A Study of the Combined Effect of Post-Harvest Fermentation, Turning and Drying of Cocoa (Theobroma Cacao L.) On Beans Quality

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***Abstract*—The effect of a combined post-harvest processing treatment, including fermentation, turning and drying was tested on beans from 2 cocoa clones, for quality characterization. Fermentation, associated with turning of cocoa beans when applicable, was done in wooden boxes, jute bags and heaps, for 5 days period. Drying consisted of 8 hours daily sun exposure of cocoa beans on black tarpolin and bamboo mats. Treatments were organized in a complete randomized blocks experiment, with 3 replications. Quality evaluation of cocoa in fermentation was based on recording of temperature variation inside bean mass while quality during drying process was appreciated based on weight loss of beans. Dry beans were checked for quality on water content, acidity, polyphenol content, mould infection and ochratoxin A occurrence. Cocoa powder was evaluated on astringency, bitterness, acidity, and aroma. Data were analyzed using SAS software and means separation used Duncan's multiple range method. Results obtained showed no significant difference (p >0,05) among effects of fermentation types on temperature. However, regardless of the fermentation system, a sharp increase in temperature inside the bean mass of all fermentations systems, was initiated from the 2nd day, that reached a peak averaging 50 C on the 3rd day of fermentation. Cocoa had a significant (p<0,05) weight loss during drying as a result of absence of turning of fermenting beans. High and significant water contents were recorded in cocoa fermented in jute bags (40,23%) and heaps (40,05%), versus (27,50%) found in cocoa fermented in boxes. A high acidity was developed in beans from all fermentation materials when turning was avoided. Fermentation in jute bags and in heaps exhibited higher polyphenol levels, 179mg/g and 175 mg/g respectively as opposed to the low level in boxes (119mg/g). The highest presence of polyphenols was caused by the lack of turning. Instead, stirring of beans significantly (p<0,05) reduced mould development and favored minimum ochratoxin A occurrence. The aroma of cocoa was improved when fermentation of beans was assoctiated with turning and followed with drying of products on tarpaulin, which equally reduced astringency, bitterness and acidity of cocoa powder as well.**

Keywords—Theobroma cocoa, fermentation; turning, drying, beans, powder, quality.

1. INTRODUCTION

Cocoa constitutes one of the aricultural products with a high economical potential worldwide. It is produced by over 50 developing countries and it accounts for about 57 % of the exported crops of west African countries (Anonymous, 2014). About 2,5 millions cocoa producers from these countries, and their families mainly rely on cocoa as essential revenue source. Cameroon ranges among the six countries providing about 90% of the world cocoa production (Anonymous, 2016). However, cocoa from Cameroon is disadvantaged by its decreasing quality. This is reported to originate from absence or inappropriate pre-harvest cultural operations as well as inadequate post-harvest processing practices such as fermentation and drying of cocoa (Mounjouenpou et al., 2001). Fermentation contributes to the development of a pleasant aroma and to reduction of undersirable astringency, bitterness and acidity of cocoa beans (Tagro et al., 2010). The interruption of fermentation favors aerophilic bacteria and molds development on beans (Ganeswari et al., 2015) and also formation of dark, brown, breakable and brittle beans with poor taste and tough and acidic aroma. Turning of cocoa during fermentation reduces increased titrable acidity in the beans and prevents negative fermentation processes (Tagro et al.,2010). That fermentation handling option equally promotes oxygenation of cocoa beans and acetic fermentation which followes the alcoholic phase of that process (Cempaka et al., 2014). Citric acetic and lactic acids formed during fermentation are eliminated in the course of that process (Assiedu, 1992; Camu et al., 2008) in addition to water content reduction occurring in the beans, from 60 to 8 % (Duncan and Pettipher, 1989). The acid outflow takes place through liquid diffusion from inside to bean air exposed surfaces (Camu et al;, 2008). A slow sun drying of cocoa is known to induce and sustain biochemical reactions inside the beans especially polyphenol oxidation by polyphenolase enzyme to form tannins, identified as insoluble and harmful compounds to cocoa quality (Tagro et al., 2010; Albertins et al.,2015; Meritza et al.,2016). Inappropriate drying conditions are reported to cause water resorption and subsequent molds development in the beans (Copetti et al.,2010; Mounjouenpou et al.,2012; Abrokwafk et al.,2013). Moreover, contamination of beans with organic and toxic substances and other dust and dirt materials occurs through contact of cocoa with bitumen during tared road side drying of that product (Kanmogne et al., 2012; Coulibali, 2016). The objective of this research is to evaluate the influence of different types of fermentation combined with turning and various drying methods on physical, biochemical and organoleptic characteristics of cocoa beans.

2. MATERIALS AND METHODS

Cocoa beans from 2 clones, Forastero UBA and Trinitario ICS, respectively developed at IRAD research center of Nkoemvone (South Cameroon) and Ekona (South west Cameroon), and cultivated in village farms of Santchou (West Cameroon). Upon extraction from the pods, fresh beans were grouped in samples of 10 kg before application of a combined post-harvest including fermentation, turning and drying through a randomized complete block design experiment with 3 replications. Fermentation was done in 3 different structures: wooden boxes, jute bags and heaps. Boxes otherwise called sweat boxes were provided with holes of 15 cm in diameter at a spacing of 8 cm in the bottom of the box for pulp drainage. Boxes were placed on support materials to avoid direct contact with soil and to allow air flow. Fermentation in bags used recycled and cleaned jute bags of 60 cm in depth and 30 cm in width. Heap fermentation consisted of mounding cocoa beans on fresh banana leaves, Turning was achieved by manually and thoroughly mixing cocoa beans, every 2 days during the 5 days of fermentation. Following fermentation, cocoa was without delay placed in drying for 10 days, on 2 types of materials comprising black tarpolins and bamboo mats. Drying was through daily sun exposure of beans, for 8 hours, with frequent turning, for all samples, to accelerate and homogenize the process.

The quality of beans was followed and observed during fermentation with recording of temperature inside the mass of beans, and evaluation of weight loss during drying. In addition, water content, acidity level, polyphenols content, molds infection, ochratoxin A (OTA) occurrence as well as organoleptic quality were recorded after drying. Temperature variation was obtained by a daily introduction for 3 mn of a thermometer probe in the mass of fermenting cocoa beans. Weight loss in cocoa was assessed by successively weighing to the nearer g empty and cocoa bean full bags, using an electronic balance.

Water content of dried cocoa beans was also determined by the double weighing method, before and after a stay of 10 h ± 1 mn in an oven, at 103 ± 2°C. The acidity level was measured by mixing 3 g of cocoa powder with 30 ml of boiled water, followed by cooling and filtration, before recording of pH, using a pH electronic probe.

The polyphenol content inside the bean was determined with reference to Niemenak et al. method (2006) for lipid extraction, consisting of mixing of 2g of cocoa powder with 1à ml of hexan followed by filtration. Fat-free cocoa was dried for 2 h in ambient temperature, before extraction of total crude polyphenols through mixing of 2 mg of ground defatted cocoa powder with 2 ml of chloridric acid (0,1N) and centrifugation of mixture at 2000 rpmn for 20 mn. Determination of polyphenols was thereafter done using Folin and Ciocalteu reagent (Ribereau, 1960), where 2 ml of dionised water followed with 10µl of polyphenol extractes and 200 µl of reagent were introduced in test tubes, before homogenization and disposition under 40 °C temperature for 20 mn. The reaction was stopped with the introduction of 0,5 ml of sodium carbonate (20 %) in the mixture. Polyphenol content was quantified using a spectrophotometer, after cooling under ambient temperature.

Moulds beans were identified and enumerated based on the cut test method. Cocoa beans were longitudinally cut and fully opened and examined for identification of defects including moldy beans, germinating beans, flat beans, violet and compact beans.

The occurrence of ochratoxin A (OTA) in cocoa samples was recorded by mixing and homogenizing for 1 mn of 50 g of cocoa powder with 50 ml of calcium carbonate (1 % and 3 % respectively), followed by filtration and addition of 10 ml sodium bicarbonate and methanol to the filtrate as buffer solution. Mycotoxin were then isolated and collected by introduction of the mixture in a chromatographer, and that sample was then analyzed in a spectrophotometer for OTA presence determination (Roberts et al., 2014).

The organoleptic quality of cocoa was assessed by a sensory test involving 10 panelists (Watts et al.). Cocoa samples were evaluated based on bitterness, astringency, acidity and aroma attributes. They were therefore scored according to the following scale: 0 (absence), 2 (very low), 3 (moderate), 4 (strong), and 5 (very strong)

Data expressed in percentage were transformed using the angular formular:

Y= arsinv x

Statistical analysis was performed with SAS software (SAS, 1982) and means were separated using Duncan's multiple range test method with the level of significance at 0,05 %.

3. RESULTS

There was no significant (p >0,05) different recorded on temperature variation among effects from the various treatments tested (Table 1). Throughout the 5 days of fermentation. However, During the second day, a rise was initiated that brought temperature average to 39 C for all samples (Fig.1). The increased observed was constant and reached the peak of 50 C on day 3 for cocoa fermented in jute bags and in boxes. Thereafter, temperature steadily declined. Fermentation method was shown with no significant (p >0,05) impact on weight loss of cocoa beans. When products were turned while in fermentation, a significant (p<0,05) drop of 4,9 % in weight was recorded for all samples of concern, compared to the result obtained (4 %) in absence of turning. Weight reduction was rather at a constant rate for all treatments. (Fig. 2). Fermentation type has significantly (p <0, 05) influenced water content in cocoa beans. Products fermented in sweat boxes exhibited a significantly (p <0,05) lower water content (27,50 %) than that obtained with fermentation in jute bags (40,05 %) and in heaps (40,23 %). Likewise, the incidence of turning was significant (p<0,05) and thus brought water content to a reduced level of 32,45 % against 44 % recorded for control products. Drying also significantly reduced the humidity level of beans with a higher impact from the use of black tarpolins which led to a remaining humidity of 32,60 % versus 43,90 % from drying cocoa on bamboo mats. The acidity level significantly varied as a result of fermentation turning. A lower pH level (5,7) was developed in the beans in absence of turning, as compared to a higher pH (6,2) brought about by a frequent mixing of cocoa beans.

Table 1. Effects of the fermentation, turning and drying processes on weight loss, water content, pH, polyphenol level, moulds, ochratoxin and organoleptic quality of cocoa beans.

Treatment		Weight loss (%)	Water content (%)	pH level	Polyphenol level (mg g^{-1})	Mouldy bean proportion (%)	OTA occurrence (ug kg^{-1})	Organoleptic quality
Fermentation	Boxes	4.0a	27.50b^{y}	6.1a	119b	3.0b	1.2a	2c
	Bags	4.0a	40.23a^{z}	5.9a	178a	4.0a	1.4a	4a
	Heaps	4.0a	40.05a	5.8a	175a	3.8a	0.5b	3b
Turning	Turning	4.0a	32.45b	6.2a	119b	2.0b	0.1b	1a
	Control	3.9b	44.00a	5.7b	235a	5.4a	2.1a	4b
Drying	Tarpolins	4.1a	32.60b	5.9a	118b	3.5b	1.12a	3b
	Mats	4.1a	43.90a	6.0a	167a	4.0a	1.35a	4a

y: Data are means of 3 observations.

z: Means within treatments not followed by the same letter are significantly different at 5% level by Duncan's multiple range test

Fermentation turning and drying significantly (p < 0,05) influenced polyphenols presence in cocoa beans. The use of wooden boxes was effective in to obtain a reduced polyphenol content of 120 mg/g in comparison to the highest level (179 mg/g) recorded in jute bags and in heaps likewise. Turning promoted the obtention of the lowest polyphenols content (119 mg/g), whereas that record almost doubled when turning was avoided. Black tarpolins also contributed to minimize the polyphenols threshold while a higher proportion (165 mg/g) was found in cocoa that was dried on bamboo mats. A significant attenuation (p <0,05) in moulds development in cocoa beans derived from fermentation in sweat boxes. Only 3 % of mouldy beans were recorded with the use of that materials versus 4 % and 3,8 % identified when jute bags and heaps were used respectively.

Turning the mass of cocoa in fermentation was significantly efficient in prevention mould infection and development inside beans, since it brought the presence of moulds to a minimum of 2%. Instead, a higher proportion of moulds infected products came out in cases of absence of turning, regardless of the fermentation materials.

The occurrence of ochratoxin A also significantly varied among fermentation types that were evaluated. Cocoa from heaps had a lower level of OTA (0,5 µg/kg) than that from boxes (1,2 µg/kg) or from jute bags (1,4 µg/kg). A comparatively low level of OTA (0,2 µg/kg) was promoted by turning cocoa beans, as they were fermented, but the mycotoxin occurrence considerably increased to 2,1 µg/kg for control cocoa. Drying did not produce a detectable impact on OTA occurrence.

The organoleptic quality of cocoa beans was significantly improved with the use of wooden sweat boxes for fermentation mostly when associated with a frequent turning of cocoa mass. Products from almost all samples that went through turning practice obtained the best appreciation records on bitterness, astringency, acidity and aroma.

4. DISCUSSION

The temperature rise observed, almost asymptotic, from the second day of fermentation was expected, as reported in previous results (Camu et al., 2008; Tagro et al.,2010; Albertins et al. 2016), although with no significant difference detected among treatments tested effects. That increase up to 50 C in temperature from the day 3, reveals the exothermic reactions occuring inside cocoa fermenting mass, indicates satisfactory fermentation conditions. Evolution phases involved are lactic then acetic fermentation by lactic and acetic bacteria (Tagro et al., 2010; Kresnowat et al., 2013; Cempeka et al., 2014; Ganeswari et al., 2015). The polyphenol oxydase enzyme involved in polyphenol degradation is known to be activated and stimulate oxidation of polyphenolic compounds during that exothermic phase (Maritza et al., 2016).

The constant weight reduction recorded during drying of cocoa regardless of the type of material (Fig. 1) is certainly the result of the above mentioned biochemical reaction persisting during drying. Some of the end products as well as acetic acid present in the beans are lost through diffusion in beans cotyledons, through volatilization in the air and through exudation from beans along with water loss, which enormously contributes to weight loss in cocoa during drying process (Camu et al., 2008). The markedly higher weight loss in cocoa that was turned during fermentation was ceatertainly favored by stirring and subsequent water evaporation (Abdoulaye, 1984). Similarly, the higher level of water loss obtained with fermentation using sweat boxes reflects important water content reduction down to 27,5 %, caused by drainage of water containing pulp through holes at the bottom of boxes. Instead, jute bags and banana leaves not provided with such drainage outlet led to less pulp out flow. Hence remaining water content of 40,25 % and 40,5% were found in beans from jute bags and heaps respectively.

Beans in the fermenting cocoa mass left without turning developed a relatively higher acidity with a pH of 5,7 as opposed to low acidity (6,2 in pH) obtained when turning was regularly applied. Avoidance of mixing and absence or inadequate aeration of beans in fermentation favored the development of anaerobic conditions, thus the onset of bacterial activities producing organic acids in the beans, which reduced the pH to the lower point of 4,5 (Rohsius et al., 2006). Turning brought the pH in the beans to level (6,2) favorable for the production of the expected good quality cocoa. Thery (1995) indicated that from pH 5,5 aroma precursors are formed and that cocoa powder with pH between 5 and 8 is likely to yield good chocolate. Wooden boxes provided an adequate oxygenation due to turning practice. This inhibited anaerobic respiration of related acidic bacteria and favored elimination through volatilization of acetic acid in beans cotyledons. Tarpaulins also contributed to volatilization of similar and related compounds during drying (Tagro et al., 2010).

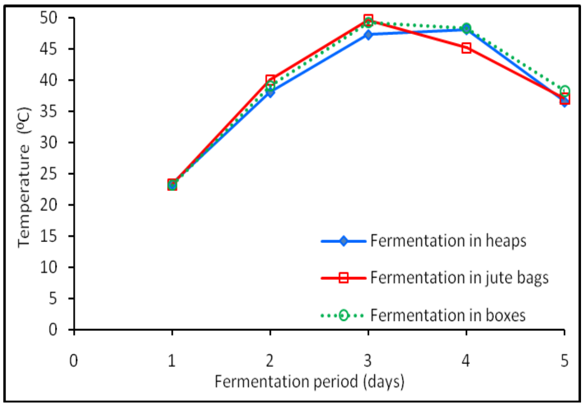

Figure 1 Temperature variation of cocoa beans during fermentation in heaps, jute bags and boxes.

Higher proportions of moulds found in cocoa fermented in jute bags and heaps without turning were certainly favored by an increased acidity that developed in the beans, resulting from accumulation of less diffusable and non volatile lactic acid produced during lactic fermentation (Tagro et al., 2010, Ganeswari et al., 2015). Contrarily aeration brought by turning of fermenting of cocoa mass as well as during drying has prevented anaerobic conditions and corresponding bacteria and acidity development and thus reduced mould infection in bean cotyledons (Camu et al., 2008).

The lack of turning cocoa in fermentation has led to CO_2 accumulation, development of lactic bacteria activities and resulting acidification inside the beans. Such conditions were favorable for mould growth and multiplication, particularly Aspergillus spp which produces ochratoxin A (Mounjouenpou et al., 2011; Mounjouenpou et al., 2012; Abrokwafk et al.,2013).

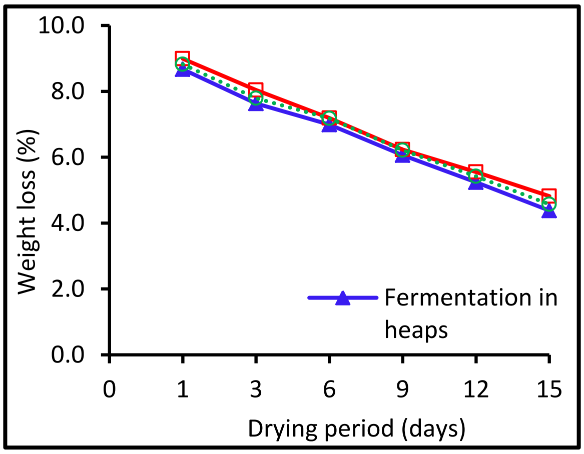

Figure 2 Weight loss variation of cocoa beans during drying in heaps, jute bags and boxes.

The high occurrence of ochratoxin A (2,1µg/kg) recorded in beans from cocoa mass fermentation deprive of turning are therefore justified. They are slightly above the authorized standard level of 2 µg/kg (Mounjouepou et al., 2011; Coulibali et al.,2012). The low occurrence of ochratoxin A (0,5µg/kg) in cocoa beans that were turned in fermentation was brought by the reduced acidity originating from prevention lactic acid formation and elimination of acetic acid through turning aeration, thus increasing pH at level unfavorable to mycotoxin producing moulds.

Cocoa powder from all samples submitted to turning was appreciated and was attributed high quality records by panelists. Fermentation of cocoa associated with turning and followed with drying on black tarpolins further contributed to reduce bitterness and astringence and to improve aroma of cocoa. That treatment combination certainly favored elimination of excess polyphenols which produced expected and satisfactory organoleptic quality in cocoa beans.

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