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Effect of Turning Beans and Fermentation Method on the Acidity and Physical Quality of Raw Cocoa Beans

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Abstract: This study concerns the influence of turning and fermentation method on quality of raw cocoa. Fermentation trials were conducted in wooden box, plastic box and in heaps with or not turning. Cocoa fermented in boxes during 4 days without stirring presented pH values above 5.0 while cocoa fermented in heaps presented pH 4.92. For fermentation with turnings, beans treated in wooden box were less acidic than beans fermented in plastic box, which recorded pH 4.75. Cocoa issued from all fermentations methods lasted 5 days without mixing presented pH above 5. Cocoa fermented in plastic box with turnings became acidic with pH 4.73 while acidity disappeared in beans fermented in heaps. A similar trend was observed in the titratable acidity of the dried beans. Cocoa fermented during 4 days without turnings presented a high percentage of purple beans nearly 40% whatever the method. Percentages of defective, slaty and mouldy beans were below 4% with or without turnings. Cocoa beans fermented with turnings recorded about 10% of defectives beans whatever the process. Percentage of purple beans decreased to about 12% for cocoa fermented in wooden box. Naturally, percentage of brown beans increased for cocoa fermented both in wooden box and in heaps. All the beans showed no sign insect damages, and negligible levels of internal molding whatever the turning and the methods of fermentation.

Key words: Beans, cocoa, fermentation, quality, raw, turning

INTRODUCTION

Cocoa is consumed widely in the form of chocolates and consumption rate is rising due to the increasing popularity of chocolate confectioneries worldwide. Other applications of cocoa can also be found in beverages, cosmetics, pharmaceuticals and toiletries products (Tafuri *et al.*, 2004). Commercial cocoa is obtained from the beans originated as seeds from the ripe pods of the plant *Theobroma cacao*, which is native to the Amazon region of South America and cultivated in the tropical regions of the world (Ardhana and Fleet, 2003). The processing of cocoa beans consists of two major steps namely fermentation and drying (Hii *et al.*, 2009). Cocoa fermentation as the first stage in the preparation of chocolate begins immediately after the beans embedded in mucilaginous pulp are removed from the pods. Fermentation needed in the development of various flavor precursors in the beans. The beans and associated pulp are subject to microbial fermentation, which is generally conducted as traditional, indigenous (Ardhana and Fleet, 2003). Microbial fermentation, which takes place, induces numerous chemical reactions leading to a deep modification of the biochemical characteristics of beans (Timbie *et al.*, 1978; Gill *et al.*, 1984). Indeed, sugars in the mucilage are converted into alcohol by yeasts, which proliferate after the sterile mass from the pods is exposed to the surrounding air (Ardhana and Fleet, 2003). This is made by providing heat, numerous organic compounds such us ethanol, lactic, acetic acids and others organic acids leading to the death of the seed embryo (Lagunes-Gálvez *et al.*, 2007, Kostinek *et al.*, 2008) and inhibiting the later germination which assures proper curing of the beans (Lopez and Dimick, 1995). The mass then becomes runny and drops away from the beans. Enzymatic reactions take place contributing to the formation of flavour and the colour changes in cotyledons from purple to brown. These biochemical changes inside the beans contribute to the reduction of bitterness and astringency and the development of flavor precursors (Lagunes-Gálvez *et al.*, 2007). Fermentation reactions have been reviewed by Fowler (1999) and Beckett (2000). Methods

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of fermentation strongly determine the commercial quality of cocoa beans produced not only especially the chocolate flavor, but also the risk of the moulds contamination. Indeed, Renaud (1954) have shown that the fungal contamination of cocoa beans could be under the control of the duration and fermentation processing. Different methods of fermentation are followed in the cocoa growing countries (Rohan, 1963; Wood and Lass, 1985). The manner of cocoa fermentation varies considerably on the cocoa-producing region. Many of traditional methods of cocoa fermentation such fermentation in banana leaves lined holes in the ground or in the baskets were used but today improved methods using wooden and plastic boxes have been performed. In total, cocoa's fermentation methods could be classified into five categories: fermentation on drying platforms, fermentation in heaps, fermentation in baskets, fermentation in trays and fermentation in boxes (Lopez and Dimick, 1995). Beans are piled in either heaps, boxes or trays, covered with plantain leaves and left to ferment for 5-7 days (Fowler, 1999). For example in Malaysia, fresh cocoa beans are usually fermented using the heap or box methods for 5-7 days depending on the condition of the beans (Hii *et al.*, 2009). In Côte d'Ivoire, most cocoa is produced on small holdings under rural conditions by fermenting it in heaps but only few big holders ferment their product using wooden and plastic boxes without turnings beans during either 4 or 5 days on weather conditions and time during the cocoa season (Guehi *et al.*, 2007). Indeed, fermentation generally takes shorter at the start and peak of the cocoa crop but longer towards the end of the crop when there is less mucilage available for fermentation. At the end of fermentation, Ivorian cocoa producers spread freshly fermented beans on a meshed wooden tray with area about 30-90 cm and raised 1 m above ground level, mats, polypropylene sheets or the concrete floor of a cocoa house each day to a depth of not less than 5 cm and mixed constantly to promote uniform drying and to break agglomerates. Sun drying is the preferred method for Ivorian producers. As chocolate quality depends strongly on the cocoa fermentation process (Schwan, 1998; Schwan and Alan, 2004) many previous studies on the impact of cocoa post harvest processing have been carried out on the quality of raw cocoa such delay of pods breaking (Barel, 1987), the development of filamentous fungi (Mounjouenpou *et al.*, 2008), the changes in Key aroma compounds (Ziegleder, 1981; Jinap *et al*, 1998; Frauendorfer and Schieberle, 2008), the content of polyphenols (Nazaruddin *et al.*, 2006), the microbial development during the fermentation (Camu *et al.*, 2007, Cleenwerck *et al.*, 2007, Kostinek *et al.*, 2008). Nevertheless there have been no studies on the effect of fermentation processing and turnings beans on the commercial value of raw cocoa. Since liberalization of Ivorian cocoa chain in 1999, the quality of raw cocoa undergoes degradation and many defective quality traits are currently associated to the cocoa sourced from Côte d'Ivoire. Therefore it is important to identify the factors that reduce commercial value by studying the chemical and physical quality properties of Ivorian beans resulted from different methods of fermentation.

The purpose of our study was to evaluate the effect of fermentation method conducted either with or without turnings beans on chemical and physical quality of raw cocoa beans.

MATERIALS AND METHODS

This study was conducted from November to December 2009 on the small holdings under rural conditions in at a cocoa farm located at Kpada, a big cocoa producing village of Soubré. Soubré is the main cocoa producing region located at South West of Côte d'Ivoire, which is the major cocoa producing country in the world located in West of Africa.

Cocoa: Fully ripe cocoa pods (*Theobroma cacao* L.) of mixed-hybrids were handily harvested during the 2009 big cocoa season.

Cocoa pod storage and breaking: Harvested pods were stored at the field's level and opened three days later using a piece of wood billet as a bludgeon (Meyer *et al.*, 1998). The distal portion of the pod falls away and the beans remain attached to the placenta from which they were easily extracted. The beans were removed carefully from placenta and any germinated, black or diseased beans or pieces of shell or placenta fragments were excluded.

Cocoa bean fermentation methods: Three different types of fermentation processing such us (i) Fermentation in Wooden Box (FWB), (ii) Fermentation in Plastic Box (FPB) where the beans were placed in boxes measuring $40\times40\times40cm^3$, and (iii) Fermentation in Heaps (FH) where the beans were tipped onto banana leaves placed on the ground as previously described by Mounjouenpou *et al.* (2008) were studied. Each method of fermentation lasted both 4 and 5 days and was performed firstly without turning beans and secondly with 2 turning beans using 100 kg of beans. Floors of the wooden and plastic boxes have holes to facilitate drainage of acidic liquid resulted from liquefaction of mucilaginous pulp and aeration of the fermented mass of cocoa and covered with plantain leaves. Both the wooden and the plastic floors were raised above ground level, over a drain, which carries away the pulp juices liberated by the degradation of the mucilage during fermentation. The heap of wet cocoa beans was then covered in the box with other fresh banana leaves in order to insulate the top of box before

Table 1: Changes in pH and titrable acidity of dried cocoa beans on turnings and fermentation methods

	4 days of treatment				5 days of treatment			
	Without turning		With 2 turnings		Without turning		With 2 turnings	
Methods of fermentation	pH	TA (meq of $NaoH.10^{-1}g$)	pH	TA (meq of $NaoH.10^{-1}g$)	pH	TA (meq of $NaoH.10^{-1}g$)	pH	TA (meq of $NaoH.10^{-1}g$)
Fermentation in wooden box	5.66 ± 0.01^a	$1,06 \pm 0.02^a$	5.30 ± 0.06^a	$1,38 \pm 0.0^a$	5.16 ± 0.04^a	$1,14 \pm 0.02^a$	$5,34 \pm 0.03^b$	$1,31 \pm 0.01^b$
Fermentation in plastic box	5.44 ± 0.05^a	$1,10 \pm 0.0^a$	4.75 ± 0.05^b	2.37 ± 0.1^c	5.44 ± 0.00^b	$1,13 \pm 0.0^a$	$4,73 \pm 0.02^a$	$2,36 \pm 0.0^c$
Fermentation in heaps	4.92 ± 0.01^b	$2,25 \pm 0^b$	5.16 ± 0.01^a	2.09 ± 0.0^b	5.04 ± 0.01^a	$1,19 \pm 0.0^a$	$6,59 \pm 0.28^c$	$0,80 \pm 0.0^a$

Mean values having a common letter within the same column are not significantly different according to Duncan's multiple range test at the 5% level

placing the wooden cover. For the experimentation of fermentation with turnings the beans were mixed after 48 and 96 h of fermentation. Three assays of each fermentation method were performed during the cocoa season.

Drying of cocoa beans: For drying, freshly fermented beans resulted from each type of fermentation were spread on polypropylene sheets to a depth of not less than 5 cm and were natural or solar dried each day from 9 am until to 6 pm. The beans were mixed constantly to promote uniform drying and to break agglomerates until the moisture content reached 7-8% as commonly practiced by Ivorian cocoa producers (Guehi *et al*., 2007).

Physical quality assessment: Cut test, the first quality-control of cacao beans, is done for sanitary and fermentation quality of all cocoa samples as previously described (Hamid and Lopez, 2000; Hii *et al.*, 2006). One hundred pieces of dried cocoa beans were cut lengthwise through the middle using a penknife. Both halves of each bean were examined in full daylight according to the cross sectional colour of the beans. Observations were made for mould infestation, insect damage, flat and germination as well as of the colour of the beans (slate, fully purple and fully brown). Slaty bean characteristics include rubbery cotyledon, blackish colour, and resistance to cutting. Purple beans occur when the fermentation has been terminated prematurely. Defectives beans are the sum of germinated beans, insect infested beans and flat beans. Fully brown beans are well-fermented beans. Results were expressed as a percentage of each type of defective beans and all analyses were done triplicate. According to the official standard, a batch of cocoa beans with more than 60% fully brown colour beans is considered as good quality product.

Chemical assessments: Chemical assessments were restricted to the determination of pH and titratable acidity (Bonaparte *et al*., 1998). Five grams of finely ground nibs (deshelled beans) were homogenized in 45 ml boiled distilled water. The mixture was filtered with Whatman Nº4 filter paper and cooled to 20-25ºC. The resulting filtrate was measured for pH using a pH-meter (Consort P 107), which had been, calibrated with buffers at pH 4 and 7 as described by Hii *et al.* (2009). A further 25 ml aliquot was titrated to an end point pH of 8.1 with 0.01N NaOH. Titratable acidity was calculated using the formula previously used by Hamid and Lopez (2000). The values reported as meq of sodium hydroxide per 10 g of dry nibs (meq of $NaOH.10\ g^{-1}$). These measurements were performed in triplicate.

Statistical analyses: The data obtained from the physical and chemical analyses were analyzed for one-way ANOVA and Duncan's Multiple Range Test using SAS statistical software (Version 8, SAS Institute, Cary, NC, USA) at 95% confidence level.

RESULTS

Effect of turnings and fermentation methods on pH and Titratable Acidity (TA) of raw cocoa beans: The results of chemical quality assessments (pH and titratable acidity) of the dried beans on process of fermentation and turning beans are shown in Table 1. For the fermentation lasted 4 days, the pH ranged from 4.75 to 5.30 and from 4.92 to 5.66 for dried beans fermented respectively with and without turnings whatever the method of fermentation. For fermentation conducted without stirring beans, while the pH of both types beans fermented in boxes (FWBwt and FPBwt) showed pH values above 5.0 with no significant difference ($p<0.05$) the beans resulted from fermentation in heaps presented pH 4.92. For fermentation with 2 turnings, beans treated in wooden box (FWB) and those fermented in heaps (FH) presented no pronounced difference ($p<0.05$) and were less acidic than beans fermented in plastic box (FPB), which recorded pH 4.75.

The pH values of beans fermented during 5 days in FWBwt and in FHwt were more acidic with respectively 5.16 and 5.04 than beans fermented in FPBwt, which recorded pH 5.44. When two turnings were done, beans resulted from FPB became extremely acidic with ph 4,73 than beans fermented in FWB which became less acidic with pH 5.34 and acidity in beans sourced from FH tended to disappear entirely; the pH value reached 6.59.

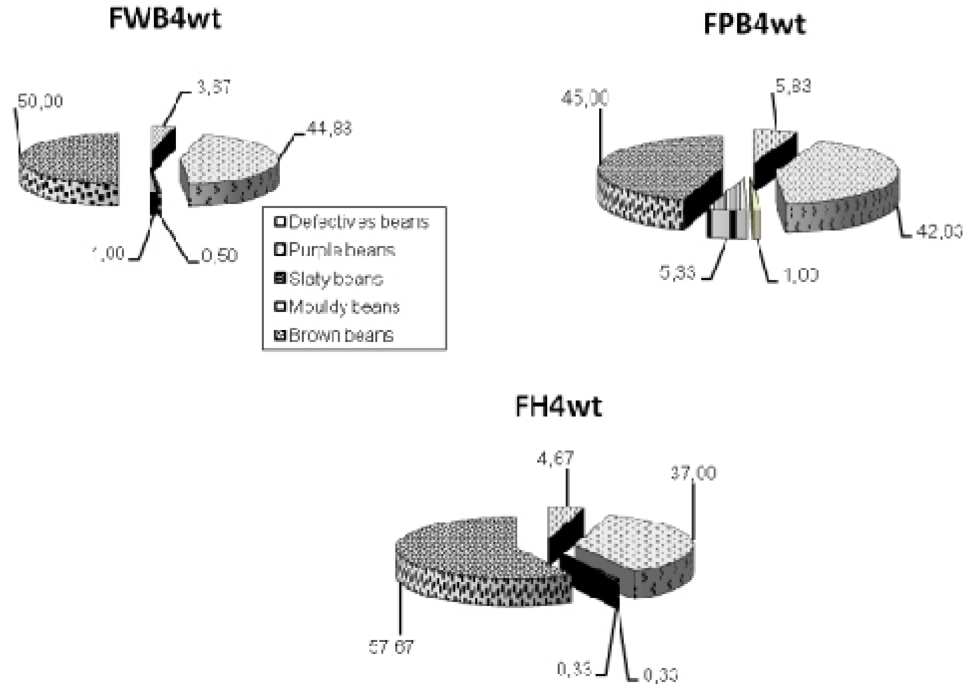
A similar trend was observed in the titratable acidity of the dried beans. Overall results showed that changes in TA, as a better indicator, follow a decreasing of pH trend for all fermentation treatments. TA ranged from 1.06 to 2.25 meq of $NaoH.10^{-1}g$ for fermentation lasted 4 days without turning while for fermentation conducted with turning beans; TA was higher with values comprised between 1.38 and 3.07 meq of NaoH.10^{-1}g. For fermentation lasted 5 days, the TA of resulted raw cocoa is similar and reached 1.19 meq of NaoH.10^{-1}g whatever the method of fermentation without turning while for the fermentation conducted with turnings, raw cocoa resulted from plastic box presented higher TA with 2.36 meq of NaoH.10^{-1}g than cocoa beans fermented in wooden box which showed TA equal 1.31 meq of NaoH.10^{-1}g and cocoa beans resulted which recorded TA equal 0.80 meq of NaoH.10^{-1}g.

Effect of turning beans and fermentation methods lasted 4 days on physical quality characteristics of dried beans: Figure 1a shows the changes in physical quality characteristics evaluated using cut test of cocoa beans on different fermentation methods lasted 4 days without turnings. Fermented cocoa beans resulted from all studied methods of fermentation presented a high percentage of purple beans nearly 40% and a low percentage of brown beans below 60%. Percentages of defective, slaty and mouldy beans were below 4%. As shown in Fig. 1b, cocoa beans fermented during the same duration but with 2 turnings of beans after 48 and 96 hours of fermentation recorded about 10% of defectives beans with no pronounced difference ($p<0.05$). Percentages of slaty and mouldy beans were below 4%. While purple beans percentage reached about 12% for cocoa fermented in wooden box, cocoa fermented in heaps contained 28% and those resulted from fermentation in plastic recorded no change (45%). Naturally, percentage of brown beans reached 77 and 62% respectively in cocoa beans fermented both in wooden box and in heaps. All the beans showed no sign insect damages, and moulds internal infestations whatever the turnings of beans and the methods of fermentation.

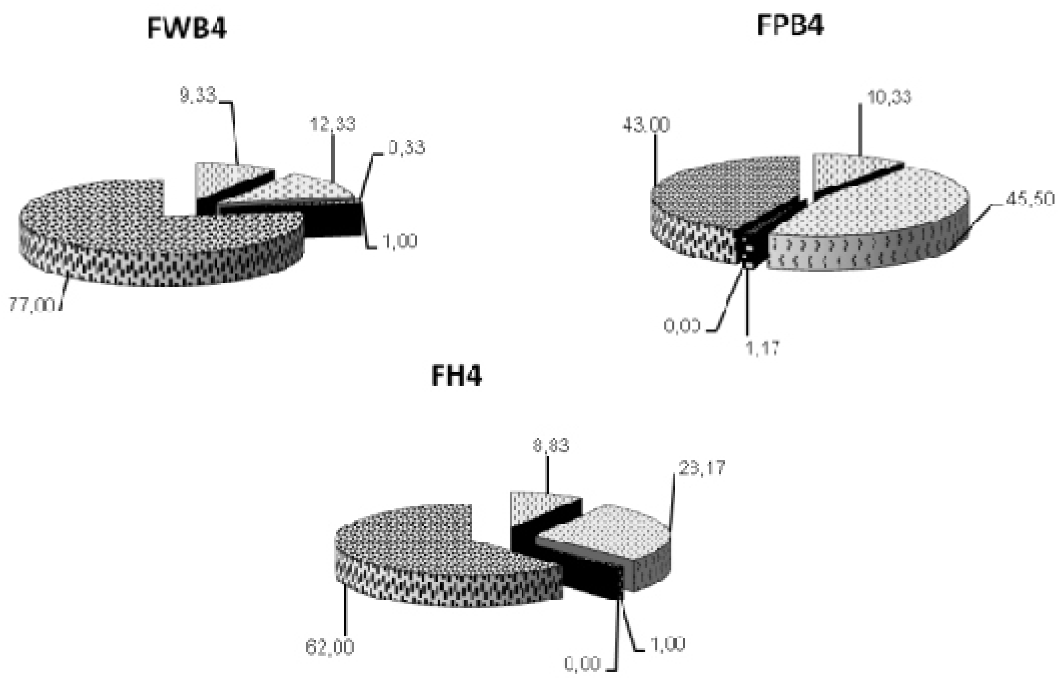
Effect of turnings of beans on physical quality properties of dried beans resulted from each fermentation method lasted 5 days: The influence of turnings of beans and fermentation methods lasted 5 days on physical quality of cocoa beans is shown in Fig. 2. In Fig. 2a cocoa beans presented around 34, 39 and 42% of purple beans after fermentation conducted respectively in heaps, wooden box and in plastic box. Only cocoa beans fermented in wooden box recorded high percentage of defective beans up 4% while raw cocoa beans resulted from both fermentations in plastic box and in heaps showed low percentage of beans below 4%. The few beans showed signs of internal mould whatever the method of fermentation and the percentage of slaty beans are very negligible. Cocoa beans treated in heaps showed higher percentage of brown beans with 62% than both cocoa beans fermented in boxes, which presented no pronounced difference in percentage of brown beans below 60%. Figure 2b indicates that after fermentation with 2 turnings of cocoa beans issued from plastic box recorded highest percentage of purple beans with 33% while raw cocoa resulted from both fermentations in heaps and in plastic box showed negligible percentage about 2%. All raw cocoa beans presented no pronounced difference ($p<0.05$) in the percentage of defective beans (8%) whatever the method of fermentation conducted with turnings of beans. Raw cocoa resulted from both fermentations in biological tools recorded 89% while fermentation in plastic box led to produce raw cocoa with slight percentage (57%) of brown beans.

DISCUSSION

Our results on acidity of raw cocoa beans are similar to those obtained by Biehl *et al.* (1990), which showed that variations in the conditions during fermentation such as duration affect the acidity of cocoa beans. The value of pH of our raw cocoa beans was found to be greater than the standard Malaysian estate beans, which is 4.4-4.7 (Nazaruddin *et al.*, 2006). The acidity of the beans obtained in this study were also slightly better than those reported by Bonaparte *et al.* (1998) after solar dried beans of pH in the range 4.78-4.81 and titratable acidity in the range of 22.38-23.03 meq NaOH 100^{-1} g. Overall results showed that changes in TA, which is a better indicator, follow a decreasing of pH trend for all fermentation treatments. Titratable acidity is a better measure of the total acids in cocoa liquor than pH, and both parameters have been correlated with taste scores or flavour acidity (Chong *et al.*, 1978; Duncan *et al.*, 1989). The fact that the pH is not significant while the TA is significant is not very clear (Bonaparte *et al.*, 1998). The pH of beans resulted from fermentation in boxes without stirring were less acidic than the pH of beans fermented in heaps due to the great production of lactic acid during fermentation in heaps. Indeed, Camu *et al.*, (2007) have observed a maximal growth of lactic acid acetic bacteria which produced respectively lactic acid and acetic acid during fermentation in heaps without mixing in cocoa chain of Ghana. In contrary to acetic acid which volatilized by the solar drying, lactic acid which is unfavourable for the quality of raw cocoa beans because it remained in to the beans due to its low volatile property (Barel, 1997). Lower acidity in beans fermented both in wooden box and in heaps is due to the greater aeration of the mass due to the turnings and the disappearance of the mucilage enable acetic acid bacteria to grow and intervene (Barel, 1997; Camu *et al.*, 2007). By oxidation, they convert the ethanol initially produced by the yeasts during alcoholic fermentation into acetic acid (Jinap, 1994) and this acetic acid produced is very volatilized during solar drying. However, during fermentation in plastic box, higher

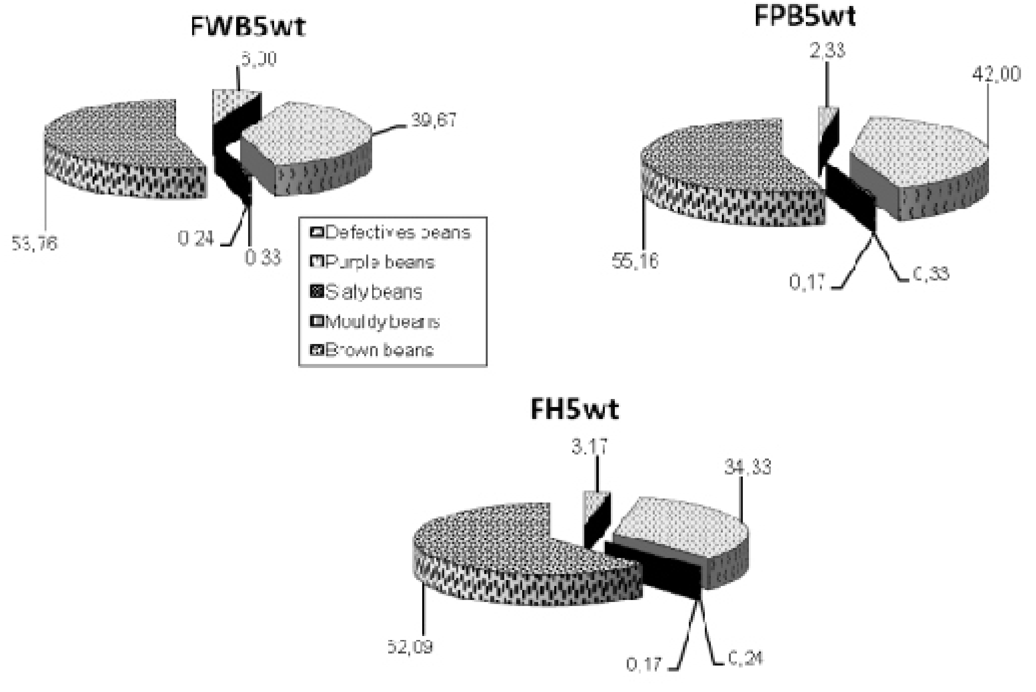


a) Fermentation conducted without turning

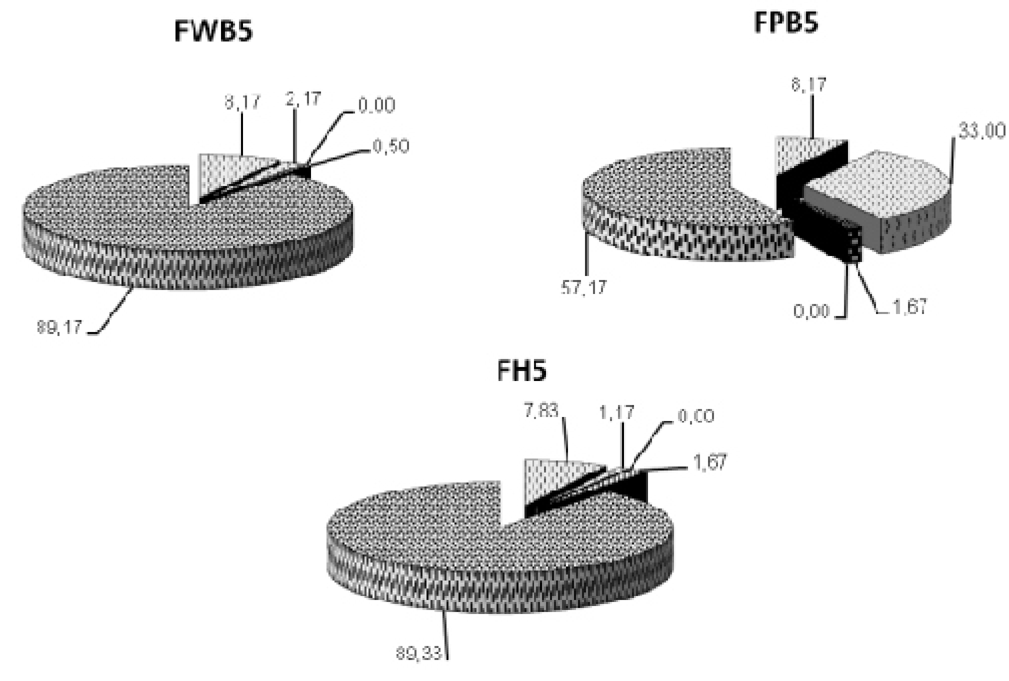


b) Fermentation conducted with 2 turnings

Fig. 1: Distribution of physical quality characteristics of dried beans resulted from each fermentation method lasted 4 days. FWB4wt: Fermentation in Wooden Box during 4 days without turning; FPB4wt: Fermentation in Plastic Box during 4 days without turning; FH4wt: Fermentation in Heaps during 4 days without turning, FWB4: Fermentation in Wooden Box during 4 days with 2 turnings, FPB4: Fermentation in Plastic Box during 4 days with 2 turnings, FH4: Fermentation in Heaps during 4 days with 2 turnings



a) Fermentation conducted without turning



b) Fermentation conducted with 2 turnings

Fig. 2: Distribution of physical quality characteristics of dried beans resulted from each fermentation method lasted 5 days. FWB5wt: Fermentation in Wooden Box during 5 days without turning; FPB5wt: Fermentation in Plastic Box during 5 days without turning; FH5wt: Fermentation in Heaps during 5 days without turning, FWB5: Fermentation in Wooden Box during 5 days with 2 turnings, FPB5: Fermentation in Plastic Box during 5 days with 2 turnings, FH5: Fermentation in Heaps during 5 days with 2 turnings

acidity might be due to the bad aeration of cocoa beans mass created by the tight closing of plastic box In general, beans fermented with turnings were less acidic than beans fermented without stirring except fermentation in boxes. Beans fermented in heaps with 2 turnings during longer time were less acidic than beans resulted from both other fermentation methods. While, beans fermented in heaps during 4 days without turnings were extremely more acidic than those issued from fermentation in boxes. This high acidity content in cocoa beans fermented without turning beans might be due to the production of lactic acid increased by anaerobic conditions. Indeed, the resulting anaerobic environment from the growth of yeasts and production of ethanol and organic acids (Schwan, 1998) favor the growth of lactic acid bacteria from 16 to 48 h of fermentation while the numbers of yeast are declining (Schwan *et al.*, 1995). The existence of lactic fermentation is not an exceptional phenomenon (Lagunes-Gálvez *et al.*, 2007) because its production is therefore often found during cocoa fermentation particularly when fermentation was conducted without aeration (Passos *et al.*, 1984). As lactic acid is not volatile, it remains into the beans and increased the acidity of raw cocoa.

High percentage of purple beans in cocoa beans resulted from all fermentation methods lasted 4 days studied might be due to the short length of fermentation and the absence of turning beans. Indeed, as shown by Biehl *et al.* (1990), variations in the condition during fermentation such as duration will affect the pH, titrable acidity and temperature during fermentation, thus influence the microbial enzymes activities. Indeed, after 48 hours of cocoa spontaneous fermentation, yeasts population was reduced and the anaerobic conditions created favour the environment suitable for lactic acid bacteria. Turning beans induced aerobic conditions become aerobic leading to the growth of acetic acid bacteria (Thompson *et al.*, 2001). The resulted reactions of oxidation of ethanol into acetic acid causes a rise in temperature up to 51ºC which activated the enzymatic reactions such oxidation of catechins and leucocyanidins in cocoa beans (Griffiths, 1957). As oxidation of polyphenols during the aerobic phase of cocoa fermentation is largely responsible for the characteristic brown colour of fermented cocoa beans (Thompson *et al.*, 2001), the percentage of brown beans increase with the aeration induced by turning beans. As shown, ethanol oxidation into acetic acid and oxidation of polyphenol compounds led to the reduction of purple beans and the increase of brown beans percentages. The few beans with signs of internal mould indicated an association with pre-fermentation defects and their mouldiness may have occurred before drying. Indeed, the rates of drying were sufficiently rapid to prevent internal moulding. These results imply that the cut test could be used to assess the degree of fermentation and hence the flavour potential of the beans as previously done (Shamsuddin and Dimick, 1986, Guehi *et al.*, 2008). No cocoa samples resulted from all fermentation methods were infested by insect. This result could be due the immediately study and analyses without storage. Indeed most of the insects, which cause damages to the cocoa beans grew during the storage step.

CONCLUSION

Methods of fermentation and turning beans determine the quality especially both chemical and physical quality of raw cocoa. Among three cocoa fermentation methods performed during this study, fermentation in heaps appeared to be better for the production of a good quality raw cocoa. Also greater aeration of the mass due to the disappearance of the mucilage, the growth of acetic acid bacteria and particularly induced by turning beans after 48 and 96 h of fermentation enables to add considerable value on the physical quality of raw cocoa material. However, the duration of cocoa fermentation may have to be controlled and could be reached average 5 days to more improve the quality of raw cocoa material and to produce best quality chocolate. Further investigations could be carried out on the influence of the duration of different methods of fermentation on the changes in the quality of raw cocoa.

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