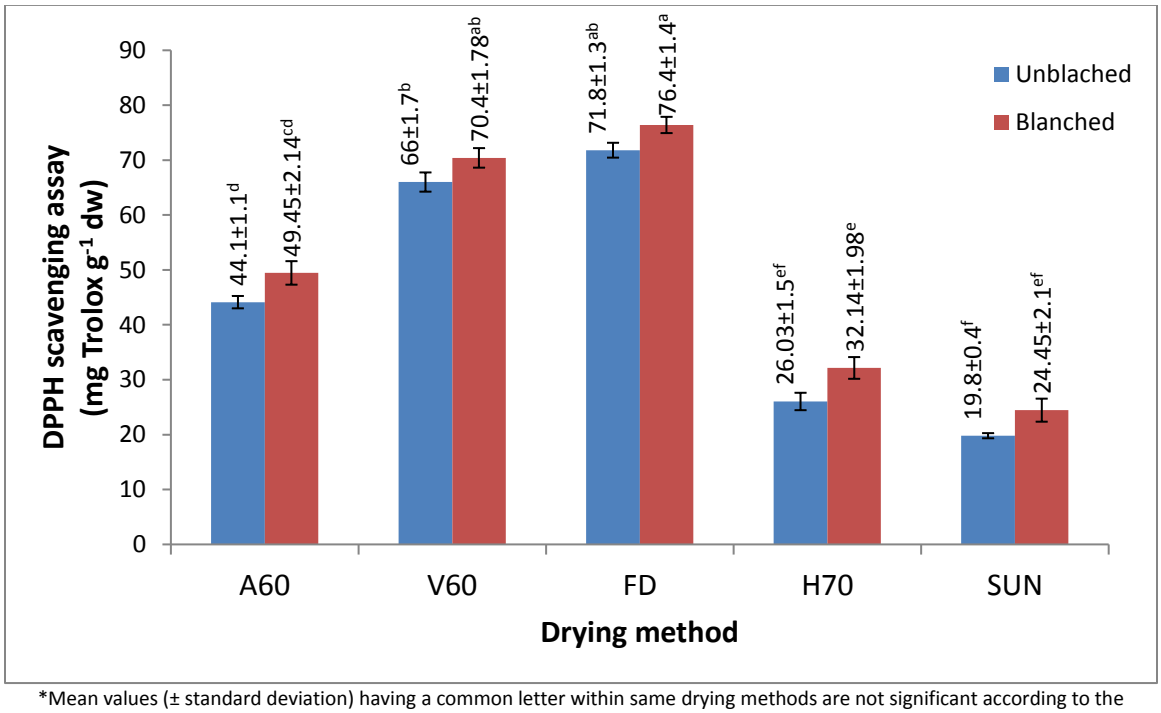
Treatment H70 showed the highest percent difference of 15.31 %. A similar work by Alan and Eva-Maria, [98] showed that steam blanched cocoa beans have high polyphenolic content (24 % more polyphenols) when it were dried at a temperature ranging from 35°C to 50°C in hot-air oven. From Table 6.3, FD dried cocoa samples showed percentage difference of -1.26 % and is quite negligible. In FD, the cocoa samples are dried in frozen conditions (ca. -18°C), and during such low temperatures the PPO enzymes are in an inactive state and cannot mediate browning process. Hence blanching (inactivation of PPO) does not contribute in enhancing the total polyphenolic recovery in freeze dried cocoa samples.

6.3.2. Antioxidant assay

The antioxidant assay results for DPPH and ABTS assays are shown in Figures 6.5 and 6.6, respectively. Both assays follow a similar trend in the results obtained and can be correlated with total polyphenolic compounds results [24–26]. The antioxidant assay reading for cocoa is a combined measurement of the potential of all the antioxidant compounds including phenolic compounds to inhibit oxidation reaction upon consumption by humans. Drying is said to cause severe reduction of bioactive compounds such as flavonoids which is a major antioxidant compound in cocoa bean [59, 62, 63]. The total polyphenolic content analysis followed in the present study was a modified form of Kim and Keeney [74]. According to Kim and Keeney, the total polyphenolic content analysis using Folin ciocalteu reagent does not take into account the anthocyanidin compounds present in cocoa beans. However, anthocyanidins are expressed in both the antioxidant assays DPPH and ABTS, respectively. Hence, any differences in the patterns of results obtained in total polyphenolic content and antioxidant assays (DPPH and ABTS) could be due to this.

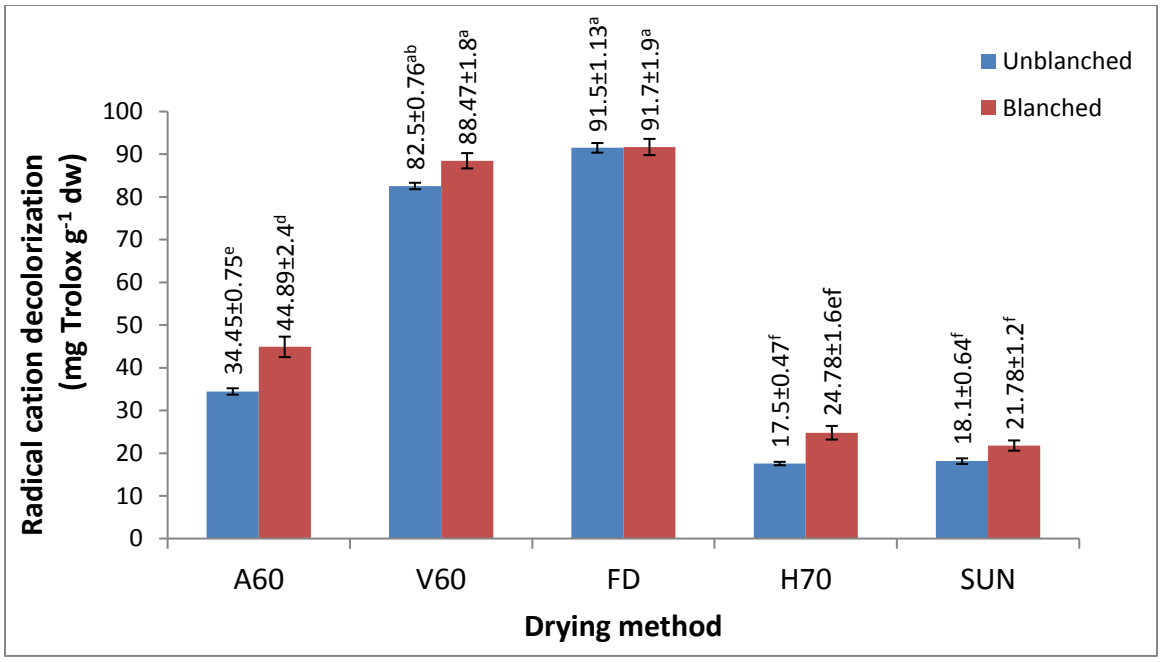
The results for the antioxidant assay for unfermented cocoa beans are relatively higher in comparison to published literatures which was in the range of 18.4-21.6 mg Trolox g^{-1} dw. [11, 90, 93, 109]. Increased antioxidant activity in fermented cocoa are due to increase in the release of antioxidant compounds (phenols and flavonoids) due to microbial hydrolysis during fermentation [104]. Samples from treatment FD showed maximum values for both DPPH and ABTS assay; 71.81 mg Trolox g^{-1} dw and 91.5 mg Trolox g^{-1} dw, respectively. This is due to the low pressure (0.015 mbar) and low

temperature (-40°C) conditions prevalent during lyophilisation process which in turn helps in retaining high traces of bioactive compounds in dried product [58, 59]. In both the assays, treatment V60 dried samples show no significant difference ($p > 0.05$) with freeze dried samples. Similar results have been noted on agricultural products dried using freeze drying, vacuum oven and hot-air oven where; catechin and flavonoids are highest retained in freeze and vacuum dried samples [54]. The significantly low values ($p < 0.05$) of antioxidant activity for treatments A60, H70 and SUN were observed for both assays (DPPH and ABTS). This could be due to the thermal degradation of antioxidants during high temperature for A60 and H70 and prolonged exposure time of drying (36 h) for SUN treatment based drying methods [105].



*Mean values (± standard deviation) having a common letter within same drying methods are not significant according to the Duncan's Multiple Range test at 5% level

Figure 6.5: DPPH radical scavenging assay of blanched and unblanched cocoa beans after drying.



*Mean values (± standard deviation) having a common letter within same drying methods are not significant according to the Duncan's Multiple Range test at 5% level

Figure 6.6: ABTS antioxidant assay of blanched and unblanched dried cocoa beans

Focusing on the effects of blanching pre-treatment on antioxidant assays, only treatment A60 (Table 6.4) method showed a significantly higher ($p < 0.05$) antioxidant activity where blanched samples recorded a value of 44.89 mg Trolox g^{-1} dw and unblanched samples recorded a value of 34.45 mg Trolox g^{-1} dw (difference of 11.4 %) for ABTS assay.

Table 6.4: The percentage differences in antioxidant assay values of blanched and unblanched cocoa beans.

Drying method	DPPH Assay Percentage difference (%)	ABTS ASSAY Percentage difference (%)
A60	7.41	11.41
V60	6.12	6.45
FD	3.76	0.21
H70	8.5	7.88
SUN	6.47	3.98

This is in contradiction with the total polyphenolic content results, where treatments A60, H70 and SUN showed significant increase ($p < 0.05$) after blanching pre-treatment. Similar results portraying a significant polyphenolic content increase after blanching process and non-significant increase/decrease in antioxidant capacity after blanching process has been reported by Oboh [106], for antioxidants of green vegetables after blanching. Results showed a decreasing trend for antioxidant activity whereas total polyphenolic contents showed an increasing trend after blanching process. This trend was reasoned by the author to be the temperature at which blanching (100°C for 5min) which increases solubility and leach away few non-phenolic lipid soluble antioxidant phytochemicals present in vegetables. In cocoa flavonoid compounds, water soluble compounds could solubilize in high temperature blanching process and thus reduces antioxidant values considerably [107]. However, Folin ciocalteu reagent which was used to measure the total polyphenolic content in this study did not take into account the anthocyanidins and shields their effects [75, 108]. The expression of these coloured pigments (anthocyanidins) could also be a factor in the treatments H70, V60 and SUN showing a non-significant ($p > 0.05$) increase after blanching on comparison with total polyphenolic content results.

6.4. Conclusion

From the initial study on effects of hot water blanching on polyphenol content and reaction kinetics of cocoa beans, a significant increase ($p < 0.05$) in the total polyphenol content (119.41 mg GAE g^{-1} dw) for fresh beans is noted after blanching at 90°C for 5 min. This blanching parameter was used for all subsequent pre-treatments for

producing polyphenol rich fresh cocoa beans. For the study on the effects of blanching on the total polyphenolic content and antioxidant activity of fresh cocoa beans, freeze drying method proved to show the highest recovery of total polyphenol contents as well as antioxidant activity. Both adsorption and vacuum dried samples showed considerably high polyphenol recovery of 104 and 112 mg GAE g^{-1} dw, respectively. The blanched cocoa bean samples showed significantly high ($p < 0.05$) total polyphenol recovery in comparison to unblanched samples when dried using adsorption, oven and sun drying methods. Antioxidant assays were performed for fresh cocoa beans with and without blanching pretreatment. The Blanched cocoa samples showed a positive percentage difference for both DPPH and ABTS assays, respectively. This confirms blanching pre-treatment to recover cocoa beans with higher antioxidant capacities. This study is highly beneficial in widening knowledge about effects of blanching pre-treatment and application of various drying methods on the production of polyphenol rich cocoa beans since literatures available on the health benefits of fresh cocoa beans are relatively scarce. In future, research should focus on extraction and application of bioactive compounds obtained using the current study. This will significantly help in making the cocoa industry a more lucrative farming option.

CHAPTER 7

FLAVOUR QUALITY OF POLYPHENOL RICH COCOA BEANS

7.1. Introduction

The typical cocoa flavour consists of many compounds whose formation depends on genetic profile of cocoa, the growth environment and the processing methods used. The influence of processing on the formation of chocolate flavour includes reactions that occur during fermentation, drying and roasting (beans, nibs or liquors) [76]. Cocoa beans are reported to contain more than 600 types of distinct flavour volatiles which are formed during processing [109]. Generally, chocolate or cocoa flavour intensity is preferred to be present in high level as it imparts the distinct flavour in chocolate products. In the current era of health conscious consumers, dark chocolate with high polyphenolic contents have gained immense popularity. With the demand of polyphenol rich chocolate and other cocoa based products, it is important to look into the flavour characteristics of polyphenol rich cocoa beans after drying. The drying methods used for cocoa beans play a major role in the development of cocoa flavour and has been reported in several literatures [3-6]. The effects of drying parameters could influence specific or a combination of several flavour attributes depending on the complex enzymatic reactions which occurs during drying [51]. Sun drying is reported to be optimal for producing the best flavour in fermented cocoa beans. This is due to the low temperature and sufficient drying time for cocoa to dry evenly which leads to chocolate flavour development [40].

However, the drawbacks of using sun drying method are long drying time and influences of environmental and climatic factors. Therefore, the applications of new artificial drying or the combination of sun and artificial drying techniques have been used in recent times to improve cocoa flavour. For example, Jinap *et al*. [17] reported that flavour attributes of Malaysian cocoa beans improved through a combination of air-blown and hot-air drying and were similar to that of sun dried cocoa samples. Hii *et al.* [18] that reported sensory profile scores of cocoa beans dried using heat-pump dryer and the results were comparable to that of Ghanaian cocoa samples which were of superior flavour quality. The disadvantage of using artificial drying methods are the high drying temperatures which leads to faster drying rates, this could lead to high acidic flavour due to high retention of acetic acid [110].

Several researches have been performed on the effects of sun and conventional hot-air drying methods on cocoa flavour quality. However, very little research has focused on the effects of non-conventional drying methods such as adsorption drying, vacuum drying and freeze drying. Cocoa beans with high polyphenolic content are usually high in astringent flavour attribute [75]. Astringency and bitterness flavours have a tendency to subdue cocoa flavour in samples and is hence usually not preferred in conventional chocolate manufacture. Therefore, the current study will emphasize on four major flavour attributes of cocoa, namely cocoa (chocolate), bitterness, astringency and acidic (sourness) of fresh cocoa beans after various drying methods (adsorption, vacuum, freeze, oven and sun drying). The sensory scores of cocoa samples which were subjected to blanching pre-treatment methods were also analysed.

7.2. Materials and methods

7.2.1. Sample preparation

Dried samples from fresh unfermented cocoa beans were used for analyzing the flavour attributes. The cocoa beans were processed (blanching and drying) according to the parameters as shown in Table 7.1. Cocoa beans after drying were cut open and the shells were then removed manually to obtain the inner nibs for roasting. Roasting was carried out by heating the nibs at thin layer on a flat aluminium sheet inside an oven (UN 55 natural convective oven, Memmert, Germany) at 140°C for 35 min [8]. Upon roasting, the nibs were ground using an end runner mill (Pascal Engineering, England) into a homogenised paste (cocoa liquor).

Table 7.1: The drying and blanching parameters used in sensory analysis.

Blanching parameter	Drying method	Drying time (h)	Drying Parameters
90°C for 5 minutes	H70	30	T= 70°C, RH= 50%, air flow rate= 0.01 m s^{-1}
	A60	24	Zeolite adsorbent, (T=ca. 60°C), RH=ca. 9-10%, air flow rate=4.1 m s^{-1}
	V60	24	T= 60°C, P= 150mbar
	FD	24	Main drying: T= -30°C , 24 h; Final drying: T=-50°C, 4 h P= ca. 0.015mbar
	SUN	36	Direct sun light exposure (7 am to 7pm), T= 26°C to 36°C, RH= 65- 75%, air flow rate= 1.3 m s^{-1}

7.2.2. Sensory analysis

Sensory evaluation was carried out by five expert panels from Malaysian Cocoa Board (Nilai, Negeri Sembilan, Malaysia) and Ghanaian cocoa liquor was used as the reference sample. Rating was carried out by using a descriptive scale ranging from 0 to 10 (from

undetectable to extremely strong intensity) where the flavour attributes assessed were cocoa, bitterness, astringency and sourness. The evaluation form is shown in Figure B1 in section Appendix B of the thesis. The flavour scores of Ghanaian cocoa reference samples are shown in Table 7.2.

Table 7.2: The flavour scores of Ghanaian cocoa reference sample

Flavour	Score
Cocoa	7
Bitter	2.5
Astringent	3
Acidic	1.5

7.2.3. Statistical analysis

The statistical analyses were carried out as mentioned in Section 3.5 of Materials and Methods (Chapter 3).

7.3. Results and discussion

7.3.1. Cocoa flavour

The intensity of cocoa flavour attribute is shown in Table 7.3. Generally cocoa is a highly desirable attribute in chocolate manufacturing industry. The quality of cocoa beans is optimum when the cocoa flavour is at its maximum while acidic flavour is at its minimum after processing. The astringency and bitterness are also preferred to be at low levels (score range of 3-3.5). However, it should not be totally eliminated as it could affect the general taste perception of finished chocolate [76]. The current study was compared with Ghanaian cocoa samples as a benchmark which is regarded as the best

flavoured cocoa [40]. On comparing the effects of cocoa flavour variation among the drying methods used irrespective of pre-treatments, SUN treatment recorded the highest cocoa flavour score (3.5). This is because sun drying has low temperature condition and longer drying time which facilitates the development of cocoa flavour precursors, especially in the unblanched samples. Treatments FD and V60 records the lowest cocoa flavour scores (2.3 and 2.0, respectively) and are significantly different ($p < 0.05$) from treatments A60, H70 and SUN. This is due to the lack of oxygen in FD and V60. In low oxygen conditions, the oxidation of enzymes responsible for cocoa flavour development does not occur.

Table 7.3: Effect of blanching pre-treatment and drying on cocoa flavour attribute

Drying method	Cocoa Flavour	
	Unblanched	Blanched
FD	2.3 ± 0.5^{c}	1.5 ± 1.0^{d}
A60	3.0 ± 0.5^{b}	2.5 ± 0.7^{bc}
V60	2.0 ± 1.0^{c}	2.1 ± 1.1^{c}
H70	2.6 ± 0.5^{bc}	2.3 ± 0.8^{c}
SUN	3.5 ± 1.4^{a}	3.1 ± 1.1^{ab}

*Mean values (± SD) having a common letter among drying methods in same column are not significant according to Duncan's multiple range test at 5% level

The results show a rather low value of cocoa flavour (1.5 to 3.5) attribute with published literature. The general variation in cocoa flavour scores for fermented cocoa beans after processing are in the range of 4.5 to 7.0 [11, 12]. Generally sufficient fermentation and drying method is mandatory for the development of cocoa flavour attributes [34]. It is understood that during fermentation the microbial activity by organisms such as yeast increases the acidity levels and temperature within the beans. This degrades the internal bean structure by enzymes such as invertase, glycosidases and proteases [31].

The process mentioned above is necessary for the development of cocoa flavour precursors which would be established during sufficient drying. Looking into blanched beans, A60 and SUN shows significantly high cocoa scores ($p < 0.05$) 2.5 and 3.1, when compared to FD, V60 and H70 treatments. Since the samples used in this experiment are not fermented, low values for cocoa flavour are expected [18].

7.3.2. Bitter flavour

The bitterness flavour attributes are shown in Table 7.4. It is reported that presence of alkaloids develop high bitterness scores in cocoa beans [111]. Alkaloids are stimulant compounds (eg: caffeine, theobromine, etc.) which also provide bitter taste characteristic to other commercial beverage such as tea and coffee [12]. After fermentation process, it was reported that there were a 50 % decrease in the overall alkaloid compounds in cocoa beans, however it is usually not affected by drying methods [65].

Table 7.4: Effect of blanching pre-treatment and drying on bitter flavour attribute

Drying method	Bitter Flavour	
	Unblanched	Blanched
FD	5.0 ± 1.0^{a}	5.1 ± 0.9^{a}
A60	3.6 ± 0.2^{bc}	4.8 ± 1.1^{ab}
V60	4.0 ± 0.5^{b}	4.4 ± 1.3^{b}
H70	4.1 ± 0.5^{b}	3.8 ± 0.7^{bc}
SUN	2.6 ± 0.5^{d}	2.7 ± 1.5^{d}

*Mean values (± SD) having a common letter among drying methods in same column are not significant according to Duncan's multiple range test at 5% level

SUN treatment shows a considerably lower value in comparison to other drying treatments and is significantly different ($p < 0.05$) from treatments A60, H70, V60 and FD. During sun drying, the acidity and astringency flavour scores are relatively low, this

helps in boosting the cocoa flavour attribute which masks the bitterness from the samples. It is also worth mentioning that FD treatment shows the highest bitter flavour attributes. Freeze drying in the current research is used as a benchmark for quality evaluation of cocoa, the high tannin residues in the bioactive compound rich FD samples are shown to exhibit bitter taste and are statistically different ($p < 0.05$) from other drying treatments [80]. The general scores of bitterness are in range of 2.8 to 3, however Malaysian cocoa samples are reported to have high bitterness trait [18]. This reason along with the fresh unfermented nature of the beans used in current study justifies the high bitterness flavour (2.6 to 5.1). Bitterness is a desirable attribute in cocoa beans although high level of bitterness would mask the cocoa flavour taste in cocoa beans [110].

7.3.3. Astringent flavour

The intensity of cocoa flavour attribute is shown in Table 7.5. Typically for well fermented cocoa the astringency scores in the range of 2.5 to 5.0 [18]. As mentioned earlier, polyphenolic compounds imparts the astringent flavour note to cocoa beans. This is verified in the current results with astringent flavour attributes ranging from 3.5 to 5.5 due to the fresh unfermented nature of cocoa sample. For unblanched cocoa samples, treatment FD shows the highest astringency flavour score of 5.5, which is significantly different ($p < 0.05$) from treatments H70 and SUN.

Table 7.5: Effect of blanching pre-treatment and drying on astringent flavour attribute

Drying method	Astringent Flavour	
	Unblanched	Blanched
FD	5.5±1.3[a]	5.2±1.3[ab]
A60	4.9±0.8[b]	5.5±1.0[a]
V60	4.8±1.8[b]	5.3±0.8[ab]
H70	4.0±1.3[c]	5.2±1.0[ab]
SUN	3.5±.03[d]	4.2±0.8[c]

*Mean values (± SD) having a common letter among drying methods in same column are not significant according to Duncan's multiple range test at 5% level

On comparing the cocoa flavour scores after blanching pre-treatments, treatments A60, H70 and SUN showed significantly higher value ($p < 0.05$). The results from total polyphenolic contents from section 6.3.3 (Chapter 6) substantiates this finding where the highest percentage differences of polyphenols were noted for A60 (12.46 %), H70 (15.31 %) and SUN (10.47 %). Similar to earlier results, treatment FD records the highest astringency scores which are due to the high polyphenols compounds recovered after drying. After blanching, significantly high ($p < 0.05$) scores were noted for treatments FD, A60, V60 and H70, on comparison with SUN treatment. This proves that blanching is an excellent pre-treatment method in recovering high polyphenolic contents in cocoa beans after drying.

7.3.4. Acidic flavour

Acidic flavour scores are shown in Table 7.6. The acidic flavour attribute are produced during fermentation process. The occurrence of high acid constituents in cocoa will lead to a sour taste in the cocoa sample. Usually during fermentation process, the sugars present in fresh cocoa beans are broken down into acid mainly acetic and lactic acid [17]. The acidity of cocoa beans during the course of fermentation is said to increase

during the activity of microbes in the outer layer of the bean. The outer mucilaginous coating of cocoa liquefies (known as sweating) and the highly acidic medium helps the microbes penetrate into the cocoa body which further degrades the sugars present in storage cells of the bean. Thus, the acid formed are contained within the bean [30]. These acidic compounds are reduced by evaporation due to the volatile nature of acids during drying process. High temperature drying and fast drying lead to the case hardening of cocoa shell. This leads to entrapment of the acid constituents to remain inside bean which increases the acidic flavour [76]. Generally the acid flavour scores for fermented cocoa are in the range of 2 to 3 [12, 15]. The results from current study show acidic flavour scores ranges from 0.5 to 1.5. The low scores are primarily due to fresh nature of cocoa beans used. Fermentation process increases the acidity in cocoa beans by converting the sugars (glucose and fructose) into lactic acid and acetic acid by microbial activity (LAB and AAB). The slight traces of acid flavour scores could be due to the citric acid residues which are present in the mucilaginous outer pulp of fresh cocoa beans [31].

Table 7.6: Effect of blanching pre-treatment and drying on acidic flavour attribute

Drying method	Acidic Flavour	
	Unblanched	Blanched
FD	1.0 ± 0.7^{bc}	0.5 ± 0.5^{d}
A60	1.0 ± 0.5^{bc}	1.0 ± 0.5^{bc}
V60	0.5 ± 0.5^{d}	0.5 ± 0.5^{d}
H70	1.2 ± 0.7^{ab}	1.4 ± 0.7^{a}
SUN	1.0 ± 0.7^{bc}	1.5 ± 0.9^{a}

*Mean values (± SD) having a common letter among drying methods in same column are not significant according to Duncan's multiple range test at 5% level

7.3.5. Selection of cocoa beans with enhanced flavour attributes

Since this study focusses on the production of cocoa beans with high polyphenolic compounds, cocoa beans with high astringency scores is a desirable attribute. Upon comparing the results with Ghanaian cocoa reference samples, the cocoa flavour scores for both unblanched and blanched cocoa samples at various drying treatments are much lower. Blanched cocoa beans shows higher astringency and bitterness scores (Tables 7.4 and 7.5) and can be confirmed to be an optimal pre-treatment method. Among blanched samples, treatment A60 can be chosen as treatment with optimal sensory properties. Treatment A60 shows high astringency (5.5) and bitterness flavour (4.8) scores. High astringency and bitterness flavours are indicators of cocoa beans with high polyphenolic contents and alkaloid contents, respectively. The acidic flavour score for A60 after blanching were also found to be 1.0, low acidity in beans are found to be a desirable attribute. All the drying treatments (unblanched and blanched) show high astringency and bitterness scores which will mask the development of cocoa flavour attribute. Since the scope of this study is not restricted to producing cocoa beans for commercial chocolate production, the low cocoa scores development in samples can be accepted. The flavour scores of treatment FD in unblanched and blanched samples show the highest astringency scores. However, FD treatment in current research were mainly used for benchmarking purposes and the large scale production of cocoa beans using FD is not economically feasible.

7.4. Conclusion

Results have indicated that unfermented cocoa beans rich in polyphenols after drying showed a different trend in comparison to sensory evaluations of fermented cocoa beans after sun drying and artificial methods. There has been no research that has focused on the sensory evaluation of fresh beans and its effects on blanching pre-treatment for the production of high polyphenols cocoa beans. In the traditional sensory evaluation analysis, bitterness and astringency flavour attributes are usually found to be undesirable. However, in the current study both bitterness and astringency scores are important as it justifies the high polyphenolic content and antioxidant capacities of cocoa beans recovered through blanching pre-treatment after various drying treatments. The score ranges for bitterness and astringency flavour attributes are 2.6 to 5.1 and 3.5 to 5.5, respectively. These values were higher than that reported in published literature and further justifies the total polyphenols content and antioxidant assay results obtained in Chapter 6. Blanching pre-treatment can shows high astringency scores (polyphenol recovery) in cocoa beans after treatments H70, A60, V60 and SUN and among them, A60 after blanching can be chosen as an optimal treatment for producing cocoa beans with optimal flavour characteristics. The current study would benefit the pharmaceutical and nutraceutical industries for the development of high polyphenols cocoa based product with proven health benefits.

CHAPTER 8

CONCLUSION AND FUTURE WORKS

8.1 General Conclusions

The present study represents an investigation on the production of high polyphenols cocoa beans using blanching pre-treatment and various drying methods with emphasis on fresh cocoa beans. A comprehensive comparison of blanching pre-treatment and various drying methods for cocoa have not been reported elsewhere to date. Artificial drying methods such as oven, vacuum, adsorption and freeze drying were compared with conventional sun drying method. Comparisons of the drying kinetics, total polyphenols content, antioxidant capacities and sensory analysis for each cocoa bean drying method were analysed. Cocoa beans with high polyphenolic contents can be introduced into market as a specialty cocoa.

The studies have reported the following significant findings:

I. Fermented cocoa beans dried in hot air oven at 70°C setting, showed the maximum polyphenols recovery (range of 56.3 to 67.1 mg GAE g^{-1} dw). The effective diffusivity values obtained from the study were in accordance with that of published literatures (2.36 X 10^{-10} to 2.86 X 10^{-10} m^2s^{-1}). The polyphenols degradation was found to be dependent on the temperature of drying as well as the drying duration.

II. Drying kinetics analysis showed that adsorption drying dried faster (24 h) than oven, vacuum and sun drying methods. The effective diffusivities of fresh beans

dried using the various drying methods have not been reported in any literatures are were recorded to be in the range of 1.54 X 10^{-10} to 4.13 X 10^{-10} m^2s^{-1}.

III. The polyphenols recovery of fresh cocoa beans was recorded to be much higher than fermented cocoa beans. The application of blanching pre-treatment was reported to be an optimal method for degrading polyphenol oxidases enzymes, which in turn preserves total polyphenolic compounds in cocoa beans. Blanching at 90°C for 5 min showed to have a significant ($p < 0.05$) increment in total polyphenolic compounds recovered after drying (about 10 % increment in polyphenolic recovery for fresh whole beans on comparison with control). Both adsorption and vacuum dried cocoa samples after blanching pre-treatment were reported to have high total polyphenolic content and antioxidant activity. Adsorption and oven dried cocoa samples achieved the highest percentage difference values for total polyphenolic contents, DPPH assay and ABTS assay, respectively (Total polyphenolic content: 12.46 % and 15.3 %; DPPH assay: 7.41 % and 8.5 % and ABTS assay: 11.41 % and 7.88 %, for adsorption drying and oven drying, respectively).

IV. Sensory evaluations of fresh cocoa beans have not been reported in literatures and high astringency flavour was noted for both blanched and unblanched samples. This confirmed the potential of high polyphenolic recovery in cocoa samples by using fresh cocoa beans. The astringency flavour for A60, H70 and SUN dried samples recorded significantly different values ($p < 0.05$) after

blanching. Adsorption drying after blanching showed optimal sensory characteristics among the various drying treatments used in the study.

8.2. Future works

The studies carried out have revealed that fresh cocoa beans with high polyphenolic content could be produced using blanching pre-treatment and the application of various drying methods. The potential of highly health beneficial cocoa beans thus produced could be further explored to provide an active raw material for pharmaceutical and nutraceutical industries. Therefore, the following future works are recommended:

I. Analyze the drying kinetics and effective diffusivities of cocoa samples after blanching pretreatments. Since it is already established that blanching helps in enhancing the quality of cocoa beans, the drying kinetic analysis of the blanched samples will be beneficial in determining the moisture diffusion patterns which occurs during drying.

II. From section 6.3.2 of this thesis, it is shown that the total polyphenolic content of fresh cocoa beans increases as the blanching temperature is increased from 70°C to 90°C, for a period of 5 min. It will beneficial in determining the total polyphenolic content of cocoa beans after blanching at higher temperatures (> 90°C).

III. Performance and cost analyses of the adsorption dryer when drying cocoa beans based on the drying conditions from this research as this drying methods show promising results when compared with other treatments. To conduct an analysis

on the performance of other adsorbent mediums such as silica gel, activated charcoal etc.

IV. Design and development of an automated blancher, which will help in scaling up the process effectively. A blancher prototype with automated temperature and time controls with conveyor belt to facilitate transfer of beans from and outside hot water medium can significantly reduce the manual labour requirements and risk associated with operation.

V. Development of bitter chocolates from the cocoa produced from the current study, which have high demand among health conscious consumers.

VI. Investigate the potential of microwave based blanching method on cocoa beans. Microwave blanching is reported to require the minimum amount of energy to inactivate enzymatic activity in food products. It is hence ideal to blanch large quantities of cocoa samples in a cost and time efficient manner.

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APPENDIX A

RELATIVE HUMIDITY PROFILE AND CALIBRATION CURVES

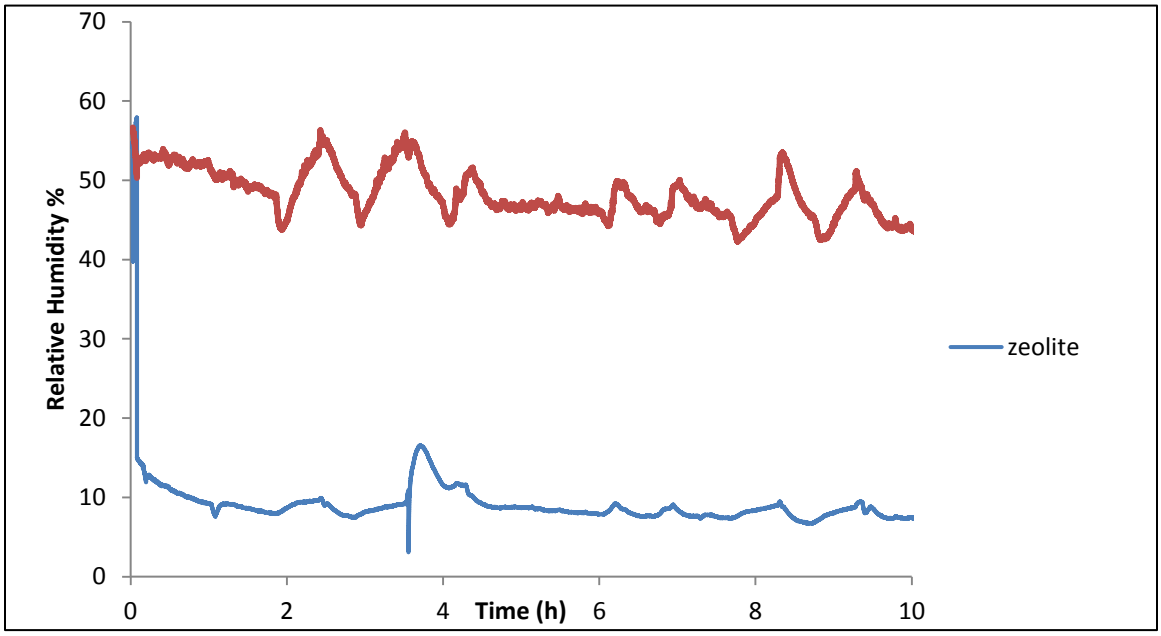


Figure A1: Relative humidity profile of adsorption dryer

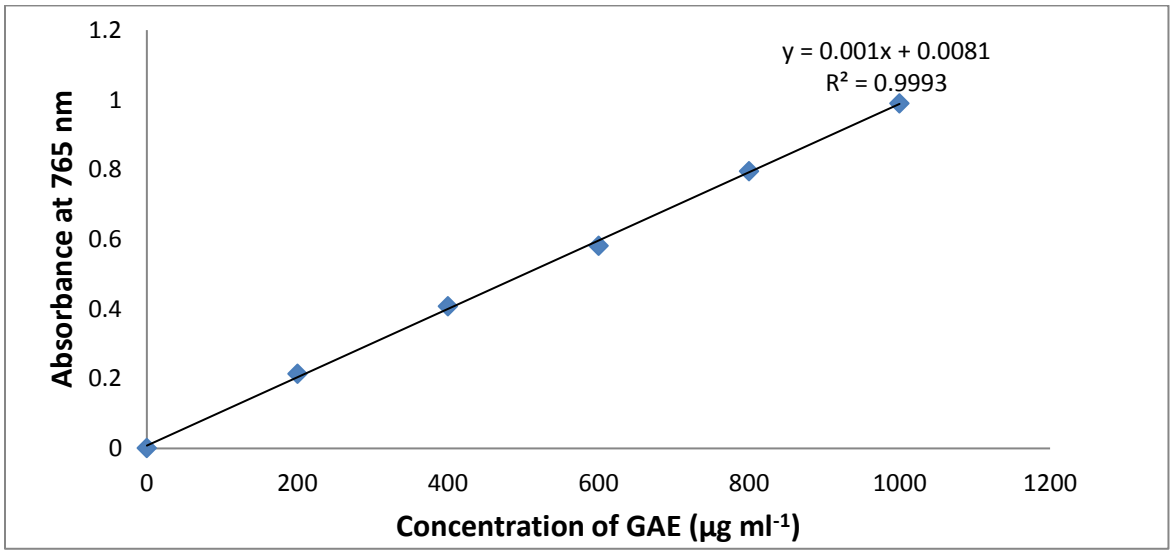


Figure A2: The calibration curve for total polyphenolic content analysis in gallic acid equivalence (GAE)

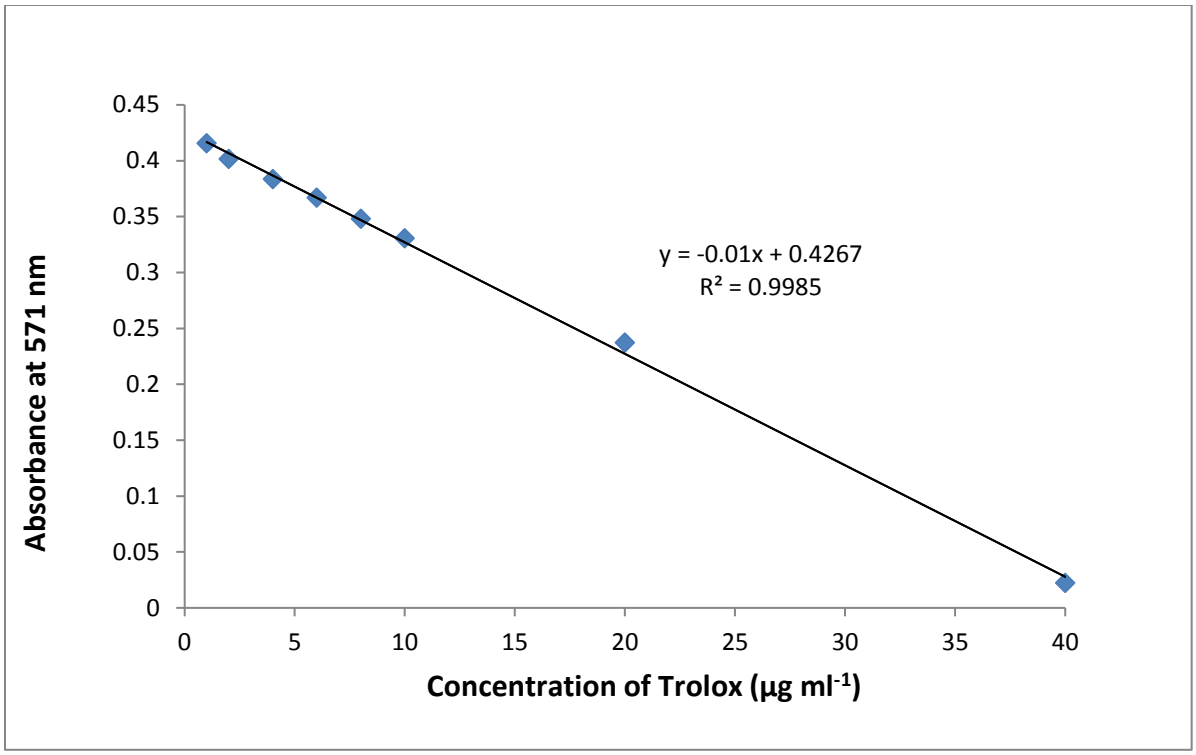


Figure A3: The calibration curve for DPPH antioxidants assay in trolox equivalence

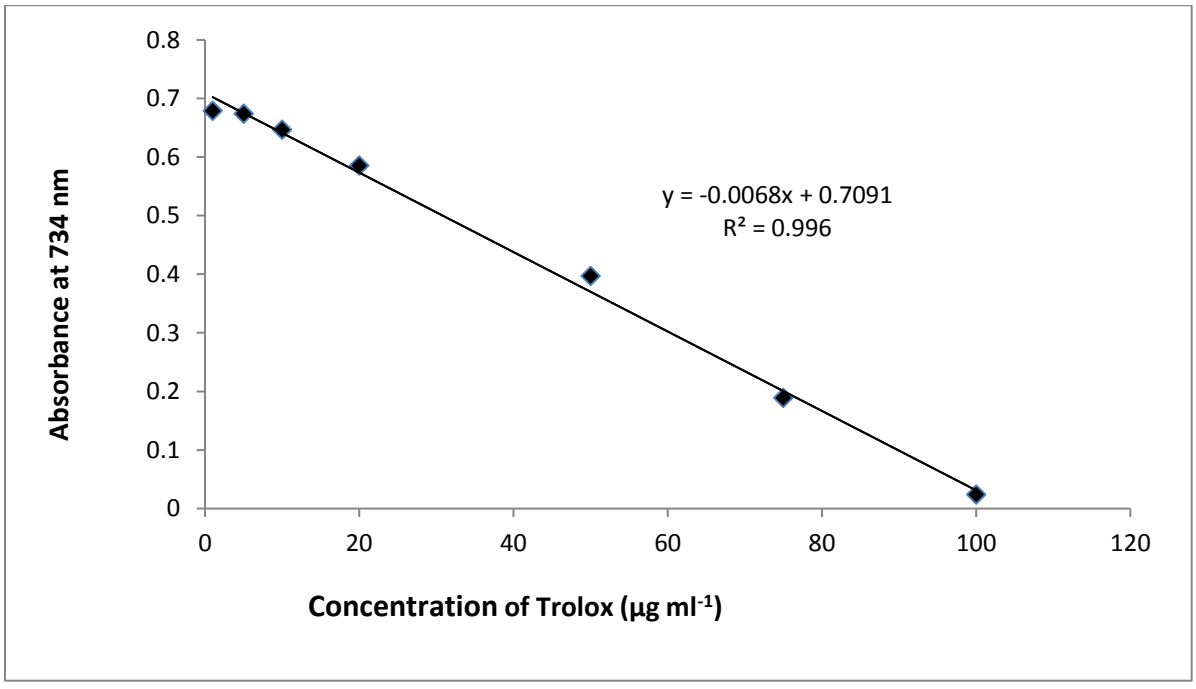
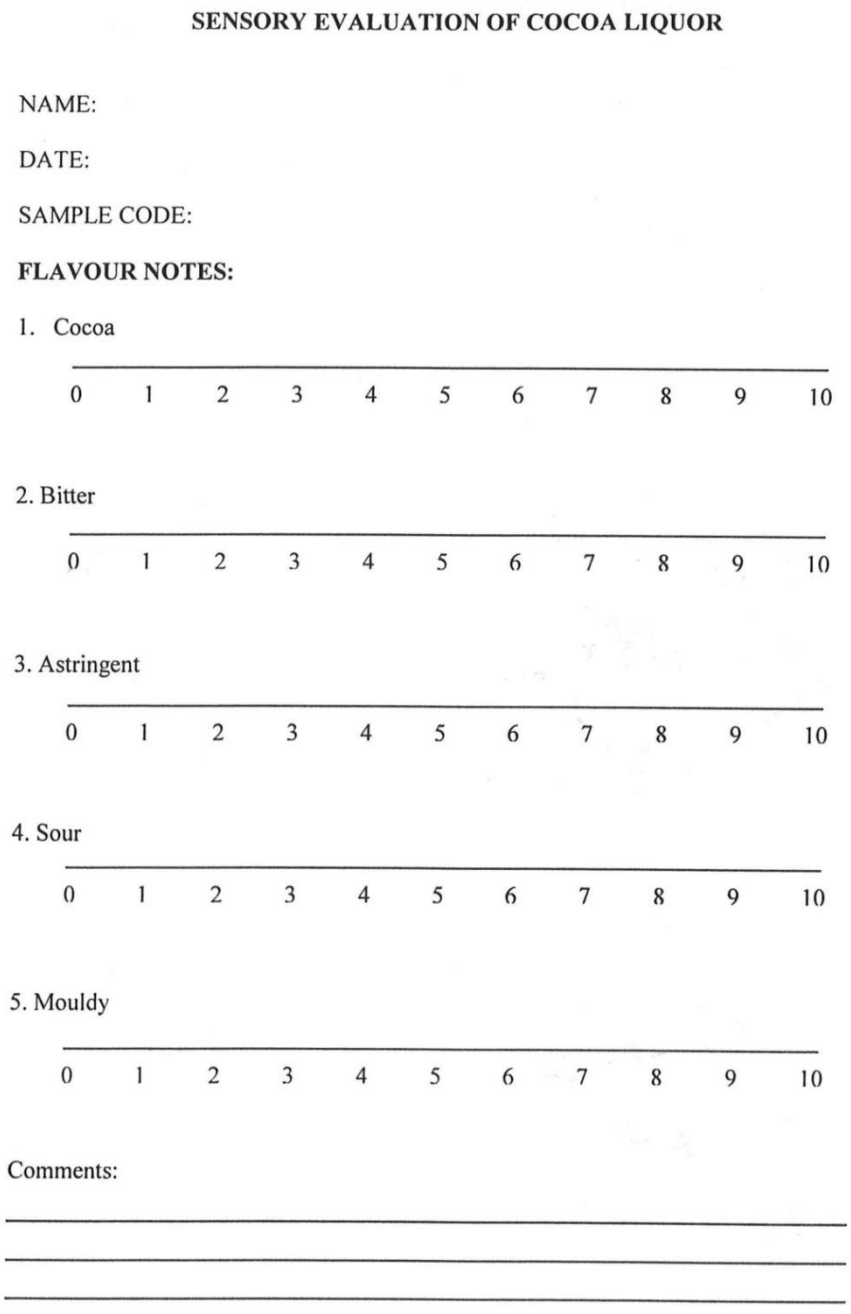


Figure A3: The calibration curve for ABTS antioxidants assay in trolox equivalence

APPENDIX B

SENSORY EVALUATION FORM



SENSORY EVALUATION OF COCOA LIQUOR

NAME:

DATE:

SAMPLE CODE:

FLAVOUR NOTES:

1. Cocoa

0 1 2 3 4 5 6 7 8 9 10

2. Bitter

0 1 2 3 4 5 6 7 8 9 10

3. Astringent

0 1 2 3 4 5 6 7 8 9 10

4. Sour

0 1 2 3 4 5 6 7 8 9 10

5. Mouldy

0 1 2 3 4 5 6 7 8 9 10

Comments:

Figure B1: The cocoa liquor evaluation form used for sensory analysis

BIODATA OF THE AUTHOR

Abhay Santhanam Menon was born on August 12, 1987 in Kerala, India. He studied in Hari Sri Vidya Nidhi School, Kerala, India until the completion of his secondary school education. He continued his higher secondary education at Govt. Model Boys Higher Secondary School, Thrissur, Kerala, India. He pursued his first degree at Anna University, Chennai, India in the field of Biotechnology and graduated with a Degree in Technology, (B. Tech) in 2010. He pursued his Master's degree (M.Sc) from 2010 to 2011 in the field of Crop Biotechnology, at the School of Biosciences, The University of Nottingham Malaysia campus. Presently, he is working as a Research Assistant and pursuing a PhD degree from December 2012 with the Department of Chemical and Environmental Engineering, The University of Nottingham Malaysia campus.