

EFFECTS OF FERMENTATION INTENSITY ON POLYPHENOLS AND ANTIOXIDANT CAPACITY OF COCOA BEANS

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ABSTRACT

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Cocoa beans were fermented for 1 day, 2 days, 3 days up to 6 days. Result showed that, polyphenols content of the fermented beans dropped from 16.11% on day 0 to 6.01% on day 6. The antioxidant capacity also dropped from 96% (IP) on the first day of fermentation to 79% (IP) on the sixth day of fermentation. Epicatechin content of the beans also dropped as fermentation progressed. Mineral content of fermented beans contained 0.006%, 0.014%, 0.14%, 0.29%, 0.01%, 0.002%, 0.005%, 1.95% and 0.04% Of Ca, Mg, Na, K, Fe, Mn, Zn, N and P respectively. Outcome of this work suggests that, polyphenol is the main phytochemical responsible for antioxidant capacity in cocoa beans.

Key words: *polyphenol, antioxidant, cocoa, Nigeria, inhibition percent*

INTRODUCTION

Cocoa beans are the principal raw material of chocolate. West Africa produces more than two-third of the World's cocoa with Cote d'Ivoire and Ghana alone accounting for 40 and 20% of the World production, respectively (Anon 2005). Cocoa beans originate as seeds in fruit pods of the tree *Theobroma cacao* where each fruit pod contains 30 to 40 beans embedded in a mucilaginous pulp. Raw cocoa has an astringent, unpleasant taste and flavour, and has to be fermented, dried and roasted in order to obtain the characteristic cocoa flavour and taste (Thompson *et al.* 2001).

The fermentation of cocoa is a spontaneous process. Following opening of the pods the mucilaginous, acidic and sugar rich pulp surrounding the cocoa beans is contaminated with a variety of microorganisms originating from workers hands, containers used for transport, knives, pod surfaces, etc. (Roelofsen 1958; Thompson *et al.* 2001; Jespersen *et al.* 2005).

Cocoa bean and its products are food sources rich in phenolic compounds. Dreosti (2000) reported that 60% of the total phenolics in raw cocoa beans are flavonol monomers (epicatechin and catechin) and procyanidin oligomers (dimer to decamer). These compounds were reported to be potential candidate to combat free radicals, which are harmful to our body and food systems (Adamson *et al.* 1999). Studies had demonstrated that the consumption of cocoa or chocolate reduced the risk of cardiovascular disease (Keen *et al.* 2001). Moreover, extracts prepared from cocoa powder and cocoa beans were shown to exhibit antihyperglycaemic effect on streptozotocin-induced diabetic rat.

Fermentation of cocoa beans normally takes between five and seven days. The actual fermentation takes place in the pulp surrounding the beans. The pulp is rich in glucose, fructose and sucrose (total content 10–15%) and the initial pH is relatively low (pH = 3.3–4.0), primarily due to a high concentration of citric acid (1–3%) (Roelofsen 1958). In the initial phases of the fermentation growth of yeasts is favoured due to the high sugar content, low pH and limited oxygen availability in the pulp (Thompson *et al.* 2001). The primary activity of the yeast is the production of ethanol from carbohydrates and also assimilation of citric acid and degradation of pectin have been reported as important activities (Jespersen *et al.* 2005).

Fermentation is one of the steps involved in the production of cocoa beans. This step is crucial in determining the quality of cocoa aroma. The production of aroma precursors during fermentation is important for producing the full aroma of chocolate (Rohan & Stewart, 1966). There are internal and external fermentation stages involved during cocoa fermentation. External fermentation primarily involves the catabolism of the sugar pulp by microorganisms, while internal fermentation encompasses the biochemical changes in the cotyledon of the beans (Biehl & Voigt, 1982).

Research has shown that chocolates produced from unfermented beans have no chocolate flavor and are excessively astringent and bitter (Biehl & Voigt, 1982). Fermentation will reduce the level of bitterness and astringency of the cocoa bean which could be attributed to the loss of polyphenols during fermentation (Kim & Keeney, 1984). Oxidation of polyphenols to insoluble tannins during fermentation was responsible for the formation of flavour precursors for chocolate processing (Jinap & Dimick, 1990). Catechin has a bitter taste with a sweet aftertaste or is described as bitter and astringent (Bonvehi & Coll, 1997). Stark *et al.* (2005) showed that catechins, which include epicatechin, catechin, procyanidin B2, procyanidin B5 and procyanidin C1, were the major compounds responsible

for bitterness and astringency of roasted cocoa. During fermentation, between days two and three, epicatechin content was observed to decrease sharply, which could indicate that it is either being used up for the formation of large tannins or lost in the fluids that drain away (Kim and Keeney, 1984).

It has been observed through personal interaction with cocoa farmers that, some farmers being under pressure from the buyers of cocoa beans, do not allow their beans to undergo adequate fermentation. Hence some of them just allow a few days fermentation while a few do not allow fermentation at all. Considering the importance of fermentation in cocoa processing and its contribution to nutritional value and flavor, this work was carried out to evaluate the effect of various fermentation duration on the polyphenol content and antioxidant capacity of beans.

MATERIALS AND METHODS

Ripe but matured Cocoa pods (Amelonado) were collected from an old cocoa plantation belonging to Cocoa Research Institute of Nigeria, Ibadan (7°14 N, 3°51E). The pods were broken with wooden stick and the beans were separated into seven groups. Each of the group of cocoa beans was wrapped with polyethylene net and inserted into a heap of cocoa beans to be fermented. This was done so that enough heat will be provided for the experimental beans. The samples were then subjected to various days of fermentation starting with day 0 up to day 6. At the end of each fermentation period, the tied polyethylene net with its content were brought out of the heap of cocoa and sun-dried. This was done consistently until the expiration of the fermentation procedure. The fermented cocoa beans were sun-dried for seven days under intense sunlight.

Chemical Analysis

Cocoa beans were grinded with ceramic mortar and pestle after which the milled beans were defatted. The powders obtained were directly used for the minerals analysis, polyphenols and antioxidant capacity. The methods and protocols used for these analyses are in accordance with AOAC (1998). The minerals were determined using atomic absorption spectrophotometer.

Polyphenol and Antioxidant capacity

The scavenging activity was estimated according to the method of Lai *et al.* (2001). An aliquot of cocoa extract was mixed with 100mM Tris-HCl buffer (800μl, pH 7.4) then 1ml of 500μM DPPH previously prepared in ethanol was added. The mixture was shaken vigorously and left to stand for 20 min at room temperature in a dark room. Absorbance was read using a spectrophotometer at 517nm. Ferric reducing antioxidant assay was determined based on the reduction of Fe³⁺ to a blue coloured Fe²⁺ (Benzie & Strain, 1996). The ferric reducing antioxidant power reagent was prepared by mixing 300mM acetate buffer (pH 3.6), 10mM TPTZ and 20mM FeCl₃.6H₂O in a ratio of 10:1:1 at 37°C for 4min. Each sample was run in triplicate. Absorbance was measured at 593nm. The ferric reducing antioxidant power was calculated using the equation described by Benzie and Strain (1996). In the FRAP assay, the antioxidant potential of sample was determined from a standard curve plotted using FeSO₄.7H₂O at a concentration range between 200 and 1000μM. Total phenolic content was determined according to the method of Singleton & Rossi (1965). Each sample (200mg) was extracted with 70% aqueous ethanol at room temperature for 2hr using an orbital shaker at 200 rpm. The mixture was centrifuged at 2000rpm for 15min. The supernatant (200μl) was mixed with 1.5ml of Folin-Ciocalteu reagent and allowed to stand at room temperature for 5min; then 1.5ml of sodium bicarbonate solution (0.566 M) was added to the mixture. After 90min, absorbance was read at 725nm. Results were expressed as as ferulic acid equivalent. The concentration used was in the range between 0.02 and 0.1mg/ml.

RESULTS AND DISCUSSION

Table 1 showed some nutrients content in fermented cocoa beans. Mean calcium content of the beans was 0.006%. The average value for calcium in the beans (60mg/kg) was higher than the value documented (25.5mg/kg) by Olaofe *et al.* (2006). Magnesium content of the beans was 0.014% (14 mg/kg). The mean value of magnesium obtained in this work was lower than the value (5179 mg/kg) obtained by Olaofe *et al.* (2006). The low magnesium content of the beans could be a consequence of magnesium deficiency in the soil of the plantation where the investigated cocoa beans were collected. The soil magnesium deficiency was earlier reported by Ipinmoroti *et al.* (2009). The fermented beans contained an average potassium value of 0.29%. The mean value obtained is higher than 2,480mg/kg magnesium reported by Olaofe *et al.* (2006).

Iron content of the beans range recorded a mean value of 0.008% (80mg/kg). Manganese was on the average of 0.002% in the beans while Zinc content was on the average of 0.005%. A mean nitrogen content of 1.95% was found in the fermented cocoa beans and phosphorus content of 0.04%. These values were higher than the Manganese, zinc, nitrogen and phosphorus content of fermented cocoa beans reported by Olaofe *et al.* 2006.

Table 1. Mineral contents of fermented cocoa beans

Mineral	Concentration (%)
Calcium	0.006±0.02
Magnesium	0.014±0.01
Sodium	0.140±0.03
Potassium	0.280±0.02
Iron	0.010±0.01
Manganese	0.002±0.03
Zinc	0.005±0.02
Nitrogen	1.970±0.01
Phosphorus	0.040±0.02

Result showed a decrease in total phenol content of cocoa beans as the duration of fermentation increased. During fermentation, the polyphenol content of the beans decreased (Fig. 1). The total content of polyphenols at the beginning of fermentation was 16.11% (wt/wt). At one day of fermentation, the concentration of polyphenols had dropped to 15.5% (wt/wt). After two days of fermentation, the concentration of polyphenol had reduced to an average of 12.9. At three, four, five and six days after fermentation the polyphenol values were 10.7, 8.2, 7.6 and 6.01% (wt/wt) respectively. Result then suggests that, fermentation of cocoa beans leads to gradual loss of polyphenol in the fermented beans. Wollgast & Anklam (2000) reported that, unfermented Forastero cocoa beans contain 120–180 g kg⁻¹. During cocoa fermentation, polyphenols are subjected to biochemical modification through polymerization and complexation with protein, hence decreasing solubility and astringency (Bonvehi & Coll, 1997). Report of Misnawi *et al.* (2002) stated that unfermented cocoa beans contain 135 g kg⁻¹ of polyphenolic compounds in which three groups of the polyphenols can be distinguished: namely catechins or flavan-3-ols constituting 37%, anthocyanins 4% and proanthocyanidins 58%. Polyphenol content in dried fermented cocoa bean was 63 g kg⁻¹ (Misnawi *et al.* 2004) Polyphenols in cocoa products are mostly responsible for the astringent sensation and they also contribute to the bitter taste along with alkaloids, some amino acids, peptides and pyrazines (Bonvehi & Coll, 1997; Luna *et al.* 2002; Misnawi *et al.* 2005). Kyi *et al.* (2005) demonstrated that the high temperature used during drying of fermented cocoa beans had reduced polyphenol contents as a result of enzymatic oxidation. Non-enzymatic oxidation of polyphenols could also occur at this stage. As cocoa powder is derived from fermented, dried, and roasted cocoa beans, the loss of phenolic compounds is higher than that of cocoa liquor (De Brito *et al.* 2000).

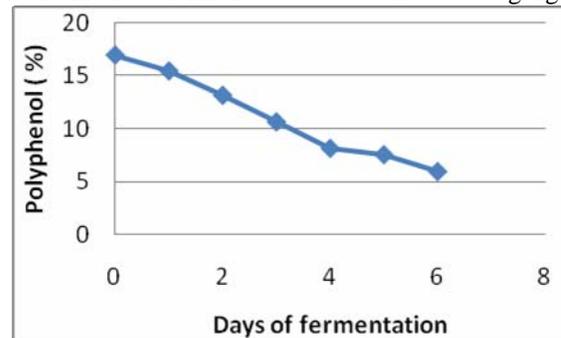


Figure 1: Effect of fermentation on polyphenol content of cocoa beans

Result showed a decrease in the epicatechin content of the cocoa beans as the fermentation duration increased (Fig. 2). Approximately 10 to 20% of epicatechin and other soluble polyphenols are reduced during fermentation. This could also be due to the diffusion of polyphenols into fermentation sweating (Kim and Keeney, 1984). Caligiani *et al.* (2007) reported that (-) epicatechin and (+)-catechin increased in the order of fully fermented (brown color), partly fermented (violet color) and unfermented (slaty color). In addition, high temperatures and prolonged processing times will decrease the amount of catechins (Wollgast and Anklam, 2000). In this work, concentrations of catechin (0.17mgg⁻¹) did not change during fermentation, whereas epicatechin concentrations at the beginning was 12mgg⁻¹ in the beans and showed a linear decrease from the start of the fermentation (Fig. 2). After six days of fermentation, 60% of the initial concentration of

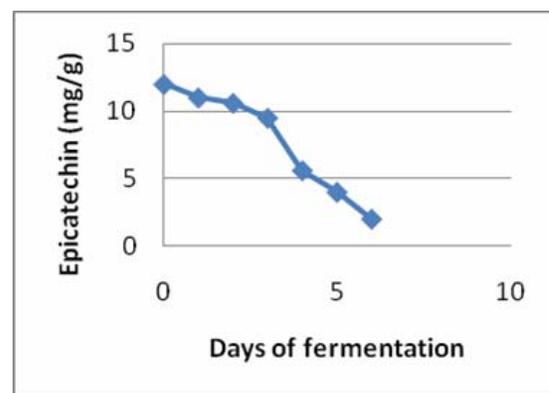


Figure 2: Effect of fermentation on Epicatechin content of cocoa beans

epicatechin was lost. Epicatechin levels decreased during fermentation due to diffusion out of the bean cotyledons and polyphenol oxidation and condensation.

The evaluation of total antioxidant activity of the phenolic extract from cocoa beans, obtained with methanol using Soxhlet apparatus, was then performed using DPPH method. The scavenging properties of the methanolic extract of phenolic pigments from cocoa extract (1:500 diluted in methanol) showed an inhibition percent (IP%) 96%, 93%, 91%, 88%, 84%, and 79% at 1, 2, 3, 4, 5 and 6 days after fermentation. Result showed a decrease in the percent inhibition activity of the polyphenol content of the fermented cocoa beans. The trend of reduction in antioxidant capacity of the fermented cocoa beans followed that of polyphenols. This result then suggests that polyphenols are likely to be the main component of cocoa beans which is responsible for the antioxidant capacity of cocoa beans. This is further confirmed by the report of Steinberg *et al.* (2002) who reported that most of the antioxidant activity in the chocolate comes from polyphenol content. All fractions of cocoa bean polyphenols have been identified to have antioxidant property. However, Jinap & Misnawi (2002) found that roasting of cocoa liquor up to 120°C for 45 min did not significantly reduce its polyphenol antioxidant activity.

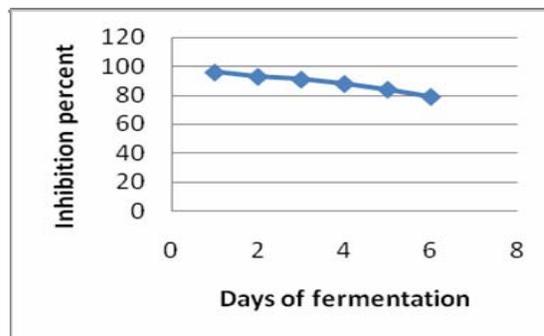


Figure 3: Effect of fermentation on antioxidant capacity of cocoa beans

CONCLUSION

The various results from this work had proved that, polyphenols is the major chemical specie in cocoa that is responsible for the antioxidant capacity of cocoa. Polyphenols in cocoa reduced during fermentation and hence, the antioxidant capacity of cocoa also reduced upon fermentation. Catechin content of the beans did not reduce upon fermentation. This work further confirmed the fact that, cocoa is rich in polyphenols though lost during fermentation, the concentration left in the beans was still high enough to produce high antioxidant capacity in the beans. To this effect, cocoa will remove free radicals capable of damaging cells and tissues from human system.

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