INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 16, No. 1, p. 355-362, 2020

RESEARCH PAPER OPEN ACCESS

Comparative physico-chemical study of fermented and unfermented cocoa beans from Côte d'Ivoire

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Key words: Cocoa almond, Fermentation, Nutrition

http://dx.doi.org/10.12692/ijb/16.1.355-362 Article published on January 30, 2020

Abstract

Theobroma cacao (Cocoa) is a shrub whose cultivation is widespread in some tropical countries. This study on almonds from cocoa was intended to establish their physicochemical characteristics before and after fermentation and to evaluate their antiradical activities. Standard methods for physicochemical dosing, phytomolecule dosing and determination of antiradical activity (DPPH) were used. Results indicate that fermentation increases carbohydrate, total sugars and fiber. On the other hand, the contents of lipids, proteins and phenolic compounds decrease after fermentation. The best antiradical activity is observed with the methanolic extract of the fermented and dried almond (CF). This study as confirmed that cocoa bean almond can be proposed as a food additive to protect against oxidative damage.

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Introduction

Theobroma cacao (Cocoa), a shrub belonging to the family Sterculiaceae, is one of the most widely grown edible plants in some tropical countries, including Côte d'Ivoire, Ghana and Venezuela. For some of these countries, the cocoa bean is one of the main export products. In 2019, production in the word is estimated at around 5 million tons (ICCO, 2018) and Côte d'Ivoire is currently the world's largest producer. And, cocoa production is a major economic challenge in Côte d'Ivoire (Verter, 2016). Thus, its cultivation occupies the entire southern area of the country, ranging from East to West and the most cultivated variety is Forastero. Cocoa cultivation in this country involves more than 1 million producers, most often organized in cooperatives (Hütz-Adams *et al.*, 2016). The variety Forastero represents about 80% of world production (Mastroiacovo *et al.*, 2014; Afoakwa *et al.*, 2013). Consumers of the cocoa bean almond report its benefits in their body (Dillinger *et al.*, 2000). This is certainly due to its composition in essential nutrients such as proteins, fats, carbohydrates, minerals, polyphenols and other phytomolecules (Afoakwa *et al.*, 2011; Othman *et al.*, 2007). Nevertheless, cocoa almond contains anti-nutritional factors such as phytates and oxalates. Also, we know that fermentation influences the rate of antinutritional factors (Mbugua, 1988). This study aims to determine the physicochemical and chemical characteristics of the cocoa bean almond at two stages: unfermented and fermented. Antioxidant activities will also be determined at these stages.

Material and methods

Plant material and preparation of samples

The cocoa beans, belonging to the variety "Forastero", were collected in the region of Abengourou, Sub-east of Côte d'Ivoire. Two groups of samples were constituted just after collection. The first one is constituted by 5000g of cocoa beans, fermented (72 hours) and dried 24 hours in sun shine then in a laboratory oven at 50°C one night. The second one is constituted by 2000g of cocoa beans, not fermented but dried 24 hours in sun shine then in a laboratory oven at 50°C one night. Each sample is ground using an electric grinder.

Determination of physicochemical parameters

Determination of pH

The pH of the almonds is determined following the method AOAC (1975). So, 10g of bean powder are macerated 30 min in 100ml of distilled water. The pH is measured directly in the mixture using a pH-meter (Hanna instrument H8424).

Determination of the moisture content

This parameter is determined according the method AOC (1975), with some modifications. The protocol is as follows: the sample is introduced into an oven (MMM Medcenter Gmbh D-82152, Munich, Germany) at 105°C for 4 hours. After required time, it is removed and cooled to the desiccator. The lipids are extracted by Soxhlet method using pure hexane as extraction solvent. The hexane is then removed by evaporation and the residue is dried in an oven at 150°C during 2 hours and cooled to the desiccators.

Determination of the rate of proteins

The protein assay is performed by the method of Kjeldahl (AOC, 1975). The sample is mineralized, distilled and nitrated with ammonia in the presence of a colored indicator (methyl red). Total carbohydrates are calculated by difference according to the method of AOAC (1975), from the following formula:

Total carbohydrate = 100 – [% moisture +% fat +% protein +% ash]

Determination of the rate of fiber

The raw fiber almond content is determined according to the method of Van Soest (1963). So, 2g of sample powder are boiled in 50ml of sulfuric acid (1.25 N) then in 50ml of sodium hydroxide (1.25 N) for 30 minutes. The residue is dried at 105°C and then incinerated at 550°C in an oven.

Determination of the rate of ash

The almond content in ash is obtained by incineration in a Select Horn muffle furnace (P Selecta, Spain) at 550°C for 6 hours. The sample is then removed from the oven and placed in a desiccator for 30 minutes, then weighed again.

Determination of the rate of sugar

The total sugar content is determined according to the method described by Dubois *et al.* (1956). In the presence of concentrated sulfuric acid, the sugars dehydrate and react with the phenol to give a yellow complex whose intensity is proportional to the concentration of the sugars present in the medium.

Determination of the rate of phytates and oxalates

The oxalates are dosed according to the method described by Day and Underwood (1986). 1 g of sample is dried in an oven, milled and then the whole is homogenized in 75ml of sulfuric acid (3 M). The mixture is heated with magnetic stirring for 1 hour and then filtered on Wattman paper. 25ml of filtrate is hot-titrated with 0.05M potassium permanganate solution until a steady pink turn.

Determination of the rate of minerals

The minerals are dosed by spectrophotometry of atomic absorption using a spectrophotometer at flam. Protocol is as follows: 1g of ash in the sample is diluted in 10ml of hydrochloric acid (0.1N) and filtered on Wattman paper. The filtrate collected in a test tube is analyzed using a flame spectrophotometer to determine its optical density (OD).

Determination of the rate of phenolic compounds

Determination of the rate of total polyphenols

Total polyphenols are dosed according to the method described by Singleton *et al.* (1999). 1g of cocoa almond powder is homogenized in 10ml of methanol (70%) and the mixture is centrifuged at 1000 rpm for 10 minutes. The pellet is recovered in 10ml of methanol (70%) and centrifuged. The supernatants are pooled in a 50ml flask and the volume adjusted with distilled water. The assay is performed by adding 1ml of Folin-Ciocalteu reagent to 1ml of methanolic extract in a test tube. After 3 minutes of rest, 1ml of 20% sodium carbonate solution is added to the test tube. The volume is adjusted to 10ml with distilled water and the tube is then protected from light for 30 minutes and the optical density is read with a spectrophotometer (PG INSTRUMENTS) at 725nm against the White.

Determination of the rate of total flavonoids

Total flavonoids are dosed according to the method described by Meda *et al.* (2005). 1g of cocoa almond powder is homogenized in 10ml of methanol (70%) and the mixture is centrifuged at 1000 rpm for 10 minutes. The pellet is recovered in 10ml of methanol (70%) and centrifuged. The supernatants are pooled in a 50ml flask and the volume adjusted with distilled water. The assay is performed by adding to 0.5ml of sample in a test tube, 0.5ml of distilled water, 0.5ml of ammonium chloride (10%, w/v), 0.5. The test tube is left standing for 30 minutes at room temperature and the absorbance is read on a spectrophotometer at 415nm against the blank.

Determination of the rate of tannin

The tannins are dosed according to the method described by Ba *et al.* (2010). To 1ml of methanolic extract in a test tube are added 5ml of vanillin reagent at 1% (w/v). The tube is left standing for 30 minutes in the dark and the optical density (OD) is read at 415nm against the white. The amount of tannin in the samples is determined using a standard range established from a stock solution of tannic acid (2mg/mL) carried out under the same conditions as the test.

Determination of DPPH Radical Scavenging Capacity

Antioxidant activity was determined by DPPH assay. DPPH is characterized by its ability to produce stable free radicals. This stability is due to the delocalization of free electrons within the molecule. The presence of these DPPH radicals• gives rise to a dark purple coloration of the solution. The reduction of DPPH radicals• with an antioxidant causes a discoloration of the solution (Molyneux, 2004). The color change can be followed by spectrophotometry at 517nm and in this way the antioxidant potential of a substance can be determined (Popovici *et al.*, 2010, Molyneux, 2004). The reagent is prepared by dissolving 0.4µg of DPPH in methanol stored at 4°C away from light. In a series of 7 tubes each containing 100µl of extract at different concentrations (1.2 to 100µg/mL) is added 2.5ml of the DPPH solution. A tube containing the methanolic solution of DPPH serves as a control solution.

After 20 minutes of incubation at 30°C in the dark, the optical density is read at 517nm using a spectrophotometer (PG INSTRUMENTS). Vitamin C is used as a reference substance.

Statistical processing and analysis

The Graph Pad prism software version 5 (San Diego Ca, USA) is used for statistical analysis of results. The averages are always followed by their standard deviations. The comparison of means is based on analysis of variance (ANOVA) followed by the Newman-Keuls test (at the 5% threshold). Two values are significantly different if the probability from the statistical tests is less than or equal to 0.05 ($P \leq 0.05$). If not, these differences are not significant ($P > 0.05$). In the tables and in the figs, the letters a, b, c, d, e, etc., in super script indicate the averages resulting from the Newman-Keuls tests. Means marked with different letters on the same line or column is significantly different.

Results and discussion

The results of the analysis of the fermented and dried cocoa almonds (CF) and the unfermented but dried cocoa almonds (CNF) are given in Tables 1-3. Regarding the physicochemical analyzes (Table 1), we can note that the pH of the unfermented cocoa almond (CNF, pH: 4.07 ± 0.02) is lower than that of the fermented cocoa almond (CF, pH: 5.51 ±0.03). There is a significant increase ($p \leq 0.05$) of the pH during the fermentation. In Table 1, there is also a significant increase ($p \leq 0.05$) in the fiber content (CNF: 5.54 ± against CF: 6.77 ± 0.28g/100g) and the carbohydrate content (CNF: 22.89 ± 0.09 against CF: 25.98 ± 1.02g / 100g). The other parameters such as the moisture content (CNF: 6.25 ± 0.60 against CF: 6.51 ± 0.27g/100g) and the ash content (CNF: 3.19 ± 0.05 against CF: 3.26 ± 0.18g/100g) do not increase significantly. The highest increases are obtained with the pH ((+ 24.77%) the fiber rate (+ 18.17%) followed by the carbohydrate rate (+ 11.89%). Some parameters, on the contrary, see their rate decrease significantly with fermentation ($p \leq 0.05$). These are precisely the rates of proteins (CNF: 22.44 ± 0.14 against CF: 20.70 ± 1.01g / 100g), lipids (CNF: 46.30 ± 30 against CF: 43.87 ± 1.48g/100g), total sugars (CNF: 7.45 ± 0.19 against CF: 5.97 ± 0.03g/100g) and reducing sugars (CNF: 461.80 ± 5.57 against CF: 421.20 ± 12.62g/100g). Here, the largest decrease is observed with the total sugars rate (-19.86%) followed by the rate of reducing sugars (-8.79%) and the rate of proteins (-7.75%).

Table 1. Physicochemical parameters of unfermented (CNF) and fermented (CF) cocoa almonds.

Parameters	Cocoa almond powder		
	CNF	CF	D (%)
pH	4.07±0.02^a	5.41±0.02^b	+ 24.77
Moisture (g/100g of FM)	6.25±0.60^a	6.51±0.27^a	+ 3.99
Ash (g/100g de of FM)	3.19±0.05^a	3.26±0.18^a	+2.15
Proteins (g/100g of DM)	22.44±0.14^a	20.70±1.01^b	- 7.75
Lipids (g/100g of DM)	46.30±1.09^a	43.87±1.48^b	- 5.25
Fibers (g/100g of DM)	5.54±1.44^a	6.77±0.28^b	+ 18.17
Carbohydrates (g/100g of DM)	22.89±0.09^a	25.98±1.02^b	+ 11.89
Total sugars (g/100g of DM)	7.45±0.19^a	5.97±0.03^b	- 19.86
Reducing sugars (g/100g of DM)	461.80±5.57^a	421.20±12.62^b	- 8.79
Energy ((kcal/100 g of DM)	581.55± 0.55^a	592.55±0.42^b	+ 1.86

The variance analysis is followed by the Newman-Keuls multiple comparison test at the 5% threshold. The averages followed by letters a, b (in super script) are significantly different ($p \leq 0.05$). DM: dry matter, FM: fresh material; CNF: Unfermented cocoa powder; CF: fermented cocoa powder, D: variation in %.

Concerning the composition in mineral of the cocoa almond, it is noted that the unfermented almond (CNF) and fermented almond (CF) have a high mineral content (Table 2). The most important elements are: phosphorus, magnesium and calcium. However, the mineral content decreases significantly ($p \leq 0.05$) for all elements when switching from unfermented almond (CNF) to fermented almond (CF). And, the largest decreases are obtained with Copper (-17.89%), followed closely by iron (-16.53%).

Table 3 gives the contents of phenolic compounds and antinutrient compounds of unfermented cocoa almond (CNF) and fermented almond (CF). We can be noted that total polyphenols are strongly present (CNF: 131.19 ± 1.69mg/100g against CF: 102.89 ± 2.86mg/100g), followed by tannins (CNF: 68.12 ± 1, 72mg/100g against CF: 46.29 ± 1.76mg/100g). Concerning antinutritional substances, it is the phytates that are very present (CNF: 48.52 ±

0.13mg/100g against CF: 42.93 ± 1.15mg/100g). As in the case of minerals, fermentation also leads to a significant decrease in the content of phenolic compounds and antinutritionals. For phenolic compounds, the largest decreases are observed with total polyphenols (-28.83%) and tannins (-21.83%). For antinutritional compounds, it is the phytate level that decreases the most (-5.59%).

Table 2. Mineral content of cocoa almond powder.

Mineral elements	Cocoa almond powder		
	CNF	CF	D (%)
Magnesium (mg/100 g of ashes)	336.32±0,11^a	312.20±0,10^b	- 7.17
Iron (mg/100 g of ashes)	35.57±0,01^a	29.36±0,01^b	- 16.53
Potassium (mg/100 g of ashes)	93.23±0,05^a	84.62±0,04^b	- 9.24
Phosphorus (mg/100 g of ashes)	672.40±0,21^a	585.20±0,19^b	- 1.07
Calcium (mg/100 g of ashes)	148.20±0,08^a	139.4±0,05^b	- 5.93
Copper (mg/100 g of ashes)	14.76±0,00^a	12.12±0,00^b	- 17.89

The variance analysis is followed by the Newman-Keuls multiple comparison test at the 5% threshold. The averages followed by letters a, b (in super script) are significantly different ($p \leq 0.05$). DM: dry matter, FM: fresh material; CNF: Unfermented cocoa powder; CF: fermented cocoa powder, D: variation in %.

Table 3. Content of phenolic compounds and antinutritionals.

Métabolites secondaires	Poudre d'amande de cacao		
	CNF	CF	D (%)
Total polyphenols (mg/100g of DM)	131.19±1.69^a	102.89±2.86^b	- 28.83
Total flavonoids (mg/100g of DM)	26.3±1.41^a	22.02±1.52^b	- 4.28
Tannins (mg/100g of DM)	68.12±1.72^a	46.29±1,76^b	- 21.83
Phytates (mg/100g of DM)	48.52±0.13^a	42.93±1.15^b	- 5.59
Oxalates (mg/100g of DM)	7.04±0.10^a	4.93±0.23^b	- 2.11

The variance analysis is followed by the Newman-Keuls multiple comparison test at the 5% threshold. The averages followed by letters a, b (in super script) are significantly different ($p \leq 0.05$). DM: dry matter, FM: fresh material; CNF: Unfermented cocoa powder; CF: fermented cocoa powder, D: variation in %.

The scavenging activities of the DPPH radical by methanol extracts of unfermented (CNF) and fermented (CF) almonds from cocoa beans were measure (Tableau 4).

The IC_{50} values obtained (CNF, 9.75± 0.41µg/mL) and (CF, 10.87±0.24µg/mL) were lowers compared to that of vitamin C (2.15±0.53µg/mL). Fig. 1 shows variations of the percentage of inhibition of the DPPH by the methanol extracts of unfermented (CNF) and fermented (CF) almonds from cocoa beans, at different concentrations.

Table 4. Scavenging activities of the DPPH radical by methanol extracts of unfermented (CNF) and fermented (CF) almonds from cocoa beans.

Samples	CNF	CF	Vita C
IC_{50} (µg/mL)	9.75± 0.41	10.87±0.24	2.15±0.53

CNF: Unfermented cocoa powder; CF: fermented cocoa powder, Vat C: Vitamin C

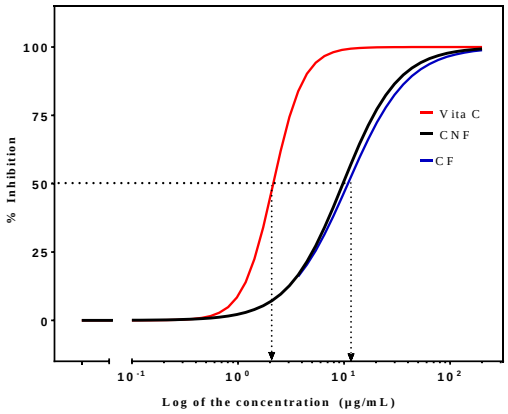

Fig. 1. Scavenging effects of methanol extract of CNF and CF, compared to that of Vitamin C extrait méthanoïque de l'amande de cacao comparée à celle de l'acide ascorbique. CNF: Unfermented cocoa powder; CF: fermented cocoa powde, Vat C: Vitamin C.

Discussion

Fermentation is a method that normally improves the nutritional value of foods and reduces their levels of antinutritional substances (Mbugua, 1988). The analysis of samples of unfermented almond (CNF) and fermented almond (CF) from cocoa has yielded very interesting results. Indeed, the measurement of the pH of CNF and CF indicate a significant increase, and therefore a decrease in acidity. Our values are close to those obtained by Ban-Koffi *et al.* (2013), which showed that cocoa fermentation gradually increases the pH value and can approach 5.5.

Under certain circumstances, it can increase continuously to reach a basic pH at the end of treatments (Koffi *et al.*, 2017). This decrease in the acidity of the almond (increase in the basicity of the almond) can be explained by the release of CO_2 produced during the fermentation; CO_2 being acidic, its elimination thus reduces the acidity of the medium. Concerning the moisture content of cocoa almond samples (CNF and CF), there is a slight increase during the fermentation (+ 3.99%). This increase is normal, since the fermentation cannot be done without hydration of the material. The moisture content of our samples goes from 6.25% to 6.51%; these results are in agreement with those of Ndife *et al.* (2013) and Torres-Moreno *et al.* (2014) who obtained respectively moisture contents of 5,95% and 6,20% in cocoa. These values are within the standard margin required by the chocolate industry (the recommended moisture content is around 7%). This is the rate needed to reduce the growth of certain bacteria and to improve the stability of cocoa derivatives (Guehi *et al.*, 2010). The levels of fat, protein, total sugars and reducing sugars decrease with fermentation. We note that the decrease in lipid level is similar to the results obtained in the literature (Amaiz *et al.,* 2012; Ajala *et* Ojewande 2014 et Djikeng *et al.,* 2017). Similarly, the level of protein in our samples is similar to that reported in the literature (Afoakwa et Paterson 2008, Afoakwa *et al.*, 2011) and well above the conventional protein value of 10% (FAO, 2004).

Concerning the mineral content of CNF and CF, fermentation has led to their general decrease. This decrease can be explained by the chemical transformations that accompany the fermentation. However, the content remains important after fermentation. The most abundant elements in both cocoa samples are phosphorus (585 to 672mg/100g), magnesium (312 to 336mg /100g) and calcium (139 to 148mg/100g). These values are similar to those reported by Zoulo et Djessou (2006) and Cinquanta *et al.* (2016).

The contents of phenolic compounds and antinutrients diminish significantly after fermentation. For total polyphenols, the contents go from 131.19mg/100g for unfermented almonds (CNF) to 102.89mg/100g for fermented almonds (CF). These results are similar to those reported by Francisco *et al.* (2007). These results also show that there are more tannins than flavonoids in the cocoa bean almond, which is consistent with data in the literature (Portillo *et al.*, 2012). The most abundant antinutritional compounds are phytates (42 to 48mg/100g) and come very far behind oxalates (4.93 to 7.04mg/100g). The decrease in the phytates and oxalates contents during the fermentation makes it possible to affirm that the latter has a beneficial effect on the nutritional value of the almond. In fact, the presence of phytate in food prevents the use of nutrients because these substances bind to minerals to form non-assimilable salts, making biologically unavailable minerals for the body (Erdman, 1979). As for oxalates, they bind to minerals and promote a risk of urinary calculus (Adeyeye, 2016).

The IC50 values show that the antiradical activities of cocoa bean almonds decrease slightly with fermentation, from 9.75µg/ml for CNF to 10.87µg /ml for CF. This reduction is too small to affect the antioxidant properties of the fermented almond. On the inhibition curve, we can see that the extracts of fermented almond and unfermented almond have about the same pace.

Conclusion

The comparative physicochemical study of fermented and unfermented cocoa bean almond shown that the fermentation has a beneficial effect on the nutritional quality of cocoa's almond, since the content of antinutritional compounds decreases by 5.59% for phytates and 2.11% for oxalates. Also, the energy value increases by about 2% with fermentation. Proceed to fermentation of cocoa beans before drying and their conditioning is beneficial to improve the nutritional quality of the beans.

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