

PHYSICAL CHARACTERISTIC, PROXIMATE COMPOSITION, POLYPHENOL AND ANTINUTRITIONAL FACTORS OF CHOCOLATE PRODUCED FROM NEW NIGERIAN COCOA VARIETY

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Abstract: Investigation was conducted on the physical characteristic, proximate composition, polyphenol and anti-nutritional factors of chocolate produced from different cocoa hybrids (TC₁, TC₂, TC₅, TC₆ and TC₇). The cocoa were fermented with tray and then sundried. Chocolate were produced from the bean's liquor of the different cocoa hybrid using standard methods. The physical, proximate, polyphenol and anti-nutritional properties of the chocolate were determined using standard methods. The average proximate composition of the chocolate samples were protein (6.29 to 7.29%), crude fat (31.96 to 34.28%), crude fiber (2.35 to 2.58%), total ash (2.24 to 2.65%), moisture (5.17 to 5.49%), dry matter (94.51 to 94.83%) and Nitrogen free extract (NFE) (48.76 to 57.73%). The vitamin A content of the chocolate sample also ranges from 233.16 to 258.55 µg/100g. The average total polyphenol of the chocolate sample were catechin (0.42 to 0.72mg/100g). Epicatechin (0.42 to 0.72mg/100g), caffeine (0.23 to 0.44%), Theobromine (0.33 to 0.64mg/100g and antioxidant (66.26 to 78.14TE/100g) respectively. The result of the physical property revealed that the samples were significantly different ($p < 0.05$) from one another. The utilization of different varieties of cocoa causes significant differences in the physical, proximate, total polyphenols of chocolate produced.

Keywords: Chocolate, Cocoa hybrids, Proximate composition, Polyphenol, Antinutritional factors, Catechin, Epicatechin, Theobromine,

I. INTRODUCTION

Cocoa (*Theobroma cacao*) originated from Amazonian region of Brazil and it is a perennial tree crop grown in tropical countries which forms the economic backbone of such countries as Nigeria, Ghana, Ivory Coast, Brazil, Malaysia, Venezuela and Indonesia with over 66% produced by smallholder farmers in West Africa (Beckett, 1994: Afoakwa *et al.*, 2011). Since the introduction of the crop into Nigeria in about 1874 (Oyedele, 2007; Oke and Omotayo, 2012), it has grown to be major export crop (Afoakwa *et al.*, 2011). The qualities of cocoa beans depend on many factors such as the genotype, the agronomic management and climatic conditions (Brunetto *et al.*, 2005). However, the production of this export crop in Nigeria has suffered a reduction in recent years owing to some factors such as low yield, inconsistent

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production pattern, disease incidence, pest attack and use of crude farm tools (Villalobos, 1989; Oluyole and Sanusi, 2009). Hybrids of cocoa was reported by Dias *et al.*, (2003) to show wide adaptability and an outstanding performance for yield and its components, compared to unimproved traditional local cultivars (Souza and Dais, 2004). A research carried out by Oyedokun *et al.*, (2011) on fourteen genotypes of cocoa showed significant differences with respect to the eight quantitative morphological variables examined. In order to solve the problem associated with old established cocoa in Nigeria, effort was put in by breeders in Cocoa Research Institute of Nigeria which brought about new varieties (CRIN TC1 to CRIN TC12) in 2013. Cocoa is widely consumed in form of chocolate with increasing popularity in the world (Tafari *et al.*, 2014)

Information has been provided on influence of certain farming practices on old grown cocoa beans and subsequent chocolate quality such as F3 Amazon, Trinitario, Criollo and Forastero. Therefore, there is a need to also establish the same for the newly grown cocoa varieties.

II. METHODOLOGY

Source of materials

One hundred matured cocoa pods of uniform sizes and ripening was harvested per variety from a new cocoa hybrid trial plots established at Cocoa Research Institute of Nigeria (CRIN), Headquarters, Ibadan, Nigeria A total of 500 pods resulting from five varieties (TC₁ TC₂ TC₅ TC₆ and TC₇) constituted an experimental unit Other materials such as sugar, lecithin, cocoa butter and foil paper were purchased from Oyingbo open market, Lagos State, Nigeria

Methods

Fermentation and drying of cocoa beans

Fresh beans removed from selected pods of each variety were bulk fermented in tray for 5-6 days in three replicates using the method of Aroyeun (2007) Fermented beans from each variety were subjected to sun drying to about 7% moisture.

Production of chocolate from fermented cocoa beans

Chocolate was produce from the fermented cocoa varieties using the method described by ICCO, 2005 and Paula, 2013 The chocolate samples (high cocoa content) were obtained using the following proportions cocoa liquor (63.0%), cocoa butter (7.0%), sugar (29.60%) and lecithin (0.4%) and were prepared in Cocoa institute of Nigeria, CRIN Ibadan, Nigeria The seeds were triturated to remove the peel and germ in order to obtain cocoa nibs The nibs were ground m a knife-grinder, with sugar added m this phase The cocoa paste was then refined in a gnnderro1ler, yielding a cocoa paste with an adequate granulation (21 µm) for making chocolate The refined paste was subjected to conching in a horizontal shell at 60 °C.

Determination of physical properties of the Chocolate

Melting point and colour were determined using a differential scanning calorimeter, heat flow type method as described by Bolliger *et al.*, (1998).

Proximate analysis of the Chocolate

Moisture, crude protein content, crude fibre, crude fat and total ash contents were determined by methods described by Tell and Hagarty, 1984; AOAC, 2000 while nitrogen free extract was determined by difference i.e [100- (% M + % CP + % ash + % CP + % CF)]

Determination of Polyphenol content and anti-nutritional factors of the Chocolate

Phenol content and flavonoid of the Chocolate were determined according to Karim *et al.*, 2000, catechin and epicatechin were determined according to ACOA, 1979. Anti-nutritional factor like Tannins was determined according to method described by SWAIN (1979), Theobromine was determined by spectrophotometric method as described AOAC, 1974 while caffeine was evaluated with reverse phase high performance liquid chromatography as described by AOAC, (2000); ACOA, (1979) and AOAC, (1974).

III. RESULT

Result of physical properties of the chocolate

Result of physical properties of chocolate from different varieties of new Nigeria cocoa is presented in Table 2. The melting point of the chocolate ranged from 51.58 to 53.76°C. TC₅ has the lowest mean score while TC₇ has the highest. The residual meter value of the chocolate ranged from 2.78 to 4.83 with TC₂ having the lowest mean score while TC₁ has the highest. The color of the chocolate samples ranged from 6.27 to 8.37 Hz. TC₂ has the lowest mean score while TC₆ had the highest. There was significant ($P < 0.05$) difference among the samples of chocolate in all the physical parameters evaluated.

Table I: Result of physical parameters of chocolate from new varieties of cocoa

Sample	Melting point (°C)	Res Meter Value	Color (Hz)
TC ₁	52.19±0.02 ^{bc}	4.83±0.02 ^a	7.97±0.03 ^{bc}
TC ₂	52.03±0.04 ^{bc}	2.78±0.05 ^d	6.27±0.02 ^e
TC ₅	52.64±0.01 ^b	4.29±0.01 ^b	7.64±0.10 ^c
TC ₆	51.58±0.02 ^c	3.79±0.03 ^c	8.37±0.11 ^a
TC ₇	53.76±0.03 ^a	3.61±0.03 ^c	7.35±0.14 ^d

Values are means ± standard deviation of duplicate determinations. Difference letter scripts in the same column indicates statistical difference ($P < 0.05$).

Keys, TC₁ TC₂ TC₅ TC₆ TC₇ (Chocolate produced from Cocoa hybrids TC₁, TC₂, TC₅, TC₆, TC₇)

Result of Proximate and Vitamin A composition of the chocolate

Result of the proximate composition of chocolate from different varieties of new Nigeria cocoa varieties is presented in Table 3. The values for crude protein ranged from 6.29 to 7.29%. TC₁ has the lowest mean score while TC₆ has the highest. There was no significant ($p > 0.05$) difference between chocolate produced from TC₁ and TC₂. While significant ($p < 0.05$) difference exist in others. Crude fat content ranged from 31.96 to 34.28%. There was no significant difference ($p > 0.05$) among the samples. Crude fiber content ranged from 2.35 to 2.58%. The lowest score was recorded for TC₁ while TC₇ has the highest. There was no significant difference between the observed samples. Total ash content of the chocolate observed ranged from 2.24 to 2.65%. TC₁ has the lowest mean score while TC₇ has the highest. There was no significant difference between the samples. The result of moisture and dry matter content of the chocolates ranged from 5.17 to 5.49% and 94.51 to 94.83% respectively. TC₆ has the lowest mean score in terms of moisture content while TC₁ has the highest. Meanwhile, TC₂ was observed to have the lowest mean score in terms of dry matter with TC₆ having the highest. However, there was no significant difference among the samples observed in dry matter content. The carbohydrate content of the chocolate varieties ranged from 48.76 to 57.73%, with TC₆ having the lowest mean score and TC₁ having the highest mean score. Vitamin A content of the chocolate was reported to range from 233.16 to 258.55% with TC₂ having the lowest mean score and TC₆ having the highest mean score.

Table II: Result of proximate varieties of cocoa

Sample	Protein (%)	Crude Fat (%)	Crude fibre (%)	Ash (%)	Moisture content (%)	Dry Matter (%)	Carbohydrate (%)	Vitamin (μ/100g)
TC ₁	6.29 ^c ±0.02	31.96±0.03	2.35±0.02	2.24±0.02	5.43 ^b ±0.02	94.57±0.02	51.73 ^b ±0.02	248.6 ^b ±0.
TC ₂	6.37 ^c ±0.07	32.15±0.11	2.38±0.02	2.31±0.05	5.49 ^b ±0.02	94.51±0.04	51.30 ^b ±0.02	235.16 ^d ±0.01
TC ₅	6.98 ^b ±0.02	33.75±0.07	2.54±0.03	2.57±0.03	5.22 ^b ±0.02	94.78±0.02	48.94 ^a ±0.02	242.88 ^c ±0.00
TC ₆	7.29 ^a ±0.03	34.28±0.01	2.65±0.01	2.63±0.02	5.17 ^b ±0.12	94.83±0.05	48.19 ^a ±0.02	253.55 ^a ±0.01
TC ₇	7.13 ^{ab} ±0.02	33.52±0.02	2.58±0.04	2.65±0.04	5.36 ^a ±0.02	94.64±0.02	48.76 ^a ±0.02	244.2 ^c ±0.02

Values are means ± standard deviation of duplicate determinations. Difference letter scripts in the same column indicates statistical difference ($P < 0.05$).

Keys, TC₁ TC₂ TC₅ TC₆ TC₇ (Chocolate produced from Cocoa hybrids TC₁, TC₂, TC₅, TC₆, TC₇)

Result of the total polyphenol composition of the chocolate

The result of total polyphenol of the chocolate from different varieties of Nigeria cocoa bean is shown in Table 4. Catechin content ranged from 0.49 to 0.67% with TC₁ having the lowest mean score and TC₅ having the highest mean score. Epicatechin content ranged from 0.24 to 0.51% TC₁ had the lowest mean score while TC₆ was reported to have the highest. Caffeine content of the chocolate ranged from 0.23 to 0.44% with TC₂ having the lowest mean score while TC₆ has the highest. Theobromine and antioxidant content of the chocolate ranged from 0.33 to 0.64% and 66.26 to 78.14% respectively. TC₁ was observed to have the lowest mean score in terms of theobromine content while TC₆ has the highest. TC₅ has the lowest mean in terms of antioxidant content while TC₆ has the highest. However, of all the chocolate samples evaluated, TC₆ was reported to have the highest mean score in terms of total polyphenol contents evaluated. There was significant ($P < 0.05$) difference among the chocolate samples based on the total polyphenol parameters evaluated.

Table III: Result of total polyphenols and ant nutritional composition of chocolate from new varieties of cocoa

Sample	Catechin (mg/100g)	Epicatechin (mg/100g)	Caffeine (%)	Theobromine' (mg/100g)	Antioxidant (TE/100g)
TC ₁	0.49 ^d ±0.05	0.24 ^d ±0.04	0.27 ^d ±0.12	0.33 ^e ±0.03	67.79 ^d ±0.06
TC ₂	0.53 ^c ±0.04	0.30 ^c ±0.03	0.23 ^e ±0.09	0.49 ^c ±0.05	76.44 ^b ±0.06
TC ₅	0.67 ^a ±0.12	0.43 ^b ±0.06	0.36 ^b ±0.07	0.45 ^d ±0.02	66.26 ^c ±0.06
TC ₆	0.62 ^b ±0.17	0.51 ^a ±0.09	0.44 ^a ±0.08	0.64 ^a ±0.03	78.14 ^a ±0.08
TC ₇	0.65 ^b ±0.06	0.39 ^{bc} ±0.06	0.32 ^e ±0.05	0.60 ^b ±0.06	75.99 ^c ±0.06

Values are means ± standard deviation of duplicate determinations. Difference letter scripts in the same column indicates statistical difference ($P < 0.05$)

Keys, TC₁ TC₂ TC₅ TC₆ TC₇ (Chocolate produced from Cocoa hybrids TC₁, TC₂, TC₅, TC₆, TC₇)

IV. DISCUSSION

The result of the physical properties of chocolate produced from different cocoa varieties is presented in Table 1. The melting point of any substance is the temperature at which the substance changes from solid to liquid at atmospheric pressure (Ramsay, 1949). Schenk and Peschar (2004) reported that the normal melting point of chocolate is -33°C, and value above this may exhibit a “memory effect” recrystallization. The melting obtained for the chocolate sample in this indicated that the result have higher melting point compared to that of milk chocolate (30 to 35°C) reported by Ouellette (2016) and this may be due to the fact that dark chocolate has a higher percentage of cocoa in proportion to milk fat. The res. meter value and color of the chocolate samples ranged between 2.78 and 4.83 and 6.27 and 8.37HZ respectively. Color is considered as a fundamental physical property of product and food stuffs, since it has been widely demonstrated that it correlates well with other physical, chemical and seasonal indicator of products quality (Segnini *et al.*, 1999). In fact, color plays a major role in the appearance quality in food industries and food engineering research (Menloza and Aguilera, 2004). The fermentation methods used cause significant decrease ($P < 0.05$) in the colour of the samples. This agrees with the reports of Rodriguez-Campos *et al.*, (2012) where it was stated that fermentation of cocoa beans used in the production of the chocolate resulted in darkening of the chocolates. Proximate composition is the true representation of the nutritive value of any food. Any food that contains these nutrients such as protein, carbohydrate, fat is defined as food with high nutritive value (Okechukwu *et al.*, 2015). The proximate composition of the chocolate produced from different new cocoa varieties is presented in Table 2. The result revealed that TC₁ has the lowest mean score while TC₆ has the highest mean score in terms of crude protein. This is in line with the values (6.67%) reported by Suzuki *et al.*, (2010) for diet dark chocolate which indicated that the crude protein content of chocolate from new cocoa beans compared favorably with that of old variety. The reduction in protein content when compared to the protein values obtained by Afoakwa *et al.* (2008) for some fermented cocoa beans may be due to the conching process which could have denature some protein in the chocolate (Ndife *et al.*, 2013). Crude fat content is an important quality index for cocoa processors during purchasing of cocoa and cocoa products (Wood and Lass, 1985, Afoakwa *et al.*, 2008). The fat content observed in this study ranged between 31.96 and 34.28%. The result of fat content obtained in this study was similar to that reported by Ndife *et al.*, (2013) (31.25 to 35.10%) for chocolate from old varieties of cocoa beans. There was no significant difference among the

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samples ($P > 0.05$) The significant increase in the fat content of chocolate sample over that of the fermented cocoa bean reported could be as a result of the contribution of ingredients such as cocoa butter Fats, especially the unsaturated fat are prone to oxidation and shorten shelf life of food products (Afoakwa *et al.*, 2007) The high fat content of some samples indicates that the chocolate samples would facilitate transportation of fat soluble vitamins and also serve as source of energy (Pamela *et al.*, 2005). Dietary fats make food tasty and they often improve the texture of food as well as flavour and smell - they make food more appealing (Pamela *et al.*, 2005) Fat also provides very good sources of energy and aids in transport of fat soluble vitamins, insulates and protects internal tissues and contributes to important cell processes (Pamela *et al.*, 2005) Fats was reported to helps to reduce the risk of coronary heart disease because the flavonoids in cocoa are capable to cause the modulation, prevent the oxidation and increase in cholesterol which could cause higher risk of heart diseases as reported by Osaakebe *et al.*, (2000) Fibre is considered an efficient protective agent for a wide variety of illness including cardiovascular diseases, colon cancer and constipation (Marlett *et al.*, 2002) Crude fibre has been proved to aids peristalsis movement of food through the digestive tract (Adeleke and Odedeji, 2010). Crude fibre contributes to the bulk density which could help in the bowel movement, lower blood cholesterol and helps prevent cancer of the colon (Hung *et al.*, 2004) The result of the crude fibre content shows the value ranged between 2.35 and 2.58% and there were no significant difference ($p > 0.05$) among the samples These values compared favorably with the values of 2.23 to 3.16% reported by Ndife *et al.*, (2013) for chocolate TC₁ has the lowest mean score while TC₇ has the highest Redgwell *et al.*, (2003) reported that dietary fibre content of cocoa products after roasting and conching will reduce, possibly due to the interaction between polysaccharides, proteins, polyphenolics, and maillard products at high temperatures This might be the reason for the significant differences observed in the result of this present study Ash is an indicator of mineral contents of foods and has been shown by Legghet *al* (2011) to be high in cocoa products The ash content observed in this study ranged between 2.24 and 2.65% with TC₁ having the lowest mean score while TC₇ has the highest The result obtained indicated that there was higher ash content in the samples when compared with the values reported by Ndife *et al* (2013) for chocolates The moisture content value obtained for the chocolate samples in this study indicated that the values fall within the standard range (5 to 7%) to reduce the eventual growth of bacteria and moulds and improve the shelf-stability of the products (Fowler, 2009, Guehiet *al*, 2010) High moisture content has been associated with short shelf life of products as they encourage microbial proliferation that leads to spoilage (Ezeama, 2007), avoiding microbial growth is then paramount The reduction in the moisture content of the chocolate may be as a result of further processing such as conching Dry matter content of the chocolate ranged between 94.51 and 94.83% There were no significant difference ($p > 0.05$) among the samples studied with sample TC₂ having the lowest mean score while TC₆ has the highest score The lowest value obtained for TC₂ indicated that it will be of lower energy level compared to other samples as reported by Jinap (1994). Nitrogen free extract is the component not analyzed and calculated in proximate analysis and does not pertain to nitrogen but focuses on sugar and starch content of the product (Free Webinary, 2018) The Nitrogen free extract (Carbohydrate) content ranged between 48.19 and 51.73% with TC₆ having the lowest mean score while TC₁ has the highest Vitamin A functions at two levels in the body The first is in the visual cycle in the retina of the eye, the second is in all body tissues systemically to maintain growth and the soundness of cells (WHO/FAO, 2001) The specific symptoms of vitamin A deficiency includes xerophthalmia and the risk of irreversible blindness while the non-specific symptoms include increased morbidity and mortality, poor reproductive health, increased risk of anaemia and contributions to slowed growth and development (WHO/FAO, 2001). The vitamin A content of the chocolate samples ranged between 253.16 and 253.55 $\mu\text{g}/100\text{g}$. Chocolate sample from TC₂ has the lowest mean score, while TC₆ has the highest which indicate that chocolate from the new varieties (most especially TC₆) will be of higher value. The result of the total polyphenol composition of the chocolate produced from new Nigeria cocoa varieties is as presented in Table 4. Polyphenols are organic compounds found abundantly in plants, have become an emerging field of interest in nutrition in recent decades (Cory *et al*, 2018). A growing body of research indicates that polyphenol consumption may play a vital role in health through the regulation of metabolism, weight, chronic disease, and cell proliferation (Cory *et al.*, 2018) Recently, cocoa bean and its products total polyphenol have attracted a lot of attention because of their potential benefits on human being The health promoting effects of polyphenols are believed to be the result of the relatively high antioxidant activity of these compounds, which protect people from chronic diseases by reducing oxidative damage (Redovnikovic *et al.*, 2009). The total polyphenol content of cocoa and its products also reduced the risk of cardiovascular diseases (Stephen, 2015) as well as improvement in the insulin level and insulin resistance. The result obtained from this study shows that catechin content ranged between 0.49 and 0.72%. The catechin values from this research were higher than that documented by literature for cocoa beans (0.17%). It was also documented by Woligast and Anldan (2010) that

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high temperatures and prolonged processing times may decrease the amount of catechin. Catechin has been reported to be present in many dietary products, plants, fruits such as chocolate, cocoa (Anand *et al.*, 2014). It affects the molecular mechanisms involved in angiogenesis, extracellular matrix degradation, the regulation of cell death, and multidrug resistance in cancers and related disorders (Anad *et al.*, 2014). Epicatechin is a major component of the polyphenols in cocoa and its products and it is a major monomer of procyanidins (Othman *et al.*, 2010). Epicatechins have proven diverse benefits to human health, reducing the risks of diabetes mellitus and cardiovascular diseases. Their pharmacological effects are anti-hyperlipidaemic, anti-inflammatory, antioxidative, anticarcinogenic, and cytoprotective (Nakayama *et al.*, 1993).

The Epicatechin content of the chocolate samples ranged between 0.24 and 0.51%. There was a decrease in the epicatechin content of the chocolate samples when compared to that documented for cocoa beans by Kim and Keeney (2010). Caffeine is known to be naturally present in cocoa and its products and is better xanthanoid, a stimulant and diuretic. The level of caffeine in chocolate is dependent on the degree of cocoa bean type and its fermentation degree (Nazaruddin *et al.*, 2006). The caffeine content of the chocolate ranged between 0.23% and 0.44% with TC₂ having the lowest mean while TC₆ has the highest value. There were significant ($P < 0.05$) differences among the evaluated samples and the variation in the caffeine content of the samples may be attributed to the different hybrid of cocoa beans used and the degree of their fermentation (Nazaruddin *et al.*, 2006). Theobromine is the characteristic chemical compound found in cocoa and its products which is responsible for the bitter taste and may also possess mild stimulation properties. Furthermore, theobromine is known to stimulate heart, muscles relaxes bronchial smooth muscles in the lungs and plays an important role in the transmission of intercellular signals (Shively *et al.*, 1984). The theobromine content in this study ranged between 0.33 and 0.64% and there was significant difference ($p < 0.05$) among the evaluated samples. TC₁ has the highest mean value of theobromine while TC₆ has the lowest. African cocoa beans have been reported to contain less caffeine and more theobromine than cocoa from South America (Matissek, 1997) and this result shows decreased in theobromine content of the chocolate samples. Appreciable percentage of anti-oxidant (67.99 to 78.14%) was also reported in this study which could slow down the damage to cells caused by free radicals.

V. CONCLUSION

This study had shown significant effect of different variety of new cocoa on the quality parameters evaluated. TC₇ hybrid was the best in most of the parameters assessed while TC₆ was the best in term of proximate composition and total polyphenols with highest mean score compared to other samples.

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