Review Article

Free Fatty Acids and cocoa butter quality traits: causes of generation in cocoa beans, assay methods, means for lower contents and effects on the chocolate consumers health.

ABSTRACT

Cocoa butter (CB) technological quality and financial value depend widely on the Free fatty acids (FFA) content. This is why the legal Free fatty acids content was fixed at 1.75 % in BC by EU Directive 2000/36/EC. Free fatty acids are carboxylic acids, generated from triglycerides via the hydrolysis of their ester bounds by enzymatic and/or chemical reactions. This review dealt about on the Free fatty acids formation mechanisms, the Free fatty acids levels assay and reduction methods. Furthermore, the impact of high Free fatty acids content on the technological and chemical qualities and on the health of consumer of cocoa derived products were described. The results highlighted that the Free fatty acids formation in cocoa beans was ascribed to the endogenous and exogenous lipases activities. Excreted exogenous lipases by contaminated molds during poor post-harvest processing are most active for Free fatty acids generation in cocoa beans. In addition, the germination, poor storage conditions such as high water activity and moisture content provoke high Free fatty acids content in cocoa butter. Although alkalization, deodorization and other refining treatments are efficient methods for reduction of Free fatty acids content, chocolate manufacturers recorded high losses in cocoa butter. Excessive consumption of diets enriched of saturated fatty acids induce increases in both low-density lipoprotein (LDL) cholesterol level and atherosclerotic lesions.

Keywords: Cocoa butter, free fatty acids, lipase enzymes, post-harvest processing, biological control.

1. INTRODUCTION

Fermented and dried cocoa is produced from the fresh seeds extracted from the ripe fruits called pods of the Theobroma cacao plant. The industrial processing of raw cocoa beans results in three main products such as cocoa liquor, cocoa powder and cocoa butter. Cocoa butter (CB) is one the most important ingredients produced during the industrial transformation of fermented and dried cocoa beans. Economically, CB is the most expensive cocoa derived product. The fat component of chocolate constitutes the continuous phase affecting variable good and interesting rheological, physical and chemical characteristics during processing [1], storage and consumption of the final product. The content of cocoa butter and the nature of fatty acid in chocolate depend on the growth conditions of the cocoa trees. The relative proportion of saturated triacylglycerols (TG) compounds determines the rheological properties including hardness, crystallization and melting of CB. These specific and unique characteristics of CB are very useful in the manufacture of cosmetic, pharmaceutical and chocolate industries. Chocolate is a complex suspension of cocoa powder and other ingredients in the continuous lipid phase constituted mostly and currently by cocoa butter [2]. During the production of cocoa butter, its crystallization properties play an important role in the final quality of the product. The desired luster and the consumers' appreciation of the "crack" of chocolate require perfect crystallization and high-quality CB [3]. However, CB could differ in TG composition and content of minor components due to various factors such as the environmental conditions (geographical region), the level of ripeness of cocoa pods, the post-harvest treatments in farms and the microbiological aspects. The crystallization of CB could be affected by the minor concentration of specific components, including monoglycerides (MG), diglycerides (DG), free fatty acids (FFA), phospholipids, hydrocarbons, sterols and sterol esters, tocopherols and many others [4]. This crystallization and melting range behavior are linked directly to TGs composition of CB. Nevertheless, fats with the same TG profile show differences in their crystallization kinetics, in their equilibrium of solid fat content (SFC) and microstructure. These differences could be ascribed to the minor component profiles of CB [5]. Recently, the effects of FFA content on fat have been reviewed, however the focus was exclusively on either FFA content reduction or their effects on the crystallization kinetics of CB [3]. Jacobsberg and Ho [6] observed that an increase in FFA content in palm oil leads to lower SFC values and lower melting points caused by eutectic formation. Pontillon [7] found that FFA concentration above 2% prolonged the CB crystallization time. In addition, human excessive consumption of dietary saturated fatty acids could increase plasma cholesterol levels and more particularly low-density lipoprotein (LDL) cholesterol level and the atherosclerotic lesions risk development [8]. Many studies have shown that palmitic acid is relatively the most hyper cholesterolemic [9] in case of excess intake [10] in contrast to stearic acid [11] which is converted to oleic acid by desaturation [12]. Physical and chemical methods are currently used for the reduction and removing FFA content in CB by alkaline neutralization, deodorization by distillation and other refining treatments. In addition, it has been shown that accumulation of soaps in un-deodorized, alkalized CB is correlated with decreased crystallization rates as well as longer induction times during isothermal crystallization [13]. Fat migration at the surface of stored chocolate products is a major physical quality default affecting the chocolate industry. The phenomenon is adversely manifested in products either as softening of coatings or fat bloom at the surface [14]. High FFA content could result from rotten pods, germinated beans, and some presence of molds [15]. Several researches have shown that high FFA contents in CB were ascribed to the lipolytic enzymes activities during fermentation resulting from poor post-harvest treatments [16]. The actions of endogenous lipases are due to the cocoa seed germination leading to the increase of lipoxygenase activity as phyto-response to the stress or catabolic action to lipolytic activities for production of energy to support the growth of plant [17]. Exogenous lipases activities could also come from various fungal contaminations namely molds [16], yeasts [18]. The objective of this review was to investigate literature to determine the causes of FFA occurrence in cocoa butter, their impact on the physico-chemical properties of cocoa derived products such chocolate and CB; finally on the consumer health.

2. WHAT ARE FREE FATTY ACIDS (FFA)?

Free fatty acids are carboxylic acids with a long carbon chain. They are resulted from triglycerides via hydrolysis through either lipase activity or oxidation due to lipoxygenase activity. Therefore, for each free fatty acids molecule formed there will be mono or di-glycerides created also in the same proportions. FFAs are naturally present in small proportion in CB. High Free fatty acids content of CB could be resulted from rotten pods, germinated cocoa seeds, and some lipolytic microorganisms such as molds [19]. Although, the risks of oxidation occurence in CB are very low due to its low unsaturated fatty acid content [20] and high content of polyphenols, other cocoa beans natural antioxidants [21]. The legal maximum limit of free fatty acids content in cocoa butter was set at 1.75 % EU Directive 2000/36/EC. Some Free fatty acids can be removed or reduced by deodorization and degumming but for cocoa butter containing high free fatty acids content other processing techniques were used to do it.

3. MECHANISMS OF FORMATION OF FREE FATTY ACIDS IN COCOA BUTTER

Free fatty acids are mainly derived from the hydrolysis of triglycerides in cocoa butter under the action of lipases enzymes. Lipases can be endogenous (naturally present in the cocoa seed) or exogenous (produced by the microorganisms). It is likely that high free fatty acids content measured in cocoa beans are due to the action of enzymes resulting from poor post-harvest practices. Some research concluded that the main formation of free fatty acids was due to endogenous or exogenous lipase activity during fermentation [22].

4. MAIN FACTORS INFLUENCING FREE FATTY ACIDS PRODUCTION IN COCOA BEANS

4.1. Intrinsic factors

According to Minifie [23], lipases activities in cocoa are responsible of free fatty acids content increase. These enzymes become active due to both the changes in moisture content of the fermented and dried cocoa beans and the increasing of temperature during the storage environment. Recently, Hu and Jacobsen [20] found that there is a low risk of cocoa butter oxidation due to their low content of free unsaturated acids. This assumed stability in terms of oxidation was confirmed during shelf life of cocoa butter but has not been proven during fermentation. Indeed, enzyme lipase is responsible of lipid break down in plants to produce the energy required for the embryo growth [24]. The research of Samsumaharto [25] related to the partial characterization of cocoa bean lipase of Theobroma cacao. L showed that the optimum temperature of partial purified cocoa lipase was comprised between 30 and 40°C and the optimum pH between 7.0 and 8.0. Permana et al. [26] dealt with the purified lipase of germinated cocoa beans reported that the optimum temperature of lipase activity was near of 38° C and the enzyme was stable at temperature ranged from 30 to 40° C. So, indigenous lipase of germinated cocoa bean was presented as an alkaline lipase having optimum pH9 and stable at pH7-10.

4.2. Exogenous factors

4.2.1. Cocoa bean post-harvest operations

The occurrence of free fatty acids in fermented and dried cocoa beans was ascribed to the activities of the endogenous and fungal lipases. Other factors such as over fermentation delay, storage time, level of water activity and cocoa bean germination were reported to favor lipase activity in fermented and dried cocoa beans [27]. Fungal diseases and infestations due to the insect actions constituted the great conditions for the molds development and growth [28], which in turn would lead to free fatty acids formation [15]. The cocoa beans extracted from the damaged pods (Figure 1A) stored for more than 10 days opening delay lead generally to Free fatty acids levels exceeding 1.75 % while those from healthy pods (Figure 1B) recorded lower Free fatty acids contents. This suggests that in order to obtain well-fermented cocoa beans with acceptable free fatty acids content, cocoa pods should not be stored for more than 10 days after harvesting [27] so as not to suffer from various ailments. Thus, both pod storage and fermented cocoa beans drying conditions influenced the souring or nib acidification process (pH and non-volatile acidity, flavor precursors development sugars degeneration and protein degradation) and free fatty acids levels in fermented cocoa [29]. High Free fatty acids content could result from diseased pods or due to lipases activities producing mold strain growth on the surface of cocoa beans and hydrolysis of cocoa butter. Fungal growth in cocoa beans is reported to be due to extended storage in poor conditions [30], prolonged fermentation, or inadequate drying process [31, 32] confirmed that cocoa beans fermentation duration seemed to have a critical effect on the increase of the chances for free fatty acids generation.

Fig 1. Fresh cocoa beans extracted from the damaged pods (A), Fresh cocoa beans extracted from healthy pods (B)

4.2.2. STORAGE CONDITIONS OF FERMENTED AND DRIED COCOA BEANS

According to Ewe [33], there was a great link between free fatty acids content, packaging materials and storage duration of fermented and dried cocoa beans. Wood and Lass [34] compared the Free fatty acids content of cocoa beans packaged in jute bags and stored for the period ranged between 30 and 120 days in glass or plastic bottle, polyethylene bags (Fig. 2A). The results of these research showed that cocoa beans stored in jute bags were all below the maximum acceptable limits of 1.75 %. However, all cocoa beans stored for the same period in glass or plastic bottles, polyethylene bags recorded high free fatty acids contents. This difference could be due to better aeration of the beans in the jute sacks and absence of molds contamination. Generally, beans kept in jute sacks and stored for 120 days recorded the most desirable quality product in terms of free fatty acids content (Fig. 2B). Most studies have shown that high moisture contents in fermented and dried cocoa beans could favor mold growth and development leading to the fungal infection [30] and changes in the organoleptic properties of raw cocoa [35]. Molds grew via fungal conidia germination on moist surfaces and under high humidity that are suitable for their life. However, low moisture content can limit the increase in free fatty acids due to the negligible fungal activities. According to Robert et al. [30], the accumulation of free fatty acids in raw cocoa beans could be mainly attributed to the presence and action of molds strains including Rhizopus oryzae, Absidia corymbifera and genus Aspergillus. Lagunes-Galvez et al. [18] reported the presence of lipolytic yeasts species such as Yarrowia lipolytica, which might cause also the increase or a change in the total lipid phase. However, high and increasing Free fatty acids contents found in defective raw cocoa beans were due probably to the microbial activities resulted from fungal diseases of cocoa pods. These molds strains could produce lipases activities which induced free fatty acids formation from triglycerides in broken cocoa nibs [15, 16, 18]. Insect damage to the stored fermented and dried cocoa beans resulted in penetration of molds in the cocoa pod and then in seeds. The significant increase in the free fatty acids content during cocoa beans storage suggests that infestation and damage of insects could be considered as the main factors causing the occurrence of free fatty acids in the cocoa butter. Several researches have reported that common storage insect pests of fermented and dried cocoa beans included Tribolium castaneum (Herbst), Cryptolestes ferrugineus (Stephens), E. cautella, Lasioderma serricorne and Araecerus fasciculatus [36, 37]. Jonfia-Essien and Navarro [38] highlighted the role of iinsects the hydrolysis of the triglycerides of cocoa butter leading to the formation of high free fatty acids content in the fermented and dried cocoa beans stored in poor conditions. The increase in the concentration of free fatty acids therefore has a direct impact on the fat content and causes negative changes in cocoa flavor [39]. It was why the EU regulation 2000/36/EC and Codex STAN (86-1981) limited the maximum free fatty acids content to 1.75% oleic acid equivalent in cocoa butter. Strong increasing of Free fatty acids contents from 6.23 % to 11 % were observed in poor quality fermented and dried cocoa beans such as clustered beans. In addition to their pronounced influence on cocoa butter crystallization, high free fatty acids content could result in a rancid off-flavor [40].

Fig. 2. Poor storage conditions of fermented and dried cocoa beans bags (A), Good storage conditions cocoa beans (B)

5. ANALYTICAL METHODS FOR THE FREE FATTY ACIDS QUANTIFICATION

The Free fatty acids extracted from cocoa butter were determined using ISO 660:2020 method. Usually, five grams of extracted cocoa butter are weighed (W) and dissolved in 50 mL of petroleum ether/absolute ethanol mixture (1:1; v/v) neutralized by adding KOH (0.1, M) in the presence of phenolphthalein. The mixture was titrated against 0.1 M sodium hydroxide or potassium hydroxide in ethanol solution and the end-point taken and recorded for the free fatty acids calculation. Some studies used other solvents to dissolve cocoa butter for the determination of free fatty acids. Ewe [33] studied the effect of packaging materials and storage periods on the quality of reference cocoa beans using the mixture of diethyl ether / alcohol 95 % (1:1 v/v) whereas Gawel [22] used absolute ethanol as a dissolving solvent for cocoa butter in his work, which focused on cocoa butter & starch inclusion complexes. Free fatty acids content could be measured also using different chromatography techniques. Lau et al. [41] used GPC-TLC to assess free fatty acids content of palm oil. Other authors have determined the free fatty acids content using only TLC technique through lipase reactions on acqueous plant extracts as lipase enzyme sources such as peanut, shea, jatropha, balanite and moringa seeds [42].

6. SATURATED FREE FATTY ACIDS AND HUMAN HEALTH

Bounded to glycerol or free saturated fatty acids such as lauric, myristic, and palmitic acids raise total and LDL cholesterol concentrations. There is some evidence that these same fatty acids increase coagulation, inflammation, and insulin resistance phenomena. In accordance with these effects, a high exposure to all saturated fatty acids and particularly to lauric, myristic, and palmitic acids is associated with higher risk of coronary heart disease (CHD), cardiovascular disease (CVD), and type 2 diabetes. The milk fat–derived odd-chain saturated fatty acids (pentadecanoic and heptadecanoic) are associated with lower risk of type 2 diabetes, CHD, and CVD. It becomes evident when considering these findings that not all saturated fatty acids have the same effects on human health, most likely because each type of saturated fatty acid has unique effects on cells and tissues. Hence, general statements about “health effects of saturated fatty acids” seem inappropriate, and a stronger focus on fatty acid–specific health effects is needed, at least within the scientific and medical community [43]. However, according to Visioli and Poli [44], saturated fatty acids (SFA) are very heterogeneous and their effects most depend on the food matrix and the proportion of other fatty acids classes present in the diet. The debate is still going on whereas International scientific societies recommend keeping their intake below 10 %. However, the consensus that saturated fat consumption must be limited [45] is progressively losing [46]. At least two meta-analyses reported no relation between saturated fat intake and overall mortality [47]. The critical issue is that an unrestricted reduction of saturated fats leads to the ban of otherwise healthful food, such as dairy foods. Additionally, the recent paper from the Global Burden of Disease group does not include saturated fats among the first 15 most important dietary determinants of human death [48]. So the pathologies caused by the exceed consummation of poor cocoa derived products could be avoided by the reduction of free fatty acids content in cocoa butter used for their formulation. In addition, the labels of cocoa-derived products such as chocolates must clearly indicate the free fatty acids content of the cocoa butter used.

7. FREE FATTY ACIDS CONTENT AND THE QUALITY OF COCOA BUTTER

Solid fat content (SFC) of cocoa butter is a very important attribute for the chocolate manufacturer. Hence, total fat solids help to increase heat stability and hardness; and to delay onset of age-related fat bloom during the storage of chocolate [5]. Pontillon [7] showed that free fatty acids content affected crystallization kinetics and SFC of cocoa butter. Specifically, this research found that a free fatty acids content above 2 % strongly delayed the cocoa butter crystallization time. According to Ayala et al. [49], SFC at various temperatures such as 20, 25 and 30°C increased inversely with decreasing free fatty acids content. Deodorization of naturel cocoa butter derived from cocoa beans sourced from Côte d’Ivoire lead to reduce free fatty acids content from 2.01 to 0.96 % at 160°C and to 0.07 % at 200°C. This treatment helped to increase crystallization kinetics and equilibrium solid fat contents between 20 and 30°C [50]. Several studies showed the strong linear correlation between SFC and FFA content measured at these temperatures [3]. However, Smith et al. [51] explained that there was no clear definition of a minor component concentration. Depending on the mechanism by which a given minor component acts, it could help to determine it concentration affecting the crystallization of cocoa butter (Fig. 3). Many minor components may also hinder crystal growth by blocking sites on crystal surfaces for further growth. Delayed crystallization noticed in the cocoa derived-products containing high free fatty acids content would favor larger crystals and greater ability for liquid fat to migrate at the surface creating potential bloom formation during products storage [5]. In contrary, low content of Free fatty acids positively influenced the crystallization kinetics and the formation of the crystal network, resulting in differences on a macroscopic scale [52]. That helped to increase crystallization kinetics and equilibrium SFC between 20 and 30°C. The SFC profile of cocoa butter indicates its melting behavior at different temperatures and influences the heat stability, consistency and sensorial qualities of chocolate [53]. Removal of Free fatty acids is easy as is shown by deodorization of cocoa butter but DG levels remain unchanged following such low-pressure treatment. While there is a few information on the effect of intrinsic ratio MG/DG on the crystallization of cocoa butter, higher FFA content was correlated intimately with long crystallization induction time, low crystallization rate and low SFC [49]. Furthermore, Metin [54] showed that lower-melting fractions contained higher contents of DG such as 1,2-DG produced by 1,3 specific lipases that are more common in the nature than 2 specific or non-specific lipases. For example, DG may either promote or delay crystallization depending on composition and free fatty acids content of CB [55]. Foubert et al. [40] reported that the cocoa butter containing higher levels of DG crystallized at a slower rate, prolonged the solidification of fat whereas Hernqvist and Anjou [56] showed that the crystal size growth was retarded in the presence of DG. As cocoa butter is one of the most expensive edible fats, oftentimes-refining oil losses could cause significant financial loss to cocoa butter and the chocolate manufacturers [5].

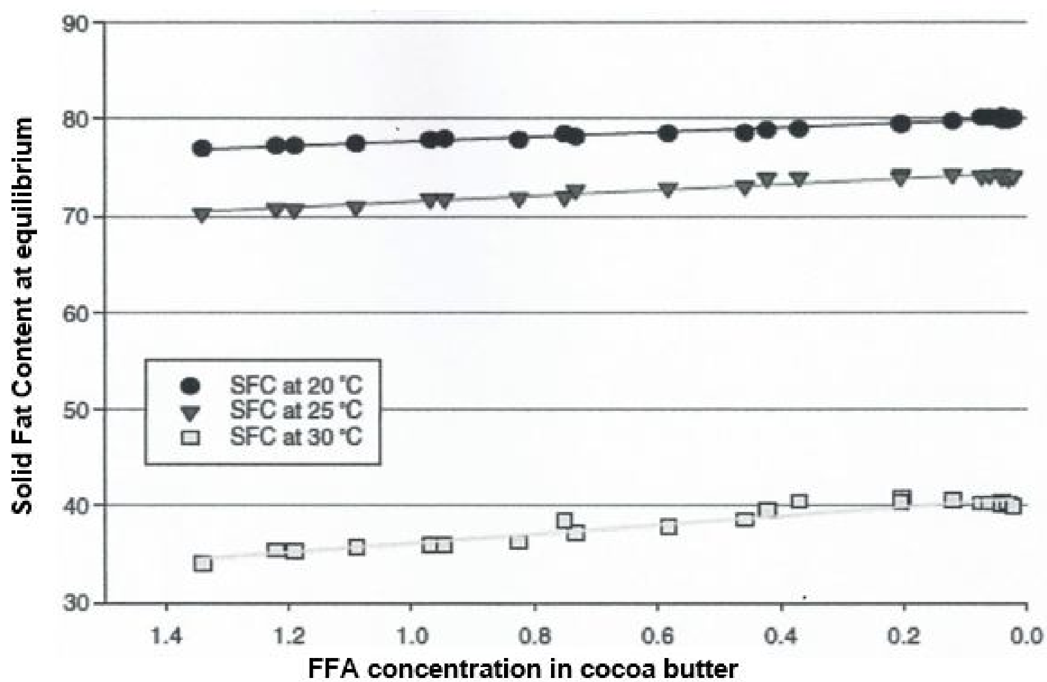

Fig 3. Solid fat content of cocoa butter, as a function of free fatty acids. Calliauw et al. [57]

8. FREE FATTY ACIDS CONTENT AND THE STRUCTURE OF COCOA BUTTER

Microstructure crystal network is also an important attribute when assessing quality of cocoa butter. It was reported that chocolate manufacturers desire a network with robust nucleation and fine well-packed crystals. The type of network would help to create a finished chocolate with high solids that would have good shine, strong snap and would help to inhibit fat bloom bloom [58]. Less spherical nuclei and crystals were found most in liquid fat in cocoa butter samples with higher contents of free fatty acids. Dewettinck et al. [59] have reported that free fatty acids form possibly eutectics in cocoa butter, and that 20°C isotherm may result in a deeper sub cooling for the deodorized samples. Also, the free fatty acids are incorporated into the crystal lattice, as they note an increased intensity and consistently more compact long spacing for α crystals in lower free fatty acids cocoa butter. So initial crystallization from α to β′ form held more quickly as more removing of free fatty acids. Induction times of the β′ form event generally decreased with lower free fatty acids content. This proves that removal of free fatty acids helps to reduce molecules that interfere in the reorganization of cocoa butter crystallization [57]. Fat bloom, defined as the appearance of white spot on the surface of the chocolate, is the primary defect affected chocolate and chocolate products (Fig. 4) [60]. It causes financial losses for chocolate manufacturer [61]. This physical default is only superficial, but it makes the chocolate less appealing for the consumers [62]. Fat bloom has three main sources: the composition, the processing, and the storage conditions of chocolates [63]. Abusive storage temperatures could cause the solid-state transformation of cocoa butter from the βV to βVI-crystallized forms (3-5) [64]. Foreign fats with lower SFC than cocoa butter could interfere with the formation of the stable βV form during tempering leading to the bloom development acceleration. Adenier et al. [65] argued that the liquid TG phase at ambient temperature due to its relative importance and quantitative fluctuation is the main mechanism of bloom occurrence. Schlichter-Aronhime and Garti [66] suspected that the passage of cocoa butter from the βV to βVI forms caused fat bloom. During the storage, elevation and fluctuation of temperatures increase the rate of transformation [67].

Fig 4. Freshly produced (left) and heavily fat bloomed chocolate pralines. Dahlenborg [60].

9. METHODS FOR CONTROL GENERATION OF FREE FATTY ACIDS IN COCOA BUTTER

9.1. Agricultural methods at the farm level

Several post-harvest treatments were reported to degrade the quality of cocoa beans. Different studies mentioned that some post-harvest treatments leading to the high free fatty acids content in cocoa butter. Oyewo and Amo [68] have shown that poor storage management of cocoa beans resulted in mouldy infestation leading to an increase in free fatty acids levels in cocoa butter. Two methods are currently applied to reach low free fatty acids contents in the fermented and dried cocoa beans: efficient drying of the cocoa beans, cool storage of well-dried cocoa beans at low temperature (4°C) [69]. Also, hermetic storage is successful method for the preservation of quality parameters of fermented and dried cocoa beans from insect development control [70]. Guéhi *et al*. [15] showed that free fatty acids content increased strongly during the storage of broken cocoa beans regardless of whole cocoa beans. So hermetic cocoa beans storage inhibited insect and microbial infestation. Hence, the contents of free fatty acids generated under microflora and insects development were low [71].

9.2. Methods for Free fatty acids content reduction in cocoa butter

Various physical and chemical methods such as alkalization, deodorization and other refining processes were set up for removal or reduction of free fatty acids content in cocoa butter. In the alkalization process, the nibs are treated with an alkaline solution such potassium carbonate, at fixed temperature and pressure [49]. This process affects both the flavor and the color of cocoa powders [72]. Simultaneously the Free fatty acids may be transform into soap [49]. The deodorization process of cocoa butter is quite typical to reduce both the Free fatty acids content from 2.01 to 0.07 % [50] and flavor compounds such as esters, aromatics, pyrazines, pyrroles, carbonyls, aldehydes, and ketones via distillation [50] using vacuum steam distillation. Deodorization of cocoa butter could be a batch, semi-batch or continuous process [66]. However, deodorization process needs to be done care because it could lead to darkening of the oil due to the phospholipid content, the intra or inter-esterification of triglycerides, the creation of trans fatty acids, the polymerization reactions and to the loss of desirable volatile compounds of cocoa butter [5]. Other refining processes used in addition to deodorization are bleaching, degumming, caustic neutralization, fractionation, hydrogenation, and inter-esterification. Gordon and Rahman [74] showed that bleaching and deodorizing strongly reduces the induction times of coconut oil crystallization. Bleaching removes phospholipids and polar components such as glycolipids, phosphatides, proteinaceous, and mucilaginous substances [75]. The degumming process reduces phosphatides, waxes, free fatty acids, and other impurities content by mixing cocoa butter with water, salt solution, dilute acids, or alkalis [76].

10. CONCLUSION

Genotype, climatic and agricultural conditions of cocoa plants, physiological factor such as germination, geographical origins, practices of post-harvest treatments of cocoa beans on the farm influence strongly the generation of Free fatty acids in cocoa beans and influence both cocoa butter composition and quality. Free fatty acids content above 1.75% cause various problems such as chemical, technological and rheological during the chocolate crystallization process. Different methods were developed for reduction or removing free fatty acids content in cocoa butter leading important financial losses for the cocoa industry. High content of SFA in the diet could cause metabolic damage to the health of all cocoa product consumers. In order to avoid these situations, several physical and chemical methods were used to limit the Free fatty acids formation from the hydrolysis of triglycerides and measure easily and quickly of Free fatty acids content in cocoa beans. As fungi development play an important role in the free fatty acids formation in cocoa beans, mechanical and biological methods could implemented in order to limit fungal contamination.

For example, use of antagonist bacteria such as *Bacillus* sp. strains or yeasts against lipolytic fungi on cocoa beans during improved post-harvest processing.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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