Roadmap of cocoa quality and authenticity control in the industry: a review of conventional and alternative methods

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Abstract

Cocoa (*Theobroma cacao* L.) and its derivatives are appreciated for their aroma, color and healthy properties, and are commodities of high economic value worldwide. Wide ranges of conventional methods have been used for years to guarantee cocoa quality. Recently however, demand for global cocoa and the requirements of sensory, functional and safety cocoa attributes have changed. On the one hand, society and health authorities are increasingly demanding new more accurate quality control tests, including not only the analysis of physico-chemical and sensory parameters, but also determinations of functional compounds and contaminants (some of which come in trace quantities). On the other hand, increased production forces industries to seek quality control techniques based on fast, non-destructive online methods. Finally, an increase in global cocoa demand and a consequent rise in prices can lead to future cases of fraud. For this reason, new analytes, technologies and ways to analyze data are being researched, developed and implemented into research or quality laboratories to control cocoa quality and authenticity. The main advances made in destructive techniques focus on developing new and more sensitive methods such as chromatographic analysis to detect metabolites and contaminants in trace quantities. These methods are used to: assess cocoa quality; study new functional properties; control cocoa authenticity; or detect frequent emerging frauds. Regarding non-destructive methods, spectroscopy is the most explored technique, which is conducted within the near infrared range, and also within the medium infrared range to a lesser extent. It is applied mainly in the postharvest stage of cocoa beans to analyze different biochemical parameters or to assess the authenticity of cocoa and its derivatives.

Keywords: Cocoa quality roadmap, Chemometris, authenticity control, non-destructive methods, multivariate analysis.

Practical Application:

Recent trends in the cocoa sector (increased quantity and quality demands, new technical specifications, emerging functional properties, global food quality control trends, such as fast, non-destructive online methods) mean that the cocoa industry has new analysis requirements. This work aims to guide researchers and quality control technicians to the possibilities available today to control cocoa quality and authenticity in the fastest most reliable way to make cocoa production more efficient, safe, fast and innovative.

Introduction

Cocoa (*Theobroma cacao* L.) is a commodity of high economic value worldwide. Most of its production comes from West African countries (mainly the Ivory Coast and Ghana, which account for approximately 60% of the world's total cocoa), but is usually processed in the European Union (1.3 million tons or 40% of the global processing market in 2014). Apart from the European Union, cocoa beans are also processed in Indonesia, United States and Brazil in significant quantities (CBI, 2016; Shavez, Ahmad, Jan, & Bashir, 2017; ICCO, 2019).

In the different producing areas, three main distinct varieties are produced. The most ancient and most appreciated chocolate manufacturer variety is called Criollo (which means native), and is that traditionally cultivated by the Aztecs and Mayans in Central and South America. Later a new variety that better resists diseases and pests, called Forastero (meaning foreign), was taken from Amazon regions to other cocoa-growing areas in Latin America, and was exported to other West Africa and East Asia countries. Finally, in order to combine the advantages of Forastero and the appreciated fine flavor of Criollo, a new hybrid variety was harvested, known as Trinitario. Besides these varieties, the Nacional variety, which is generally considered native to Ecuador, is receiving more attention in the cocoa market for its sensory properties (Crouzillat et al., 2000). Each variety has specific sensorial characteristics that are related to its origin, environmental conditions and fermentation (Chetschik et al., 2018; Loullis & Pinakoulaki, 2018). Forastero is considered a bulk variety, while Criollo, Trinitario and Nacional are considered fine varieties. Bulk cocoas usually possess strong harsh flavors, while fine cocoas are perceived as being more aromatic or smoother (Counet et al., 2004). Growing conditions and postharvest practices can condition the final features of cocoa pods and, thus, of cocoa products (ADM Cocoa Manual, 2006). Therefore, knowing the variety and geographical indication of the cocoa beans used as raw material to produce different cocoa products is becoming increasingly more important as it can condition the final quality and hence, cocoa prices.

Regardless of cocoa variety, cocoa beans are subjected to different postharvest and industrial processes to obtain distinct cocoa products (Di Mattia *et al*., 2014, Aprotosoaie, Luca, & Miron,

2016). The first steps include cocoa bean fermentation and drying (Suazo, Davidov-Pardo, & Arozarena, 2014). Next, fermented and dried cocoa beans undergo several industrial processes. Bean shelling provides nibs and the first subproduct: shells (Tan & Kerr, 2018). Nibs can be roasted and milled to obtain cocoa liquor (Ioannone *et al*., 2015). When cocoa liquor is pressed, two products are obtained: cocoa butter and cocoa cake (Oliviero, Capuano, Ca, & Fogliano, 2009). Finally, cocoa cake undergoes another milling step to provide cocoa powder. Optionally, another important step to develop color and flavor, called alkalization or dutching process, can be performed in different cocoa products: cocoa nibs, cocoa cake or cocoa powder (Pérez-Esteve, Lerma-García, Fuentes, Palomares, & Barat, 2016). Alkalization is normally carried out by adding sodium or potassium carbonate at high temperature and controlled pressure. According to the final pH, cocoa powders can be classified into natural (pH 5-6), light-alkalized (pH 6-7.2), medium-alkalized (pH 7.2-7.6) and strong-alkalized powders (pH > 7.6) (Miller *et al*., 2008). Light-alkalized cocoa powders are light brown, but darker than natural ones, and their flavor is less astringent and less acidic than those of natural powders. Strong-alkalized cocoa powders are very dark and have a much stronger flavor than medium-alkalized ones (Kostic, 1997). A summary of all these processes is shown in Figure 1.

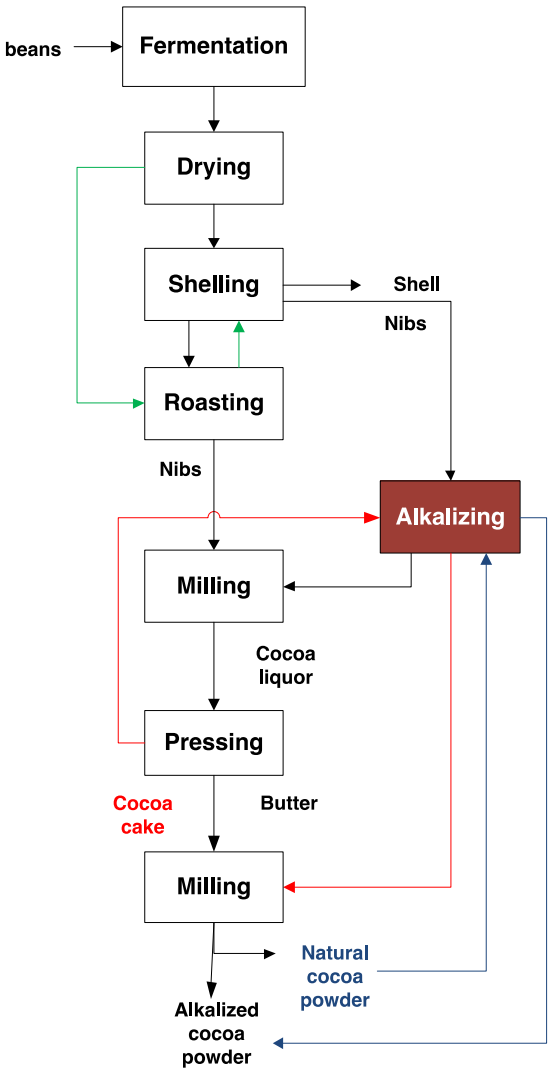

Fig 1. Cocoa and derivatives flow processing chart. Alkalization ways: black (nibs), red (cocoa cake), natural cocoa powder (blue).

If cocoa bean quality is poor, the quality of the final products will be worse. So over the years, the cocoa industry has defined different relevant aspects, such as the physical characteristics with a direct bearing on manufacturing performance or flavor which, over time, have become the commercial standards employed worldwide. These commercial standards for cocoa beans, cake or chocolate usually include parameters related to physico-chemical parameters and compositional features (see Table 1). These evaluations aim to obtain a product that combines ideal aroma, flavor, color, technological behavior and functional compounds. This goal is fulfilled by assessing the physico-chemical cocoa characteristics in raw material and its derivatives in each

processing stage (Miller *et al*., 2006). Indeed each processing stage comprises key quality control processes that should be addressed to obtain high quality cocoa products. For example, the fermentation control in the postharvest stage is crucial for the formation of aromatic compounds (Aculey *et al*., 2010). Then, further quality control points should be set to guarantee quality requirements (e.g. fat content, moisture, etc.) while drying, industrial roasting and alkalization cocoa processes.

Apart from its nutrients, pleasant flavor, aroma and color, cocoa is also known for offering many health benefits (Bonvehí, 2005) because it is an excellent source of antioxidants (Langer, Marshall, Day, & Morgan, 2011). Many different bioactive compounds are present in cocoa, such as polyphenols, mainly flavonoids (flavanols, procyanidins, and anthocyanins) and methylxanthines (caffeine, theobromine) (Talbot, Mensink, Smolders, Bakeroot, & Plat, 2018), among others. These phytochemicals can be present at different concentrations depending on diverse factors like cocoa variety and cocoa processing, which can lead to the presence of new bioactive compounds. For example, cocoa roasting is a precursor for the formation of heterogeneous high-molecular-weight polymers known as "melanoidins", which are related to antihypertensive and antioxidant properties (Quiroz-Reyes & Fogliano, 2018).

Cocoa phytochemicals are an excellent ally to prevent cardiovascular and other chronic diseases, which are the main cause of mortality in Western countries (Gianfredi, Salvatori, Nucci, Villarini, & Moretti, 2018; Martín & Ramos, 2017). It has been shown that cocoa’s lipid profile balance is beneficial given the presence of stearic acid, which is a saturated fatty acid present in high proportions in cocoa butter (ca. 35%). The behavior of this fatty acid is unusual because, despite being a saturated fat, it behaves like an unsaturated one and has a neutral effect on blood cholesterol levels (Torres-Moreno, Torrescasana, Salas-Salvadó, & Blanch, 2015).

Polyphenols, especially epicatechin, perform neuroprotective and neuromodulatory action. The former action is associated with the prevention and reduction of neurological, cognitive and functional brain diseases (Alzheimer's, Parkinson's and senile dementia). The second action is related to cognition, humor, learning and memory skills (Ishaq & Jafri, 2017). These healthy

cocoa benefits promote its employment as a basic ingredient used by the pharmaceutical and cosmetic industries (APEDA, 2015; Oracz, Nebesny, & Żyżelewicz, 2015).

Based on cocoa's, and therefore on chocolate's. sensory attributes and functional properties, it is not surprising that global cocoa demand is increasing. Demand for cocoa is predicted to rise by 30% by 2020, which equals the present production output of the Ivory Coast (1 million tons) (Afoakwa, Quao, Takrama, Budu, & Saalia, 2013; Shavez et al., 2017). The extent of this growth is such that without empowering and investing in small-scale farmers, the industry will struggle to provide sufficient supply. This increasing cocoa demand, volatile prices and the uncertain global cocoa production, which is at risk due to climate change, can lead to cases of cocoa adulteration.

In this context, the development of new and faster analysis methods it is not only essential for guaranteeing quality specifications and costumers requirements, or for process control purposes; but also important to explore new properties of cocoa products and to detect new frauds attempting food safety and cocoa authenticity. Therefore, the goal of this review is to provide a comprehensive insight into both traditional and fast non-destructive technologies that might be used in the cocoa industry to assess cocoa composition and quality, to study new cocoa properties and to detect frequent and emerging frauds.

2. Determination of cocoa components

2.1 Major components

Cocoa compounds, such as fat, nitrogenous compounds, protein, moisture, ash and fiber, are usually evaluated by proximate analyses. Fat is determined by the AOAC 963:15 Method, which consists in a Soxhlet extraction method, moisture is determined by the AOAC 931:04 method, protein by measuring the nitrogen content with the Kjeldahl method (AOAC 970:22), ash by the AOAC 972:15 method and fiber by AOAC 991.43. An example of a recent application of these methods is a study about the effect that solar heat has on cocoa beans (Abdullahi,

Muhamad, Dzolkhifli, & Sinniah, 2018). Automation improvements of these methods have been incorporated into both industry and R&D laboratories. For instance, fat can be determined by the Soxtec™ AVANTI 2050 system (Servent *et al*., 2018; Sess-Tchotch *et al*., 2018), while nitrogen content can be determined using an automatic Kjeldahl apparatus (Hue *et al*., 2016) or a micro-Kjeldahl apparatus, which allow microquantities to be established (Hashimoto *et al*., 2018). With these analyses, it is possible to establish the nutritional information of cocoa and derivatives, which is usually included on product labels.

By applying these techniques, it can be concluded that fat, nitrogenous compounds, sugars and polyphenols are the main constituents of cocoa products. Cocoa fat is roughly 57%, 6.6%, and 11%, and total nitrogen content is ca. 2.5%, 3.2% and 4.3% for nibs, cocoa shells and cocoa powder, respectively. The percentage of water is ca. 3.2% in nibs, 6.6% in cocoa shells and 3% in cocoa powder (Afoakwa et al., 2013; ICCO, 2012). Cocoa powder also contains a relevant polysaccharide content (comprising cellulose, hemicellulose, and pectin), noncarbohydrate lignin, nonstructural polysaccharides like gums and mucilage. It also contains considerable amount of flavanols and organic acids (ca. 4% among lactic and acetic acids), which are responsible for cocoa color (Shavez et al., 2017). Table 2 summarizes the main components of cocoa powders.

2.2 Bioactive compounds: polyphenols and methylxanthines

Polyphenols are the most relevant bioactive cocoa compounds found to date. They can be divided into at least 10 different classes depending on their basic structure in the plant kingdom (Wollgast & Anklam, 2000). In cocoa, the flavonoids family constitutes the most important single group, which can be further divided into several classes. The main classes of flavonoids found in cocoa are summarized in Figure 2. The most abundant flavonoids in cocoa comprise monomeric flavanols (or flavan-3-ols) and their oligomeric and polymeric forms (known as procyanidins) (Lacueva et al., 2008). Monomeric flavanols include epicatechin (reported as the major monomeric flavanol in cocoa that represents ca. 35% of total phenolic content (Lacueva et al.,

2008), catechin (found in smaller amounts) and also traces of gallocatechin and epigallocatechin (Wollgast & Anklam, 2000). Procyanidins, also known as condensed tannins, are mostly flavan-3,4-diols, which are $4 \rightarrow 8$ or $4 \rightarrow 6$ bound to condensed dimers, trimers or oligomers with epicatechin as the main extension subunit (Wollgast & Anklam, 2000). In cocoa, procyanidins with a degree of polymerization (DP) up to decamer have been identified. Oligomers (procyanidins B1, B2, B5, and C1) and polymers account for 90% of total polyphenols, while monomers account for 5–10% (Lacueva et al., 2008). Another flavonoid class is anthocyanins, which is the most important group of water-soluble plant pigments responsible for the color of flowers and fruits of higher plants (Wollgast & Anklam, 2000). The main anthocyanins identified in cocoa beans are cyanidin-3-arabinoside and cyanidin-3-galactoside, which represent ca. 4% of the total polyphenol content of cocoa beans, but they can be hydrolyzed during the cocoa fermentation process (Forsyth & Quesnel, 1957; Wollgast & Anklam, 2000).

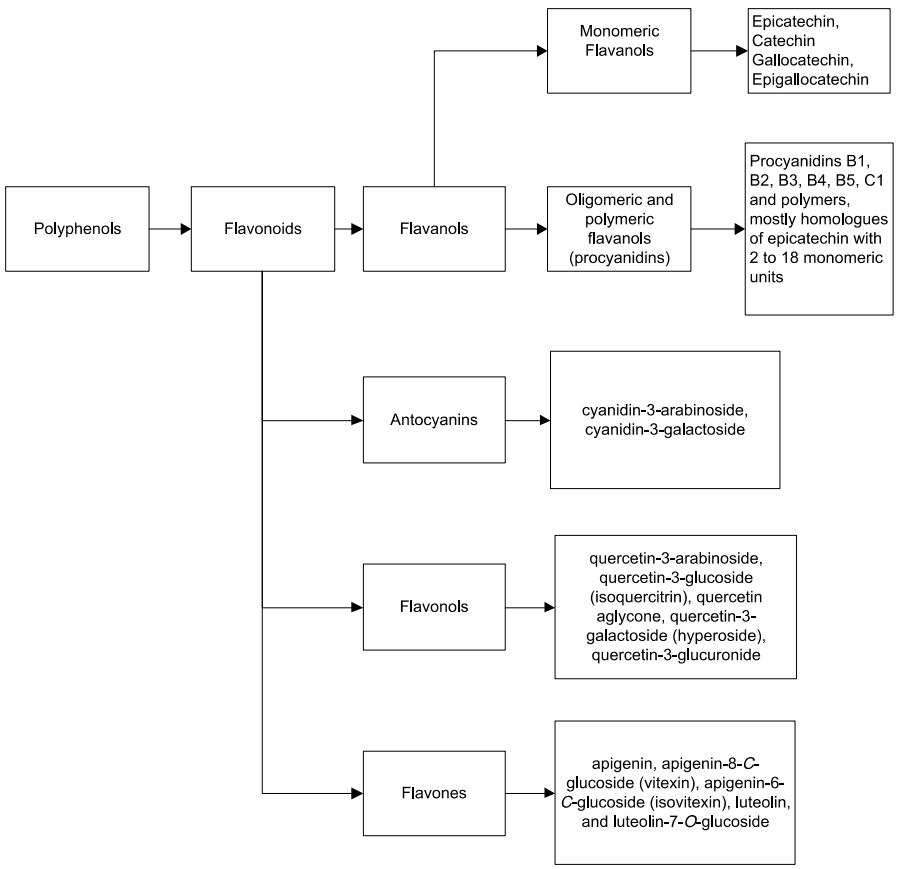

Fig 2. Summary of the main polyphenol classes found in cocoa.

Other important bioactive compounds found in cocoa and cocoa products are methylxanthines (Li et al., 2012). The main methylxanthines present in cocoa include caffeine and theobromine, but low levels of theophylline have also been found. These compounds are related to psychoactive properties that lead to better daily human life (i.e., more efficient thinking, exploring, hunting, etc.) without the serious side effects of drugs of abuse (Franco, Oñatibia-Astibia, & Martínez-Pinilla, 2013).

Both polyphenol and methylxanthine compounds are responsible for the astringent and bitter taste of cocoa, which affects cocoa stability and digestibility (Li et al., 2012). Moreover, they are generally determined to control the quality of the cocoa products obtained from raw beans in all the processing steps until end (ready-to-eat) products are obtained. Therefore, their determination is very important for the cocoa industry.

Phenolic compounds are usually extracted from cocoa matrices using different solvents, and methanol is considered the most efficient one (Belščak, Komes, Horžić, Ganić, & Karlović, 2009), although other solvents solutions, like acetone, water and acetic acid, are also widely used. The polyphenol content of cocoa is usually evaluated by total polyphenol content (TPC), antioxidant capacity (which can be obtained by different assays, which are described below), and by also quantifying the different individual polyphenols present in samples. TPC is usually determined by the Folin-Ciocalteu colorimetric assay, which is based on the Folin-Ciocalteu reagent's ability to react with phenolic hydroxyl groups (Manzano et al., 2017).

Antioxidant capacity can be established by methods based on both hydrogen atom or electron transfer reactions. The first category includes methods like ORAC (oxygen radical absorbance capacity), TRAP (total radical trapping antioxidant parameter), Crocin bleaching assay, IOU (inhibited oxygen uptake), inhibition of linoleic acid oxidation and inhibition of LDL (Low Density Lipoprotein) oxidation. The second category includes assays such as TEAC (Trolox equivalent antioxidant capacity), FRAP (ferric ion-reducing antioxidant parameter) DPPH (diphenyl-1-picrylhydrazyl), copper (II) reduction capacity, etc. (Di Mattia et al., 2014). The

heterogeneous methods (different reagents) used to determine antioxidant activity make the comparison of the obtained results difficult. However, the most frequently used assays are ABTS, DPPH, ORAC, TRAP, and FRAP. These methods can provide discordant results depending on the most abundant antioxidant molecules in the system and their interactions (Di Mattia et al., 2014).Individual determinations of both polyphenols and methylxanthines are usually performed by HPLC-UV, but the concomitant identification of other unknown compounds, mainly flavan-3-ol derivatives (Fayeulle et al., 2018), has led to the proliferation of innovative, miniaturized and/or two-dimensional HPLC methodologies (Toro-Uribe, Montero, López-Giraldo, Ibáñez, & Herrero, 2018). For this purpose, other detectors like mass spectrometry are widely used (Cádiz-Gurrea et al., 2014; Pedan et al., 2016; Rodríguez-Carrasco, Gaspari, Graziani, Sandini, & Ritieni, 2018).

Many articles have been published in the literature about the determination of and/or the changes produced in the different types of polyphenols and methylxanthines among several distinct cocoa products (Gabbay Alves et al., 2017; Machonis, Jones, Schaneberg, Kwik-Uribe, & Dowell, 2014; Manzano et al., 2017; Risner, 2008), in cocoa processing steps (Elwers, Zambrano, Rohsius, & Lieberei, 2009; Lacueva et al., 2008; Li et al., 2012, 2014; Miller et al., 2008; Payne, Hurst, Miller, Rank, & Stuart, 2010; Pedan, Fischer, Bernath, Hühn, & Rohn, 2017; Quiroz-Reyes & Fogliano, 2018), between different cocoa clones or varieties (Elwers et al., 2009; Niemenak, Rohsius, Elwers, Omokolo Ndoumou, & Lieberei, 2006), etc. Therefore, some of these studies are reviewed below.

Risner (2008) determined both methylxanthines (theobromine and caffeine) and flavan-3-ols (catechin and epicatechin) by HPLC in different cocoa products, including standard reference material baking chocolate 2384, cocoa powder, cocoa beans, and cocoa butter. Miller et al. (2006) published a study in which antioxidant capacity (the ORAC method), vitamin C equivalence antioxidant capacity (VCEAC), TPC and procyanidin contents were determined and analyzed by principal component analyses (PCA) to identify their behavior in different cocoa derivatives, such as natural cocoa powders, unsweetened baking chocolates, semisweet baking chips, milk and dark

chocolates and chocolate syrups. The highest levels of antioxidant activities, TPC and procyanidins were found in natural cocoa powders, followed by baking chocolates, dark chocolates, baking chips, and by finally milk chocolate and syrups.

In another study, the influence of alkalization on TPC, methylxanthines, flavan-3-ols and other components, such as volatiles, free amino acids, and sugars, was studied in commercial cocoa powders (Li et al., 2012). The results showed that the content of both methylxanthines and flavan-3-ols lowered as the degree of alkalization increased, while a higher degree of alkalization decreased TPC. Similar results were found by Miller et al. (2008), who also studied the influence of alkalization on the antioxidant capacity (ORAC method), TPC and flavanol content of cocoa powders. For all the samples, the highest contents of all the determinations were found for natural powders.

The influence of the alkalization process on the content of both monomeric flavanols (catechin and epicatechin) and flavonols (quercetin-3-glucuronide, quercetin-3-glucoside, quercetin-3-arabinoside, and quercetin) in cocoa powders was studied by Lacueva et al. (2008). The authors concluded that the marked reduction found in the flavonoid content of natural cocoa powder, together with the change observed in the monomeric flavanol profile that resulted from alkalization treatment, could affect the antioxidant properties and the polyphenol bioavailability of cocoa powder products.

Li et al. (2014) studied the effects of alkalization treatments on color, colorimetric fractions, TPC, and anthocyanin contents of cocoa powders. They concluded that the color qualities of cocoa powder can be improved by optimizing alkalization parameters. For example, cocoa powders alkalized with K_2CO_3 displayed darker colors and lower TPC than the powders alkalized with NaOH. High temperature and basic pH conditions gave a darker color due to sugar degradation, Maillard reactions and anthocyanin polymerizing.

In addition to the changes that take place during alkalization, further studies have studied the influence of other processing steps. One such case is the work published by Quiroz-Reyes et al.

(2018), who evaluated the effect that roasting and fermentation steps had on TPC, and antioxidant capacity and proanthocyanidins, melanoidins and flavan-3-ols contents on two cocoa bean varieties (Forastero and Criollo). The results showed that the Forastero variety was characterized by the highest melanoidins content, antioxidant capacity (DPPH Quencher assay) and TPC values under severe roasting conditions, while severer thermal treatments lowered the concentration of TPC and proanthocyanidins in both varieties, and also influenced the flavan-3-ols profile. Thus it can be concluded that a proper roasting process design and adequate cocoa variety selection can optimize the cocoa health potential, especially melanoidins and phenolic compounds.

In another study (Payne et al., 2010), the impacts of fermentation, drying, roasting and alkalization processes on catechin and epicatechin contents were evaluated in both unfermented and fermented cocoa beans. The results showed that unripe cocoa beans had a 29% higher level of epicatechin and the same level of catechin as fully ripe beans, while no significant difference in the content of both flavanols was observed during drying. A marked reduction (>80%) in catechin and epicatechin levels was observed in fermented *versus* unfermented beans. During roasting, loss of epicatechin took place along with a concomitant increase in the catechin level, probably due to the epimerization of epicatechin. Finally, alkalization led to a reduction in both catechin and epicatechin contents. Therefore, these authors proposed using the epicatechin/catechin ratio as a useful sensitive indicator for the processing history of cocoa beans.

Pedan et al. (2017) studied the influence of different lab-scale chocolate manufacturing process stages (including opening fresh cocoa pods, fermentation, drying, roasting and conching, and finishing chocolate bars) on the content of oligomeric proanthocyanidins and their antioxidant capacity by the NP-HPLC-online-DPPH methodology. For this purpose, one single batch of 5 kg of fresh Trinitario variety cocoa beans was studied in the different processing stages. The results showed that the total proanthocyanidin content continuously lowered during the manufacturing process, with only ca. 20% of the initial content present in chocolate.

As previously indicated, several studies have been conducted in which the influence of cocoa clones, variety and/or origin on polyphenols content has been studied (Elwers et al. 2009;

Niemenak et al, 2006). For example, Niemenak et al. (2006) compared TPC, flavanol (catechin and epicatechin) and anthocyanin (cyanidin-3-galactoside and cyanidin-3-arabinoside) contents of different seeds from Cameroon. The obtained results suggested that there was no qualitative difference in TPC in cocoa beans despite their genetic origin and fermentation-like process. However, a quantitative difference in epicatechin, catechin, cyanidin-3-galactoside and cyanidin-3-arabinoside, and also in three undefined substances, was found. This difference was attributed to growing conditions (microclimate, position of pods on trees, etc.). Finally, PCA and hierarchical cluster analyses classified samples according to their polyphenol and anthocyanin contents. Alternative methods for analyzing these bioactive compounds (polyphenols and methylxanthines) are included in Section 4.

2.3 Fatty acids

It has been reported that cocoa beans and cocoa liquor have around 50g/100 g of fat (Hashimoto et al., 2018). This fat, also called cocoa butter, is frequently reported to be the main vegetable fat used in chocolate manufacturing due to its rheological, textural and chemical characteristics, such as triglycerides and fatty acids (FA) composition (Guehi et al., 2008). Cocoa butter hardness depends on the ratio between saturated and unsaturated fatty acid bound in triglycerides, and on the free fatty acids (FFA) content. Whereas cocoa butter hardness increases with a higher proportion of saturated fatty acids, higher FFA content reduces this parameter. Thus Council Directive 73/241/EEC (EU, 2000) limits maximum FFA contents to a 1.75% oleic acid equivalent in cocoa butter (Guehi et al., 2008). The FA profile is also linked to cocoa aroma quality as the presence of volatile fatty acids (e.g. acetic, propionic, butric, isobutric, and iso-valeric acids) is linked to low quality products (García-Alamilla et al., 2007). Then there is stearic fatty acid (C18:0), which offers health benefits (Torres-Moreno et al., 2015). In this context, the characterization of both the quantity and quality of FA present in cocoa seeds and cocoa products is important and frequently evaluated (Guehi et al., 2008).

In cocoa butter, total FFAs are determined by measuring the amount of base needed to neutralize oleic acid (titration method) according to the official method 42-1993 (IOCCC, 1996). This method consists of dissolving 5 g of extracted cocoa butter in 50 ml of a previously hot petroleum ether/absolute ethanol mixture (1:1, v/v) neutralized by adding phenolphthalein. The mixture is then titrated with 0.1N alcoholic KOH solution. This method was used by Guehi et al., (2008) to study how storage conditions affect the FFA contents of raw cocoa beans. The above-cited authors used different samples of fermented-dried cocoa beans purchased from the Ivory Coast. The authors reported very low FFA contents (0.2-0.8%) in whole healthy cocoa. Their study also stated that FFA formation did not depend on either genotype or cocoa post-harvest processing technologies (number of fermentation days). However in defective cocoa beans, high and increasing FFA contents were found. This increased content was attributed to the activity of microflora, which has been associated with initial quality and loss of the physical integrity of cocoa beans.

The FA profile can be determined by preparing FA methyl esters (FAMEs) using method AOAC 948.22 and gas chromatography coupled to mass spectrometer detector GC-MS (Torres-Moreno et al., 2015). By the aforementioned method, Torres-Moreno et al., (2015) studied the influence of the geographical origin (Ecuador and Ghana) and processing conditions of chocolate (three roasting times: 30.5, 34.5 and 38.5 min; two conching times: 24 and 42 h) on the FA profile. For this purpose, the authors used the official method 948.22 (AOAC International, 1990b) and identified 15 FA in cocoa and chocolates. Of these, the most important FA were C16:0 (>25%), C18:0 (>33%) and C18:1 (>32%), expressed as the relative percentage of the total fatty acid content in unroasted cocoa beans and in the chocolate made from Ecuadorian and Ghanaian samples. For cocoa, differences in the FA profile were found in C12:0, C14:0, C16:0, C16:1, C17:0, C17:1 and C18:0, while differences were found only in C16:0, C18:0, C18:1 and C18:2 for chocolates. For all the samples, C16:0, C18:0, C18:1 and C18:2 were quantitatively the most important FA. Differences in the FA profile were explained mainly as an effect of the geographical origin and were not due to processing conditions in chocolate. Thus Ecuadorian

chocolate showed a healthier FA profile with larger amounts of unsaturated FA and smaller amounts of saturated FA than Ghanaian chocolate.

2.4 Amino Acids

Amino acids take part in the aroma and flavor formation of cocoa and cocoa-related derivatives (Voigt, Textoris-Taube, & Wöstemeyer, 2018). Their content is also related to human health (Stark, Lang, Keller, Hensel, & Hofmann, 2008). Thus, in addition to total protein contents, knowing the profile of the amino acids that form these proteins is essential.

High-performance liquid chromatography is the method normally used to analyze amino acids. As amino acids do not exhibit chromophore groups in their structure, they cannot be detected by UV–VIS spectrometry. Thus they have been traditionally derivatized before being analyzed. During the derivatization step, a UV–VIS nonresponding analyte can be converted into a compound with significant absorbance or fluorescence that allows determinations with greater sensitivity (Kubíčková et al., 2011).

One study that aimed to correlate amino acid content with cocoa aroma was published by Voigt et al., (2016). These authors analyzed amino acid content in cocoa beans to characterize the amino acid sequence of aroma precursor peptides. For this purpose, amino acids were converted into their ***o***-phthalaldehyde (OPA) derivatives and then separated by reversed-phase HPLC. Effluents were monitored fluorometrically. Another study using derivatization with a fluorescent chromophore to quantify the content of free amino acids in Forastero cocoa beans was conducted by Hinneh et al., 2018. In this work, the authors evaluated the influence of pod storage on the free amino acid profiles and the implications on the development of some Maillard reaction related to flavor volatiles. As a result, they found that although the concentration of free amino acids was directly proportional to pod storage duration, significant differences were observed for pod storage periods exceeding 7 days (Hinneh et al., 2018).

In relation to health properties, amino acids and their metabolites can act as functional molecules. Kynurenic acid, obtained during the metabolization of amino acids like tryptophan

through the kynurenine pathway, exhibit antioxidant capacity. Several authors have attempted to quantify tryptophan content and its derivatives in the kynurenine pathway by liquid chromatography with various detectors. One study that analyzed tryptophan and its derivatives in the kynurenine pathway in cocoa is that reported by Yılmaz and Gökmen, 2018. In their study, the authors compared the content of these analytes in several fermented food products (bread, beer, red wine, white cheese, yogurt, kefir and cocoa powder). Tryptophan derivatives were determined by ultra-high-performance liquid chromatography-tandem mass spectrometer (UPLC–MS/MS). Of these analytes, cocoa powder contained more kynurenic acid, which is a neuroprotective compound (Yılmaz & Gökmen, 2018).

The aim of another recent application of cocoa amino acids quantification was to assess the geographical origin (Asia, Africa and South America) of cocoa beans used to produce chocolate (Acierno, Alewijn, Zomer, & van Ruth, 2018). For this purpose, the authors tested the applicability of Flow Infusion-Electrospray Ionization-Mass Spectrometry (FI-ESI-MS). Among the tentatively identified compounds, the authors recognized free amino acids that could be used to distinguish the geographical origin of cocoa beans. This fell in line with other studies that have reported the geographical influence on the free amino acid concentration in raw cocoa (Rohsius, Matissek, & Lieberei, 2006).

2.5 Peptides

As with amino acids, the presence and concentration of certain peptides (e.g. N- terminal 15-kDa vicilin found in South American CCN51 samples) can be used to evaluate the origin of a particular cocoa. Kumari et al., (2018) used ultra-high-performance liquid chromatography-electrospray ionization mass spectrometry (UHPLC-ESI-MS) to analyze the proteins and oligopeptides of nonfermented and fermented beans of various geographic origins. ESI is a soft ionization method capable of providing both protonated and deprotonated molecules. Q-TOF-MS is able to combine high sensitivity and mass accuracy for both precursor and product ions and, therefore, allows the elemental composition for both parent and fragment ions to be confirmed

both quickly and efficiently. UHPLC can provide high resolutions for the separation of complicated natural products and improves the sensitivity of Q-TOF-MS detectors (Li *et al*., 2017). In this study, the authors observed how protein quantities, and their profiles derived from two-dimensional gel electrophoresis, showed striking differences for nonfermented beans depending on their geographical origin. However, in fermented beans, the detected diversity of peptides did not correlate with geographical origin, but to the degree of fermentation. These findings suggest that the variability in peptide patterns depends on the fermentation method applied in the country of origin, which ultimately indicated diversified proteolytic activities (Kumari *et al*., 2018).

2.6 Sugars

Cocoa sugars are cocoa aroma precursors that are present in higher proportions in cocoa pulp as fermentable sugars (9-13% w/w). The predominant sugars in cocoa beans are sucrose, fructose and glucose. In cocoa beans, fermentation allows reducing sugar (fructose and glucose) formation. Therefore, during the roasting process they undergo Maillard reactions and Strecker degradation, which lead to the generation of desirable flavor volatiles. Thus reducing sugars determination is important for cocoa sensorial control purposes (Kongor *et al*., 2016).

A traditional method to analyze total and reducing sugars in cocoa beans and products is that known as the phenol sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). This method allows simple sugars, oligosaccharides, polysaccharides, and their derivatives, to be detected, including methyl ethers with free or potentially free reducing groups as they give an orange-yellow color after treatment with phenol and concentrated sulfuric acid.

However, the identification and quantification of different reducing sugars require a more selective technique. One common alternative is to use gas chromatography after aqueous extraction and derivatization. Hinneh *et al*. (2018) analyzed the sugar profile of Forastero cocoa beans by gas chromatography. For this purpose, these authors obtained an extract that was then derivatized in two steps: first oximation and second the formation of trimethylsilylesters. The

study revealed that on storage day 0, cocoa pods exhibited 0.672±0.004 g/100 g of fructose, 0.264±0.001 g/100 of glucose and 0.021±0.001 g/100g of sucrose. These amounts varied with storage. After 3 pod storage days, the amount of glucose and sucrose had increased. After 7 pod storage days, these amounts lowered, while the amount of fructose increased, so the respective fructose-glucose ratios for 0 PS, 3 PS, and 7 PS were approximately 3:1, 2:1 and 4:1. This confirms the role of PS in influencing sugar degradation dynamics through nib acidification during fermentation.

2.7 Aroma and flavor

Aroma and flavor are the most appreciated cocoa bean features as they contribute to the final flavor of chocolates and other derived products. Samples can be evaluated for cocoa strength or chocolate flavor, residual acidity, bitterness and astringency, and for the presence of any off-flavor and positive ancillary flavors, such as fruity or floral. The sensory evaluation of cocoa products can be made by difference and descriptive tests. Difference tests are performed to compare samples, or samples against a standard, which include the triangle test, paired comparisons, ranking and the two-out of five test. No expert training is needed to carry out these tests (ADM Cocoa manual, 2006). Descriptive tests include the flavor profile method (FPM), the descriptive analysis test (QDA) and the free choice profiling (FCP), a variant of (QDA). Sensorial analysis methods may also include the use of a principal component analysis (PCA), which allows variable reduction according to inter-related connections. The information displayed in a two-dimensional graph provides essential information on the flavor profiles of cocoa samples based on descriptors. This method was used by Luna *et al*., (2002) to evaluate the flavor of Ecuadorian cocoa liquor, who concluded that polyphenols could be essential for the overall perception of cocoa liquor characteristics (CAOBISCO-ECA-FCC, 2015; Luna, Crouzillat, Cirou, & Bucheli, 2002).

Aroma and flavor are conditioned by different parameters that are chemical (nonvolatile and volatile compounds), biological (origin, variety) and physical (physical integrity) (Guehi *et al*.,

2008). Among nonvolatile flavor precursors, monosaccharides, disaccharides, oligosaccharides and some L-amino acids can contribute to the sweet taste of cocoa, while FA can contribute to acid taste. Tannin molecules like epicatechins, catechins, and procyanidins (total polyphenols) can confer bitterness and astringency. Alkaloid molecules (methylxanthines) can also contribute to a bitter cocoa taste (Jinap, Thien, & Yap, 1994). Thus they condition the sweetness, bitterness, acidity and astringency of cocoa and its derivatives. Volatile compounds appear in cocoa post-harvest stages, such as fermentation and drying. These steps occur in the origins of cocoa beans by generating heterogeneous materials. As previously mentioned, variety and physical integrity (that depend on postharvest practices) are important factors for volatiles to form.

During fermentation and roasting, pleasant volatiles that determine chocolate odor, (such as aldehydes, ketones and pyrazines) are formed. Jointly with esters and alcohols, these compounds are also related with sweet odor (Rodriguez-Campos *et al*., 2012). Properly dried beans usually have a long shelf life, a crisp texture and plump appearance, a well-oxidized interior and good flavor without excessive acidity, hammy, smokiness or other off-flavor notes (Jinap *et al*., 1994).

In contrast, inappropriate post-harvest handling (e.g. amount of mucilage in pods) can generate high contents of volatile fatty acids (VFA) like acetic, propionic, butyric, isobutyric, isovaleric acids (C2-C5), which cause strong acidic flavors and off odors. These off odors include rancidity, musty, stale, cheese rind, unpleasant and hammy flavors (García-Alamilla *et al*., 2007; Vázquez-Ovando, Chacón-Martínez, Betancur-Ancona, Escalona-Buendía, & Salvador-Figueroa, 2015). Nevertheless, VFA can decrease during roasting (Jinap *et al*., 1994).

The free amino acids, short-chain peptides and reducing sugars formed during the fermentation process can also contribute to cocoa flavor development during roasting in Maillard reactions. **A**ldehydes and pyrazines are produced as a result of this reaction. Tetramethylpyrazines (TMP) reach their maximum level upon medium roasting; trimethylpyrazines (TrMP) increase steadily throughout the roasting process and 2,5-dimethylpyrazines (DMP) rise under strong roasting conditions. The sensory evaluation shows that a normal roasting degree is linked to high concentration ratios of TMP/DMP and TMP/TrMP between about 1.5 and 2.5, respectively. Low

values for the above ratios are linked to over-roasted cocoa beans (Aprotosoaie, Luca, & Miron, 2016). So they contribute to high quality chocolates, and these molecules are desirable in cocoa beans (Afoakwa, Paterson, Fowler, & Ryan, 2009). A more extensive description can be found in (Aprotosoaie, Luca, & Miron, 2016).

Regarding the analysis of aroma and flavor compounds, on the one hand, part of the aroma analysis is done by determining the aroma precursors that are free amino acids, oligopeptides, and reducing sugars. The analyses of these compounds have been previously described. This section reports only the methods used to study the combination between aroma precursors and sensory attributes.

A profounder understanding of the aroma profile can be attained by the determination of individual aromatic compounds. The determination of aroma compounds is usually made by their extraction, separation and detection. Studies have been published using different extraction methods, such as headspace-solid phase microextraction (HS-SPME) (Miriam Torres-Moreno, Tarrega, & Blanch, 2014), solid-phase microextraction (SPME) (Humston, Knowles, McShea, & Synovec, 2010), aroma extraction and dilution analyses (AEDA) and solvent-assisted flavor evaporation (SAFE distillation) (Chetschik *et al*., 2018). To separate compounds, gas chromatography GC is frequently used. Flame ionization detector (FID) (Cambrai *et al*., 2010), mass spectrometry (MS) or, for more accurate detection times, fly mass spectrometry (TOFMS) (Humston., 2010) are used for detection purposes.

Many studies about the determination and/or changes produced in the flavor, aroma and taste during cocoa fermentation (Crafack *et al*., 2014), roasting (Torres-Moreno *et al*., 2014), between different cocoa clones or varieties (Liu *et al*., 2017), and between different cocoa products (Chetschik *et al*., 2018), have been recently published. Torres-Moreno *et al*., (2014) extracted volatile compounds from dark chocolate using HS-SPME followed by GC-MS to determine the influence of the roasting process on chocolate aroma formation. Variations have been found in the chocolate aroma profile and their concentrations according to roasting time and geographical origin (Torres-Moreno., 2014).

Changes in the aroma of cocoa beans with moisture damage have been analyzed in cocoa beans of different origins (Costa Rica, Ghana, Ivory Coast, Venezuela, Ecuador and Panama). SPME sampling coupled to two-dimensional gas chromatography combined with time of fly mass spectrometry (GC×GC–TOFMS) has been applied for such assessments. Twenty-nine compounds have been detected as a result of moisture damage (Humston., 2010). Similarly, gas chromatography coupled to a flame ionization detector (FID) and MS has been used to distinguish different cocoa types and their derivatives (Cambrai *et al*., 2010).

Thanks to a high sensitivity, selectivity and reproducibility of HS-SPME-GC–MS, the method is being increasingly used in combination with chemometrics. This determination technique in combination with PCA have been used to simultaneously understand the behavior of several aroma components (Cambrai *et al*., 2010)**.** Li, *et al*., (2012) detected 80 volatile aroma compounds in cocoa powders of different degrees of alkalization by the aforementioned GC-MS technique. Among these compounds, a high acetic acid concentration was determined. Moreover, a decreasing trend of this acid while increasing the degree of alkalization was reported (Li *et al*., 2012). HS-SPME-GC-MS has also been used to evaluate the inoculation effect of starter cultures and fermentation techniques on the volatile aroma and sensory profile of chocolate. As a result, 56 volatile chocolate compounds have been identified and aromatic profiling differences have been linked to fermentation technique types, but not to the used starter cultures. However, the differences were too small to change consumer perceptions (Crafack *et al*., 2014).

Other aroma extraction methods include the aroma extraction and dilution analyses (AEDA) and solvent-assisted flavor evaporation (SAFE distillation), and both can be coupled to GC-MS. Chetschik *et al*. (2018) used the SAFE method to characterize the aromas of cocoa pulp, and how they are transformed during fermentation. These authors found higher 2-phenylethanol and 3-methylbutyl acetate concentrations in cocoa pulp than in cocoa beans in several fermentation stages. Conversely, quantities of odorants, such as linalool and 2-methoxyphenol, have been observed at larger concentrations in cocoa beans (Chetschik *et al*., 2018).

In another study (Van Durme, Ingels, & De Winne, 2016), the authors used the in-line roasting hyphenated with a cooled injection system coupled to a gas chromatograph–mass spectrometer (ILR-CIS-GC–MS) to assess fermentation quality and the overall potential formation of cocoa aroma. For this purpose, data on unroasted cocoa were compared with data on conventional roasted cocoa beans obtained by headspace solid phase microextraction (HS-SPME-GC–MS). The results of this analysis revealed that similar formation trends of important cocoa aroma markers were found according to fermentation quality. These main markers of cocoa aroma were aldehyde, pyrazines, aldehydes (amyl alcohols), and pyrazines tetramethylpyrazine (TMP) and trimethylpyrazine (TrMP), which are present at high concentrations when cocoa beans are well-fermented. The aforementioned method requires no sample preparation and can be performed in short times (<1 h).

Apart from methods based on the separation and identification of compounds, new innovative, faster and robust analytical techniques to determine aromatic compounds are being proposed. Concretely, the hyphenated HS-SPME-MS-nose configuration, based on mass fingerprinting and pattern recognition, uses the hyphenated dynamic headspace-chemical sensor configuration. This equipment has a fully automated sample preparation unit for the online dynamic headspace isolation of cocoa aroma compounds. This technique has been used for the differentiation by the origin and fermentation degree of roasted fermented cocoa beans (from Indonesia, Peru, Ghana and Vietnam) by a hierarchical cluster analysis (HCA), PCA and one classification algorithm, namely soft independent modeling of class analogy (SIMCA). So a clear separation of fine flavor cocoa variety Criollo was possible, as was classifying samples according to their degree of roasting (Diem *et al*., 2015). Regarding origin, Liu *et al*. (2017) made a comparison of the aroma compounds of cocoa liquors from Asia, Africa and Oceania by gas chromatography-olfactometry-mass spectrometry (GC-O-MS). With this study, components at high concentrations were found, such as 3-methylbutanal, acetic acid, tetramethylpyrazine, and 3-methylbutanoic acid, and a relation between the aroma profile and origin was found by PCA (Liu *et al*., 2017).

2.8 Polycyclic aromatic hydrocarbons, toxins and heavy metals

Cocoa samples can also contain compounds that could be considered of risk for humans. These compounds can come from soil contamination (i.e. heavy metals (HM)), or can be generated during manufacturing practices (i.e. polycyclic aromatic hydrocarbons (PAHs) and mycotoxins). The levels of some of these compounds are regulated by the European Food Safety Authority (EFSA) (European Commission, 2011). The methods normally used and the studies carried out to control their presence are described below.

2.8.1 Polycyclic aromatic hydrocarbons (PAH)

PAHs can be generated during incomplete combustion and are widely present in the environment. These compounds can contaminate foodstuffs and are related to human toxicity (carcinogenic, genotoxic, mutagenic) (Cordella *et al*., 2012). As they are lipophilic, their determination is usually made in cocoa butter. A frequent way to analyze PAHs in cocoa samples is to extract them from the sample by the method based on the stirred saponification of 1 g of cocoa butter in KOH (1:6), 1M at 80°C. After extraction, the determination is made by HPLC coupled to a fluorescence detector. Four PAHs have been mainly determined, namely benzo(a) anthracene, chrysene, fluoranthene, and benzo(b) pyrene (Bratinova, Karasek, Buttinger, & Wenzl, 2015). Sess-Tchotch et al., (2018) used the aforementioned extraction and determination method and found limits of detections (LoDs) ranging from 0.01 µg/kg to 0.033 µg /kg for these compounds (Sess-Tchotch *et al*., 2018).

Another example of the identification and quantification of polycyclic aromatic hydrocarbons in cocoa beans was recently presented by Belo *et al*., (2017). These authors used an accelerated solvent extraction before GC-MS to determine eight PAH in cocoa beans. The evaluation of the method was made by analyzing relative standard deviations (RSD) under repeatability and precision conditions, and average recoveries. The authors found precision with RSD ranging from 2.57% to 14.13% and from 4.36% to 19.77% under repeatability and intermediate precision conditions, respectively. The average recoveries of the eight PAH ranged

from 74.99% to 109.73%. These parameters, limits and measurement uncertainties met the performance criteria set by EU regulations.

2.8.2 Toxins

Not many studies about toxins in cocoa and its products can be found. The few studies published to date show that the most widely studied toxins in cocoa and its products are ochratoxin A (OTA) (Kutsanedzie *et al*., 2018) and aflatoxins. Ochratoxin is a mycotoxin that is formed by species of *Aspergillus* and *Penicillium*. Aflatoxins are formed by *Aspergillus flavus*, *A parasiticus*, and other *Aspergillus* spp. The most important aflatoxin, due to its occurrence, is aflatoxin B1, which is classified as carcinogenic (Group 1). The presence of ochratoxins in cocoa can lead to such serious health problems that the European Commission has set a tolerable weekly intake (TWI) of 120 ng/kg body weight. However, no maximum limit has been set for cocoa and cocoa products as these products do not contribute significantly to OTA exposure in diet (European Commission, 2010). No maximum limits have been set for aflatoxin (Turcotte, Scott, & Tague, 2013).

The most widespread technique to analyze toxins in cocoa is HPLC. To analyze ochratoxin in cocoa powder, Brera, Grossi and Miraglia (2005) developed an HPLC method based on OTA extraction from samples by blending with an aqueous solution of bicarbonate, diluting with a solution of phosphate buffer saline, filtering and cleaning-up by an immunoaffinity column (IAC) that contained antibodies specific to OTA. After washing the immunoaffinity column, OTA was eluted with methanol, separated by reversed-phase HPLC and quantified by fluorescence detection. This method was validated by an interlaboratory study, and allows the detection and identification of different OTA within the 0.1-2 μg/kg range. The same method was followed with drinking chocolate and cocoa powder to also detect ochratoxin (Cubero-Leon, Bouten, Senyuva, & Stroka, 2017). In this study, the authors found that the mean recoveries ranged from 85% to 88%, the RSD values went from 13.7% to 30.7% and the resulting Horwitz ratios, according to the Horwitz function modified by Thompson, fell within the 0.6-1.4 range for cocoa and drinking chocolate, respectively.

In a recent study that aimed to determine toxins in different cocoa products, toxins extracts were cleaned by AflaOchra (IAC) columns before HPLC separation. Toxin detection was performed by a post-column photochemical reactor for aflatoxin B1 and G1 (due to derivatization) and by fluorescence for OTA. The method's limits of quantification (LOQ) were 0.16 ng/g (OTA) and 0.07 ng/g (aflatoxin B1). The OTA levels in the different analyzed samples were 1.17 ng/g in natural cocoa, 1.06 ng/g in alkalized cocoa, 0.49ng/g in baking cocoa, 0.39ng/g in dark chocolate, 0.19 ng/g in milk chocolate and 0.43 ng/g in cocoa liquor. Regarding aflatoxin, the following incidences were found: 0.86 ng/g in natural cocoa, 0.37 ng/g alkalized in cocoa, 0.22 ng/g in baking chocolate, 0.19 ng/g in dark chocolate, 0.09 ng/g in milk chocolate and 0.43 ng/g in cocoa liquor (Turcotte *et al*., 2013).

2.8.3 Heavy metals

Heavy metals (HM) are naturally present in foodstuffs. These compounds are toxic to humans. Cadmium (Cd) is a heavy metal present in several foods consumed daily and in larger quantities, including cocoa. In order to maintain and control the amount of Cd in the human diet, the European Commission has set maximum Cd limits in certain products (European Commission, 2006), for example 0.10 mg/kg in milk chocolate with < 30% total dry cocoa solids, 0.30 mg/kg in milk chocolate with ≥ 30% total dry cocoa solids or 0.60 mg/kg in cocoa powder sold to end consumers or as an ingredient in sweetened cocoa powder sold to end consumers (drinking chocolate). In this context, monitoring the presence of this and other HM in cocoa products is a growing necessity.

To ensure compliance with regulations, CODEX STAN 228 (2001) suggests some Cd analytical methods, such as atomic absorption spectrometry (AAS) after incineration or microwave digestion (using HNO_3) and Anodic Stripping Voltammetry (ASV), of which AAS is more widely used.

Such is the concern today about the presence of Cd in cocoa and derived products that many studies have been conducted in the last 5 years to determine the amount of Cd present in cocoa derivatives. Cd has been determined in cocoa beans (Chavez *et al*., 2015) and plants from Ecuador

(Chavez *et al*., 2016), in cocoa trees and leaves from Peru (Arévalo-Gardini, Arévalo-Hernández, Baligar, & He, 2017); in cocoa beans from Indonesia (Assa, Noor, Yunus, Misnawi, & Djide, 2018); in cocoa powders and chocolates in the USA (Abt, Fong Sam, Gray, & Robin, 2018), in raw cocoa and processed chocolate mass from Poland (Kruszewski, Obiedziński, & Kowalska, 2018), and in Italian cocoa powder and chocolate (Lo Dico *et al*., 2018). In the study of Chavez *et al*., 2015, the authors determined Cd in cocoa plant materials (ground leaves, shells or beans). For their analysis, samples (ground leaf, shell or bean) were digested with nitric acid (HNO_3) (Jackson *et al*., 1986). The digested samples were diluted with distilled water and filtered through a membrane filter prior to the Cd analysis. Then the Cd concentrations in plant digesters were determined by inductively coupled plasma optical emission spectrometry (ICP-OES).

In another article, the Cd concentrations in cocoa beans from Indonesia were established by atomic absorption spectrometry after digesting samples with HNO_3 and H_2O in a microwave. The Cd concentration in these samples was below the LOD of 0.100 mg/kg (Assa, Noor, Yunus, Misnawi, & Djide, 2018). Finally, Abt *et al*., (2018) determined Cd content in cocoa powder and chocolate products on the US market, and concluded that the Cd contained in these products ranged from 0.004-3.15 mg/kg.

3. Other analytical methods for cocoa bean trading along the value chain

Apart from the compositional analysis, other quality control analyses are done before and during the commercialization of cocoa beans. This section indicates how these analyses are applied. Quality control begins in the place of origin. To do so, conventional methods to assess and control correct fermentation, size, and even the integrity, of beans are widely used after sampling the total batch (FCC, 2018; ICCO, 2018). For fermentation quality assessments, the standard method applied is the cut test that involves counting 300 beans. These 300 beans are then cut lengthwise through the middle and examined to infer the physical (integrity, color) and sensorial characteristics of cocoa-fermented beans, which provides an indication of quality (Lee & Djauhari, 2013; Schwan, 1998). During the cut test, the number of defective cocoa beans can be assessed. These defects can consist of beans with mould, damage caused by insects, and

698 germinated or flat beans. The results are expressed as a percentage of 300 beans examined per
699 defect kind. The amount of defective beans determined through cut tests is an indication of flavor
700 characteristics (ICCO, 2018). Bean size is established by counting the number of cocoa beans per
701 100 g. By considering this, they are classified into three grades as follows: grade 1 (≤ 100 beans
702 per 100 g), grade 2 (101–110 beans per 100 g) and grade 3 (111–120 beans per 100 g). In bean
703 mass (or weight) terms, the standard states that bean cocoa mass should be at least 1.0 g
704 (CAOBISCO-ECA-FCC, 2015). Before commercialization, other control parameters can include
705 color, pH and titratable acidity (Hinneh *et al*., 2018).

706 During cocoa transformation, cocoa shell determination after shelling is an important factor
707 as it affects some final product characteristics, such as flavor or taste. It can also be responsible
708 for off-flavors. The fiber content in cocoa shells is very high. Thus it can be a problem for the
709 grinding process because it can cause equipment abrasion in some cases (Mendes & Lima, 2007;
710 Quelal-Vásconez *et al*., 2019). During shelling, cocoa shells (approx. 12-20% of the cocoa bean)
711 cannot be completely removed. In order to guarantee that cocoa powders have been well peeled
712 and not adulterated with cocoa shells, the Codex Alimentarius establishes that cocoa shells
713 including germ must be present, but below 5% (in fat-free dry cocoa) (Codex Alimentarius, 2014;
714 Okiyama *et al*., 2017).

715 The official methods followed to analyze cocoa shells content are methods AOAC 968.10
716 and 970.23 (Codex Alimentarius, 2014). The first method, called the spiral vessel count, consists
717 of counting spiral vessels in a defatted, ground and digested sample with the help of a microscope
718 adjusted to mold counting (field of view 1.382 mm at 100 x) (AOAC, 2006). The second method,
719 called the stone cell count, consists of counting the stone cells present in samples assisted by a
720 microscope after laborious preparation (AOAC, 1984).

721 As observed in Sections 2 and 3, all the conventional methods followed to determine cocoa
722 components or quality control during cocoa bean trading focus on destructive determinations. The
723 inability to use the analyzed raw material, in combination with very long analytical procedures,
724 high solvents utilization and waste production, and the need for highly skilled operators, mean
725 that fast non-destructive alternative technologies must be developed.

4. Fast non-destructive technologies applied in the cocoa industry

This section contains an exhaustive analysis of the non-destructive technologies applied in the cocoa industry. A review of the different studies carried out with non-destructive techniques, analyzed products, the equipment used for analyses, measurement parameters, traditional methods used as references, chemometric model calibration and validation details. The results are presented in Table 3.

4.1 Types of non-destructive techniques used

Several fast non-destructive technologies, such as electronic tongue, electronic nose, hyperspectral image, terahertz spectroscopy and infrared spectroscopy, have been widely explored and applied in the cocoa industry (Table 3). Electronic tongue has been used for the rapid identification of cocoa beans according to their geographical locations (Teye *et al*., 2014a). Electronic tongue and near infrared spectroscopy, together with a chemometric analysis, has been used for the accurate classification of cocoa bean varieties (Teye, Huang, Takrama, & Haiyang, 2014c) and for the rapid determination of total polyphenols contents in cocoa beans (Huang *et al*., 2014). An electronic nose / gas chromatography-mass spectrometry (GC-MS) system combined with artificial neural network (ANN) has been used for determining roasting degree in cocoa beans (Tan & Kerr, 2018). Electronic nose combined with pressure control-generated stimulation has been used in chocolate classification (Valdez & Gutiérrez, 2016). The hyperspectral image analysis has been used for cocoa bean quality assessments (Soto *et al*., 2015) and to predict the fermentation index, polyphenol content and antioxidant activity in single cocoa beans (Caporaso *et al*., 2018). These analyses have been done with whole cocoa beans and spectra measurements have been correlated with conventional Partial least squares (PLS) determinations with promising results. Terahertz spectroscopy has been utilized to control tempering in chocolate factories (Weiller, Tanabe, & Oyama, 2018). Terahertz (THz) spectroscopy energy corresponds to collective molecular macro-vibrations and is considered a promising potential to identify macromolecules (i.e., polymer and biomolecules). This non-destructive noncontact technique has been used to characterize polytypes of crystals formed on the basis of FA combination in the

chocolate structure. For this purpose, two chocolates are analyzed and the measurements normally taken by X-ray diffraction (XRD) are compared with optical microscopic observations and THz spectroscopy measurements, with similar results (Weiller, *et al*., 2018). Infrared spectroscopy has been used to predict major (moisture, carbohydrate, fat, protein) or minor functional compounds (theobromine, catechin, organic acids, etc.) (Álvarez, Pérez, Cros, Lares, & Assemat, 2012; Huang *et al*., 2014; Krähmer *et al*., 2015; Veselá *et al*., 2007) and for quality control (discrimination of cocoa beans according to geographical origin, prediction of cocoa powder adulterations, prediction of methylxanthines and polyphenols in alkalized cocoa powder, etc.) (Quelal-Vásconez *et al*., 2020; Quelal-Vásconez *et al*., 2019; Quelal-Vásconez, Pérez-Esteve, Arnau-Bonachera, Barat, & Talens, 2018; Teye, Huang, Dai, & Chen, 2013).

Of all of these technologies, infrared spectroscopy offers a number of important advantages over traditional chemical methods. It is non-destructive, noninvasive, requires minimal or no sample preparation, its precision is high, and it can act as a multi-analytical technique because several determinations can be simultaneously made. Infrared spectroscopy also offers the possibility of measuring physico-chemical properties (Veselá *et al*., 2007).

4.2 Infrared spectroscopy

Infrared spectroscopy (IR) involves the interaction of infrared radiation with matter. It is conventionally divided into three wavelength regions: near-infrared (NIR: 750–2500 nm or 13333–4000 cm^{-1}), mid-infrared (MIR: 2500–25 000 nm or 4000–400 cm^{-1}), and far-infrared (25–1000 μm or 400–10 cm^{-1}). The distinction made among these three regions may vary depending on the type of instrumentation used to acquire IR spectral information.

4.2.1 ***NIR spectra acquisition***

The IR method or technique is run with an instrument called infrared spectrometer (or spectrophotometer) which produces an infrared spectrum. A generalized spectrophotometer has

four parts: 1) an energy source; 2) a wavelength selection device; 3) a detector; 4) a data processing system.

The most explored technologies for cocoa studies are near infrared spectroscopy (NIR), Fourier-transform near infrared spectroscopy (FTNIR) and, to a lesser extent, Fourier-transform infrared spectroscopy (FTIR). The term Fourier-transform infrared spectroscopy originates from the fact that a Fourier transform (a mathematical process) is required to convert raw data (collected in frequencies in an interferogram) into the actual spectrum. In an NIR instrument, values are reported in nm, generally from 900 to 2500 nm, or from 650 to 2500 nm if the visible region is included (Nielsen, Snitkjaer, & Van Den Berg, 2008). The values with an FTIR instrument are generally reported in cm^{-1}, from 4000 to 10000 cm^{-1} (Teye & Huang, 2015a) or from 4000 to 12500 cm^{-1} (Sunoj, Igathinathane, & Visvanathan, 2016).

Several optical alternatives are available for IR spectroscopy: 'reflectance', 'transmittance', 'transflectance', and 'interactance' (Alander, Bochko, Martinkauppi, Saranwong, & Mantere, 2013; Cortés, Blasco, Aleixos, Cubero, & Talens, 2019). The majority of studies for cocoa powder (Quelal-Vásconez *et al*., 2018) or cocoa beans (Caporaso *et al*., 2018) use reflectance (Table 3), but transflectance has been used for semi-solids and liquids like cocoa butter or chocolate (Bolliger, Zeng, & Windhab, 1999).

4.2.2 Multivariate data analysis

Due to the complex and the large amount of hidden information in IR spectral data, particular attention should be paid to data mining with chemometrics for the IR spectroscopy analysis. Multivariate data analysis or chemometrics is the science of extracting information from chemical systems by data-driven means. It offers lots of applications and diverse natures. Specifically, it is used in IR applications to extract rich information from IR spectra, including preprocessing spectral data, reducing variables, building calibration models (quantitative) and/or classification (qualitative) analyses, and model transfer, and all this to acquire more information from data (Martens *et al*., 2003).

A multivariate analysis generally involves the following steps: data exploration, data preprocessing, quantitative or qualitative model calibrations, and finally external validation. Data exploration allows finding sample groups, the relation between variables and management with outliers samples by means of a PCA or a parallel factor analysis (PARAFAC) (Bro, 1997; Rodrigues, Condino, Pinheiro, & Nunes, 2016). Data preprocessing can be handled with preprocessing algorithms, such as smoothing methods (Savitzky-Golay, Gaussian filter, median filter, moving average), normalization and scaling, detrending (Levasseur-garcia, 2018), 1st Derivate, 2nd Derivate-Savitzky Golay (Savitzky & Golay, 1964), Standard Normal Variation (SNV) (Teye, Uhomoibhi, & Wang, 2016), Orthogonal Signal Correction (OSC) (Wold, Antti, Lindgren, & Öhman, 1998) and Multiple Scatter Correction (MSC) to build and enhance calibration models (Su & Sun, 2017). The selected preprocessing method can be related to data features to, for example, rid up multiplicative and additive effects in spectra. As seen in Table 3, datasets are usually divided into calibration and validation, except those carried out by Krähmer *et al*., (2015) and Sunoj *et al*., (2016), who performed only cross-validation. Calibration datasets are composed of a different number of samples, from 65 (Permanyer & Perez, 1989) samples in the calibration set to 190 at the time of this study (Caporaso, Whitworth, Fowler, & Fisk, 2018). High accuracy has been obtained for calibration models by employing proper multivariate linear regressions, such as PLSR, PCR, SVMR, and other statistical algorithms like artificial neural networks (ANN) (Teye & Huang, 2015a; Teye, Huang, Lei, & Dai, 2014b; Teye *et al*., 2015b), PLS with variable selection, such as Sinergy Interval-PLS (Si-PLS), Ant Colony Optimization-PLS (ACO-LS), Competitive Adaptive Reweighted Sampling - PLS (CARS - PLS), Synergy Interval-Genetic Algorithm-PLS (Si-GAPLS) (Kutsanedzie et al., 2018), Modified Partial Least Squares (mPLS) and Synergy Interval Backpropagation Neural Networks Regression (Si-BPANNR). Efficient classification results have been obtained with tools like support vector machine (SVM), discriminant partial least squares (PLS-DA) (Berrueta, Alonso, & Héberger, 2007), Linear discriminant analysis (LDA), SIMCA, SVM, QDA and Kernel nearest neighbor (KNN) (Teye, Uhomoibhi, & Wang, 2016; Teye, Huang, Han, & Botchway, 2014a), and the discriminant function analysis (DFA) (Goodacre & Anklam., 2001). To build calibration models,

all the spectra can be used, or variable selection methods also are employed to obtain computationally efficient algorithms.

However, variable selection can be performed to avoid complex models. Table 3 also shows that full cross-validation is widely used during model calibration. The evaluation of model performance is made by parameters, such as the coefficient of determination of calibration, cross-validation and validation (R^2), coefficient of correlation (R), root mean error of calibration, cross-validation and validation and the relation deviation prediction (RPD). Sometimes both bias and slope are considered.

4.3 Applications

4.3.1 Non-destructive determination of constituents and industrial processing monitoring

Very few studies done with non-destructive technologies have been applied in the cocoa industry. Of these, the most frequently used non-destructive techniques are NIR and FT-NIR (Table 3). The majority of studies have been done in the postharvest (fermentation/drying) stage of cocoa beans. Biochemical parameters like fat (Álvarez *et al*., 2012; Weiller *et al*., 2018), sugars, polyphenols, procyanidins (Whitacre *et al*., 2003), methylxanthines, moisture and pH (Krähmer *et al*., 2013; Sunoj *et al*., 2016; Veselá *et al*., 2007) have been evaluated. The aim of these studies was the quality control of end products, and/or the determination of authenticity through compositional analyses or by clustering samples from their spectral fingerprint (origin, varietal classification).

Near infrared light is sensitive to the sample's physical properties. These physical conditions can cause variations in measured spectra, and have been identified in spectra as multiplicative and additive effects. These effects, due to light scatter, are minimized using a sample of a small homogenized particle size (Barbin *et al*., 2018). Most studies have employed ground beans more than whole beans, partly as a way to minimize the aforementioned variations and effects (Barbin *et al*., 2018) (Table 3)

In relation to measurement modes, Dickens, (1999) defined four ways to implement measurement equipment into processes: (i) offline: a sample analysis run away from the production line (i.e., laboratory); (ii) at line: manual random sample extraction from the production line and an analysis performed close to the process line; (iii) online: samples separated from the production line which, after being analyzed in a recirculation loop (by-pass), are returned. (iv) inline: samples are analyzed on the running production line (*in situ*) (Dickens & Dickens, 1999; Osborne, 2000). Table 3 shows that the performance of this non-destructive analysis done in the offline mode in almost all the studies carried out by NIR in cocoa beans. Only Bolliger performed an inline application of NIR in 1999 to monitor the rheological properties (viscosity, melting enthalpy) of chocolate in the tempering stage.

In connection with cocoa bean fermentation, the degree of fermentation and flavor profile are routinely determined in both the trade and industry by a cut test (color check). Both assessments require specially trained personnel. Sensory evaluation is highly subjective depending on the sensory panel (Afoakwa *et al*., 2013). So fermentation has been the subject of different approaches, such as characterization by spectroscopic and chromatographic methods (Aculey *et al*., 2010). Accordingly, Table 3 shows that the lower value predicted by NIR is in ppm units of a metabolite (NH3) product of fermentation. NH3 contents have been found to fall within a range of 46-332 ppm with a standard error of prediction (SEP) of 20 ppm (Hue *et al*., 2014).

The fermentation of cocoa beans has been analyzed by NIR and Denaturing gradient gel electrophoresis (DGGE) to gain a better understanding of the fermentation mechanisms related to the microbiological factor. A good correlation between both measurements has been found (Nielsen *et al*., 2008). NIR integrated with an electronic tongue (ET) and multivariate analyses have been applied to perform a 100% (accuracy) classification of five cocoa bean varieties. Accurate classifications can be attributed to three functional groups (second overtone) of methylene ($–CH_2$), methyl (CH_3) and ethenyl (–CH=CH–). Theobromine, for instance, has one

887 methyl group, while caffeine has two methyl groups. These compounds may play an important
888 role in discriminating employed cocoa bean varieties (Teye, Huang, Takrama, *et al*., 2014c).

889 Bacteria (e.g. Staphylococcus aureus, Bacillus cereus) in cocoa powders have been found to
890 affect their quality grades, and these bacteria can be detected by the FT-MIR spectral system
891 (Ramalingam *et al*., 2009). The total fungi count (TFC) in cocoa beans has been evaluated by
892 Fourier transform near infrared spectroscopy (FT-NIRS) combined with synergy interval-genetic
893 algorithm-PLS (Si-GAPLS). This technique allowed a prediction coefficient of 0.975 to be
894 obtained, along with a root mean square error of prediction (RMSE) of 0.384 CFU/mL and a ratio
895 prediction deviation RPD of 4.32 (Kutsanedzie *et al*., 2018).
896
897 ***4.3.2 Authenticity and adulterations***
898 Table 4 shows details of the studies carried out by non-destructive techniques used to assess
899 the authenticity and adulteration of cocoa products. Trilcová *et al*., 2004 showed that NIR and
900 FTIR spectroscopy can be used as a very fast and reliable tool for cocoa powder authentication.
901 The term authenticity refers to the inherent quality attributes of cocoa, and has been included in
902 new food fraud authenticity policies and identified as product integrity (Manning, 2016). The
903 authenticity of cocoa and its derivatives is determined by studies that aim to identify the origin of
904 raw material, varietal purity, compositional parameters, detection of adulterants, etc.

905 The sensory characteristics of cocoa products have created an increasing consumer trend to
906 choose cocoa of a specific origin. These preferences have allowed more appreciated cocoa origins
907 whose quality is differenced by market prices. This differentiation has yielded bad commercial
908 practices, like mixing more expensive cocoa beans of the highest quality and an outstanding origin
909 with other lower quality cocoa kinds that are cheaper to obtain fraudulent economic benefits
910 (Magagna *et al*., 2017).

911 Determination of origin has been evaluated by the phenolic fingerprint (D'Souza *et al*.,
912 2017). Most of these studies have been conducted by the compositional analysis mentioned in
913 Section 2. Non-destructive technologies like NIRS have been applied to classify cocoa by its

origin. As a result, classification percentages according to the geographical origin of cocoa beans of 90.63 (LDA), 75 (KNN), 96.88 (BPANN) and 100 (SVM) have been obtained by Teye *et al*., 2013.

Cocoa products and derivative fraud are related to intentional contamination, and to noncompliance to product descriptions and adulterations. The used adulterants are low-cost raw material (van Ruth, Huisman, & Luning, 2017), such as different flours like carob or chicory, which have been processed to substitute cocoa powder (Loullis & Pinakoulaki, 2018; Salem & Ohaad Fahad, 2012). The NIR technique and the multivariate analysis have been used for the quantitative and qualitative detection of carob flour added to cocoa powder (Quelal-Vásconez *et al*., 2018). In another study, Quelal-Vásconez *et al*., 2019 quantitatively determined the presence of cocoa shells by NIR and a PLS model. These authors also classified between two categories of cocoa blends with 92.5% accuracy: (1) presence of < 5% cocoa shells; (2) presence of > 5% cocoa shells in cocoa powders.

Another adulteration type is to add different vegetal or animal fats to cocoa butter (Jahurul *et al*., 2018; Kucha, Liu, & Ngadi, 2018). These fats can come from pork, palm, Garnicia indica, Madhuca butyracea and of other vegetable origins with lower market values (Reddy & Prabhakar, 1994). These fats are considered cocoa butter equivalents (CBE) and should not exceed 5% of the final cocoa product (EU, 2000). However, these less expensive materials and their intentional additions aim to lower production costs in industry.

No specific regulation exists for the products used as raw materials for the food industry. Industries (beverages, bakery, pastries) are responsible for testing their raw materials and for searching ways to detect adulterants (Beulens, Broens, Folstar, & Hofstede, 2005; Trafialek & Kolanowski, 2017) to ensure the precedence and content of these raw materials. Traceability is one of the ways to ensure the food safety of end products. Other studies have been done to detect added molecules that are not declared in products, like vanillin and ethyl vanillin (Pérez-Esteve *et al*., 2016). Cocoa powder adulteration by identifying the fingerprints of cocoa powder

polysaccharides has been studied, and has provided the possibility of finding as from 15%, or higher, cocoa shell powder and 10% exogenous plant material (Yang *et al*., 2015).

FTIR has been applied to detect cocoa butter equivalents CBE (allowed in chocolate up to 5%: palm oil, illipe, sal, shea, kokum gurgi and mango kernel). FTIR is considered a rapid screening method to distinguish pure and vegetable fats, but a single global statistical model to predict the precise level of added fat is still not available. The large uncertainty in predicting CBE has been connected to the wide natural variability of samples (precise geographical origin). So it was difficult to detect CBE in CB mixtures (e.g. illipe) (Whitacre *et al*., 2003).

Non-destructive technologies have been used to improve processes with new control and evaluation methods (e.g. the fermentation index, the degree of alkalization) and replaced or improving the conventional analysis methods (chromatography, sensory analysis, etc.). Several results about certain features like fat, moisture, color, proteins, pH (Moros, Iñón, Garrigues, & de la Guardia, 2007) and functional compounds (antioxidants) have been obtained by only spectra measurements. As the NIR technology has demonstrated its versatility, its applications are rapidly increasing not only to control the safety of cocoa products, but also to improve their quality, and to optimize times and costs.

Despite all the successful applications regarding the use of alternative methodologies to analyze and control the above-described cocoa quality, their implementation into the cocoa industry poses challenges, such us the simultaneous presence of a variety of chemical compounds (nutrients, phytochemicals, adulterants, contaminants, etc.) in cocoa products with diverse structures and concentrations. This circumstance makes spectrometric signals very complex and difficult to analyze. However, technology is rapidly advancing and new equipment include improved signal collection and software capable of performing chemometric analyses, which are key to acquire reliable information.

5. Conclusions

The analytical methods applied to control the quality and authenticity of cocoa products and their derivatives in industry and research laboratories have mainly been conventional ones to date. They are conventional because they have been used for years and are characterized by tasks like sampling, sample preparation to extract target compounds and quantitative determination by using chemical reagents. The majority of these methods are standardized and used especially for monitoring and optimizing the process during cocoa flow production by individual analyses of attributes (color, pH, acidity and proximal analysis) by wet chemistry. Most of the advances made in these methods are related to analyte extraction to improve sensitivity, accuracy and analysis speed, also to the application of multivariate data analyses. For sensitivity and accuracy determinations, chromatographic methods like HPLC and GCMS, and its inline utilization, are the most well-developed ones, while multivariate data analyses are mainly employed to determine the authenticity parameters (i.e. origin or varietal features) of cocoa products. The most explored non-destructive technique is spectroscopy, which is conducted within the near infrared range, and also within the medium infrared range to a lesser extent. Most NIR and FTIR studies have been conducted in the postharvest stage of cocoa beans by analyzing biochemical parameters like fat, sugars, polyphenols, procyanidins, methylxanthines, moisture and pH, or for the purpose of assessing the authenticity of cocoa and its derivatives by identifying the origin of raw material, varietal purity, compositional parameters or the detection of adulterants.

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Authors' contribution section

M.A Quelal-Vásconez searched the literature, drafted the manuscript and prepared the tables and figures; M.J Lerma-García, E Pérez-Esteve and P. Talens helped to design the framework of this review and critically revised different sections of the draft; J.M Barat performed the final revision and authorized the publication.

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Table 1: General quality attributes of cocoa beans, chocolate and cocoa powders.

Quality attributes	Details	Observation
Cocoa beans		
Size (#beans/100g)	≤ 100	Standard beans
	101-110	Medium beans
	111-120	Small beans
	> 120	Very small beans
Uniformity	Variable-sized beans are harder to break and deshell	
Fermentation	5% slaty, 5% defectiveness	Good fermented
	10% slaty, 10% defectiveness	Fair fermented
Moisture	<8%	Aceptable
Chemical residues	According to authority regulations	Under limits
Chocolate and cocoa powders		
Fat content		Characteristic
Fat quality	Low in free fatty acids, show characteristic melting and solidification properties	
Aroma and flavor	Without moldy off-flavors, smoky taints, acidic off-flavors, proximity to another strong-smelling products	Characteristic
Color		Characteristic
Cocoa powder		
Solubility	95%	Good solubility
Shell content	< 5% in fat free-dry cocoa	Aceptable

CAOBISCO-ECA-FCC (2015), ADM Cocoa Manual (2006)

Table 2: Cocoa powder composition (ICCO, 2012; Krähmer *et al*., 2015; Lacueva *et al*., 2008).

Component	**Major compounds (%)**
Fat	11
Moisture	3
Total nitrogen	4.3
Nitrogen (corrected for alkaloids)	3.4
Protein	20
Nitrogen corrected for alkaloids x 6.25 %	21.2
Ash	5.5
Water soluble ash	2.2
Phosphate (as P_2O_5)	1.9
Ash insoluble in 50% HCl	0.08
	Minor compounds (mg/g)
Flavanols	
Catechin	0.6
Epicatechin	5.7
Methylxanthines	
Caffeine	6
Theobromine	28
Other compounds:	
Total procyanidins	22
Total amino acids	3.4
Total sugars	8.9

Table 3: Non-destructive research of cocoa and sub-products applied in off-line and in-line process mode

Analyzed parameters	**Conventional**	**Equipment and conditions**	**# Samples or spectra**	**Chemometrics**	**Calibration and cross-validation**	**External test set**	**Author**
Beans-Postharvest /Fermentation Total Fungi count	Cut test (Bean fermentation levels, flavor, color, fungi presence and other foreign materials)	**FT-NIR** (Antaris II model, Thermo Fisher Company in the U.S.A); wavelength range (WR): 1000-2500 nm; scans: 5, **Transmittance**	Total: 95 samples; Calibration:57; Prediction:38	PLS; (Si-PLS); (Si-GAPLS); (ACO-PLS) (CARS-PLS)	Correlation coefficient of calibration: 0.97 RMSEcv: 0.402	Correlation coefficient of prediction: Rp 0.951 RMSEP (CFU/mL) 0.384 RPD: 4.32	Kutsanedzie *et al*., (2018)
Beans-Postharvest/ Compositional analysis Fermentation index (FI), pH, and total polyphenol content (TPC)	FI, pH, TPC	**FT-NIR** spectroscopy system (MATRIX-I, Bruker optics, Germany) using integrating sphere; WR: 12500-3600 cm^{-1} or 800 to 2778 nm; resolution 8 cm^{-1}; scans: 64	72 spectra (24 samples 3 replications)	PLS	FI: R^2 of 0.88; RMSEcv=0.06; RPD: 2.74; pH: R^2 of 0.76; RMSEcv=0.26; RPD: 2.05; Total TP:R^2 of 0.84; RMSEcv=0.93; RPD: 2.53; 0.535 and 1.242, TPC 6.48 and 15.58 mg g^{-1}	NA	Sunoj *et al*., (2016)

Table 3 (Continued)

Analyzed parameters	Conventional	Equipment and conditions	# Samples or spectra	Chemometrics	Calibration and cross-validation	External test set	Author
Beans-Postharvest/ Compositional analysis -Phenolic substances, organic acids, epicatechin, lactic acid, fermentation time, pH	High performance liquid chromatography HPLC, FI, sensory test (QDA), Gas chromatography mass spectrometry GCMS, cut test	**FT-NIR** (Multi-Purpose Analyzer, Bruker Optics, Ettlingen, Germany), WR: 12500 to 3600 cm^{-1}; resolution 8 cm^{-1}; scans 32	48 samples	PLS	Leave 10% out Theobromine 1.73-3.02 mg/100g	NA	Krähmer *et al*., (2015)
Beans-Postharvest/ Fermentation - Fermented and unfermented	HPLC, FI, sensory test, GCMS, cut test	**FTNIR**	Classification: FC=26; UFC=26; Adulterated 80. Calibration 90; Prediction 42	SVM, SiPLS	Leave one out	100% classification; RMSEP:0.98, prediction since 5%	Teye, Huang, Lei, & Dai, (2014b)
Beans-Postharvest -TPC	Colorimetry (Folin-Ciocalteu), Thin layer chromatography and HPLC	**ET:** Data collection: Astree brand (Alpha MOS Company, Toulouse, France), potentiometric chemical sensors. **FT-NIR:** Antaris II FT NIR (Thermo Electron Company, USA) equipped with an indium gallium arsenide (InGaAs) photodiode detector. WR: 10000-4000cm^{-1}; Scans 32; interval 3.85 cm^{-1}; resolution 8 cm^{-1}; 25ºC; humidity 60%	110 samples (80 calibration and 30 prediction)	Fusion techniques: low level of abstraction with PCA, (Si-PLS)	NA	Optimal data fusion model: Rp 0.982, RMSEP 0.900 g g^{-1} and bias 0.013 in the prediction set	Huang *et al*., (2014)

Table 3 (Continued)

Analyzed parameters	**Conventional**	**Equipment and conditions**	**# Samples or spectra**	**Chemometric techniques**	**Calibration and crossvalidation**	**External test set**	**Author**
Beans, ground and sieved 400 µm mesh - Postharvest/ Fermentation - pH and FI	pH and FI	**FT-NIR** Antaris II (Thermo Electron Company, USA) with an integrating sphere, WR: 10000-4000cm^{-1}; scans 32; interval 3.85 cm^{-1}; resolution 8 cm^{-1}, 25ºC	Categories Fermented 80, partly fermented 25 and unfermented 25. Model 90 calibration, 40 validation	(BPANN); (Si-BPANNR)	Leave one cross-validation (LOO-CV)	pH was Rp = 0.98 and RMSEP = 0.06, while for FI was Rp = 0.98 and RMSEP = 0.05	Teye *et al.*, (2015b)
Cocoa beans, cocoa liquor - Postharvest/ Fermentation - Amount of ammonia nitrogen (NH3)	Conway technic (Conway & Byrne, 1933)	**NIR** FOSS 6500 monochromator (Foss, Silver Spring, MD) using a spin cell sample module; WR: 400-2500 nm; intervals 2 nm; scans 32. **Reflectance**	190 samples and spectra	PCA (Mahalanobish distance (H) no higher than 3), PLS	R^2c= 0.975, SEC 16 ppm, SE_{CV} 24 ppm; Range 25-441 ppm	R^2p 0.935, SEP 20 ppm Range 46-332 ppm	Hue *et al.*, (2014)
Beans, ground and sieved 500 um mesh - Postharvest/ Compositional - Fat	Fat (Soxhlet extraction apparatus - SOEP, Microwave-assisted process-MAP)	**FT-NIR** Antaris II (Thermo Electron Company, USA) equipped with an InGaAs photodiode detector. WR: 10000-4000cm^{-1}; scans 32; interval 3.85 cm^{-1}; resolution 8 cm^{-1}, 25ºC	80 (50 calibration, 30 prediction)	(Si-PLS), SVMR	Leave one out cross-validation (LOO-CV)	Si-SVMR _ RMSEP=0.015 and Rp=0.9708	Teye & Huang, (2015a)

Table 3 (Continued)

Analyzed parameters	Conventional	Equipment and conditions	# Samples or spectra	Chemometric techniques	Calibration and crossvalidation	External test set	Author
Beans - Postharvest/ Drying - Compositional analysis	AOAC Method to determine purines and procyanidins by HPLC	**NIR:** Monochromator (model 6500; Foss NIRSystems, Laurel, Maryland; WR 400- 2500 nm; intervals 2 nm, scans 32, **Reflection**	Wet chemestry: 40 NIR: 470(Fat), 342 (caffeine), 343(theobromine), 224 (epicatechin)	Neighbourhood Mahalanobish Distances PLS	25% of the samples randomly selected leave out, 4 times.	NA	Álvarez *et al*., (2012)
Beans - Postharvest/ Fermentation - Microbial profiles	Cut test (visual)	**NIR** System 6500, Inc. USA, 400-2500, silicon detector, WR: 400-1100 and lead sulphide detector, WR: 1100- 2500; intervals 2nm, 45º, scans 16, **Reflectance**	50 kg	(DGGE) PCA, PLS 2	Correlations 0.87 (bacterial derived DGCE spectra) and 0.81 (yeast derived DGCE spectra)	NA	Nielsen *et al*., (2008)
Beans - Postharvest/ Fermentation - FI, TPC and antioxidant activity (AA)	HPLC ABTS (Antioxidant activity); QUENCHER "Quick, Easy, New, Cheap and Reproducible method"	**HSI** (Hyperspectral image) system Gilden Photonics Ltd. (Glasgow, UK) SWIR camera (Specim, Oulu, Finland) with a cooled 14 bit 320x256 pixel HgCdTe detector; WR; 1000-2495 nm; resolution 6 nm	17 beans 170 beans/batch; 170 samples; 340 spectra; Calibration:240; Prediction:100	PLS	Full cross-validation, FI: R^2 =0.57 (RMSEc = 0.22; RMSEcv=0.24) TP: R^2=0.82 (RMSEc = 23.35mg ferulic acid g^{-1}, RMSEcv= 28.09; AA:R^2=0.76 (RMSEc =55.25 mmol Trolox . kg^{-1}, RMSEcv =59.23	FI: R^2 = 0.50 (RMSEP = 0.27, RPD = 1.40), TP: R^2=0.70 (RMSEP = 34.1 mg ferulic acid g^{-1}, RPD = 1.77) and AA: R^2= 0.74 (60.0 mmol Trolox . kg^{-1}, RPD = 1.91)	Caporaso *et al*., (2018)

Table 3 (Continued)

Analyzed parameters	Conventional	Equipment and conditions	# Samples or spectra	Chemometrics	Calibration and crossvalidation	External test set	Author
Cocoa butter - Sensorial -Fatty acids -Crystal structure of chocolate	X-ray diffraction (XRD) (characterization of crystal structure)	**THz spectroscopy** 1064 bn Q-switch pulsed Nd: YAG Laser as pump for Cr: For sterite lasers	2 chocolates, 3 samples prepared	NA	NA	NA	Weiller *et al.*, (2018)
Cocoa powder - Industrial processing/ compositional analysis -Fat, nitrogen, and moisture	Soxhlet extraction (Fat), Nitrogen (Kjeldahl), Moisture (in platinum dish in an aerated oven at 100ºC)	**FTIR:** Bruker IFS-55 (Bruker, Germany) with single **reflection** ATR accessory, diamond cell (Golden Gate), DTGS detector and triangular apodization function Resolution 8 cm^{-1}; Background spectrum against the air; co-added scans 128; WR: 1100-2500nm and 4000-600 cm^{-1}. **NIR**: NIR Systems 6500 Persptop Analytical Company, USA. Tugsten filament lamp; resolution 2nm; 3 replicated (36 co-added scans). Ceramic used as photometric standard; scanning speed 62 s; Internal reference was the background spectrum	100	NIR-FTIR data fusion- outer product matrix for two spectra (vectors), PLS	**NIR:** $RMSE_{CV}$ = 7.0%, R^2 =0.96 for fat, 1.7%, R^2 =0.98 for nitrogen, and 5.2%, R^2 =0.94 for moisture. **FTIR:** $RMSE_{CV}$= 10.4%, R^2 =0.94 for fat and 3.9%, R^2 =0.95 for nitrogen.	RMSEP (%): 1.2 fat, 0.10 nitrogen, 0.40 moisture.	Veselá *et al.*, (2007)
Cocoa powder - Industrial processing/ compositional analysis -Moisture, fat, sucrose	Individual determination	**NIR**; WR: 1900-2320 nm single beam NIR spectrophotometer with three tilting filters (Pacific Scientific, Gardner Neotec Division, Model Compscan 3000), **Reflection**	calibration 65 samples, 10 samples prediction	PLS	R^2c= 0.978, SEC 0.157; R^2c= 0.987, SEC 0.100; R^2c= 0.998, SEC 0.526	SEP (%) Moisture 0.034, fat 0.051, sucrose 0.68	Permanyer & Perez, (1989)

Table 3 (Continued)

Analyzed parameters	Conventional	Equipment and conditions	# Samples or spectra	Chemometrics	Calibration and crossvalidation	External test set	Author
Cocoa butter chocolate -Industrial processing/ physical properties – Cocoa butter, Viscosity, enthalpy (crystal content), and slope values. For precrystallized chocolate, analytical values such as viscosity and slope values _ detected off-line and used for calibration of NIR spectroscopy.	Rheology, Viscosity (viscometer_Searle principle), Calorimetry (temper curves)	**NIR:** Universal spectrometer (NIRVIS with the software version BCAP 4.40 CH, Bühler AG, Uzwil, Switzerland); WR: 1000-2500 nm. Light fiber probe inserted in the outlet tube of the shear crystallizer through a special adapter. Cocoa butter: **Transflection** (transmission and reflection measurement used to transparent media) probe, measuring gap width 5mm (end of the probe and a standardized reflection surface) Chocolate: **reflection** probe	In line	PCA, PLS	Correlations with measurements of viscosity, crystal content (R = 0.975) and slope (R=0.945). Precrystallized chocolate correlation (R = 0.973)	NA	Bolliger *et al*., (1999)

Abbreviations: BTS, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); ACO-PLS, Ant Colony Optimization-PLS; FTIR, Fourier Transform Infrared; FT-NIR, Fourier Transform Near-Infrared; GC-O-MS, Gas Chromatography Olfactometry Mass Spectrometry; NA, not apply; NE, not specify; PCA, Principal component analysis; RMSEC, Root Mean Square Error of Calibration; RMSECV, Root Mean Square Error of Cross-validation; RMSEP, Root Mean Square Error of Prediction; RPD, Ratio prediction deviation; Rp, Correlation coefficient of prediction; SEP, Standard Error of prediction; THz, Terahertz; Si-BPANNR, Synergy Interval Backpropagation Neural Networks Regression, Si-GAPLS, Synergy Interval-Genetic Algorithm-PLS.

Table 4: Authenticity evaluated with non-destructive methods

Analyzed parameters	**Conventional**	**Equipment and conditions**	**# Samples or spectra**	**Chemometrics**	**Calibration and crossvalidation**	**Validation test**	**Author**
Beans -Postharvest/Origin -Cocoa variety Moisture Ash Protein Fat Carbohydrates L* a* b* PH	Protein (total organic nitrogen-Kjeldahl procedure). Fat content (Soxhlet apparatus). Moisture (gravimetric method - drying 2 g of sample at 105 ºC to constant weight). Ash (muffle furnace at 550 ºC for 12 h) TPC (difference of components)	**NIR** Near -Infrared (XDS) model XM 1100 series-Rapid Content Analyzer Foss NIR Systems, Denmark; wavelength range WR: 400-2498 nm; 2nm intervals. **Reflection**	80 samples	PLS, SVM, LDA	Full cross-validation (leave one out)	The performance of SVM model was superior to LDA model, achieving an identification rate of 100%.	Barbin *et al*., (2018)
Beans -Postharvest/Varietal discrimination/Authentication -Varietal discrimination	Physical characteristics and Cut test	Handled Raman spectrometer; Raman spectrometer with a 1064 nm Nd:YAG laser; Total exposure time for a Raman spectrum: 15 s; laser power set at 250 mW; resolution 4 cm^{-1}; 100 scans	20 samples (7 national and 13 CN-51 from different locations-25 samples per location were measured)	SVM	Leave one out	NA	Popp *et al*., (2016)

Table 4 (Continued)

Analyzed parameters	Conventional	Equipment and conditions	# Samples or spectra	Chemometrics	Calibration and crossvalidation	Validation test	Author
Beans -Postharvest/-Varietal discrimination/Authentication	Liquid chromatography LC, gas chromatography GC, capillary electrophoresis, sensory evaluation or plasma atomic emission	**ET:** Data collection: Astree brand (Alpha MOS Company, Toulouse, France), potentiometric chemical sensors. NIR: Antaris II **FT NIR** (Thermo Electron Company, USA) equipped with InGaAs photodiode detector. WR: 10000-4000cm^{-1}. 32 scans, 3.85 cm^{-1} interval, 8 cm^{-1} resolution. 25ºC, 60% humidity, 9500-7500 cm^{-1}	65 calibration, 35 validation (samples)	Data fusion by normalization, PCA, SVM	NE	(NIRS and ET) has a classification rate between 83 and 93%. Data fusion (ET-NIRS) had a classification rate of 100%	Teye, Huang, Takrama, & Haiyang, (2014c)
Beans grounded -Geographical origin/Adulteration	Sensory evaluation GCMS, HPLC Colorimetry and inductively coupled plasma mass spectrometry	**FT NIR** Antaris II (Thermo Electron Company, USA) with an integrating sphere, WR: 10000-4000cm^{-1}). Rotating cup 120º, 32 scans, 3.85 cm^{-1} interval, 8 cm^{-1} resolution. 25ºC	194 samples, 130 calibration and 64 prediction	LDA, PLS-DA, KNN, BPANN, SVM	NA	Classification (%) LDA 90.63, KNN 75, BPANN 96.88, SVM 100	Teye, Huang, Dai, & Chen, (2013)
Beans grounded, cocoa liquor - Postharvest/Industrial processing/ Authentication -Total procyanidin oligomers (monomer to decamer)	HPLC - Normal-phase separations of the procyanidin oligomers.	**NIR** Systems II 6500 (NIR Systems Inc. Silver Springs, Md., USA) WR: 400 to 2500 nm, 2 nm intervals, 20 scans. The samples were presented in an open cell (liquor measured to 50ºC). **Reflection**	96 samples	mPLS	Total procyanidins (mg/g) Mean 9.89; SEC: 1.04; SECV: 1.09	10 samples prediction	Whitacre *et al*., (2003)

Table 4 (Continued)

Analyzed parameters	Conventional	Equipment and conditions	# Samples or spectra	Chemometrics	Calibration and crossvalidation	Validation test	Author
Cocoa powder - Adulteration – cocoa shell	Blue value (colorimetric test), HPLC by detection of LGC (fatty acids of cocoa shell)	**NIR** FOSS 5000 (SILVER SRPING, MD, USA); WR: 1100-2500 nm; 2 nm intervals; 32 scans. **Reflection**	90 samples, 70 calibration and 20 prediction.	PLS, PLS-DA, variable selection	Full cross-validation or leave one out; R^2c=0.975; $RMSE_{CV}$:1.91;	R^2P:0.967; BIAS:0.195; RMSEP:2.43; RPD:5.03% accuracy:92.5	Quelal-Vásconez, *et al*, (2019)
Adulteration -Cocoa powder, carob flour	Chromatographic techniques	**NIR** FOSS 5000 (SILVER SRPING, MD, USA); WR: 1100-2500nm; 2 nm intervals; 32 scans. **Reflection**	12 cocoas 234 prepared samples	PLS, PLS -DA	Full cross-validation or leave one out; R^2c=0.98; $RMSE_{CV}$:2.9; SLOPE:0.981	Coefficient of determination for prediction (R^2) of 0.974 and a root mean square error of prediction (RMSEP) of 3.2%	Quelal-Vásconez, *et al*, (2018)
Butter (diluted 1:10, analytical grade acetone)-Industrial Processing/Authentication	Chromatography (triglycerides and fatty acids)	**FTIR:** Bruker IF28 FTIR spectrometer (Bruker Spectrospin Ltd., Coventry, United Kingdom) equipped with a mercury-cadmium telluride (MCT) detector cooled N_2; WR: 4000 to 600 cm^{-1}; Acquisition rate 20s^{-1}; Resolution 4 cm^{-1}; Improve signal-to-noise ratio 256 spectra/sample. **Transmittance**	192 samples (triplicate). 14 CB (10 pure- various geographical origins and 4 commercial mixtures) 18 CBE (12 mixtures and 6 pure CBE) and 154 mixtures of CB with CBE (5 to 20%). Training data encompass the full range under study. Training 186 spectra (62 in replicate) and 183 (61 in replicate)	PC-DFA r canonical varieties analysis (CVA), ANN, PLS	Good classification (10 and 20% adulteration level) of the training set	Non successful classification	Goodacre & Anklam, (2001)

Abbreviations: BPANN, Backpropagation Neural Networks; CARS-PLS, Competitive Adaptive Reweighted Sampling -Partial Least Squares; CB, Cocoa butter; CBE, Cocoa butter equivalent; CVA, Canonical Varieties Analysis; ET, Electronic Tongue; mPLS, Modified Partial Least Squares; N2, Nitrogen; PCA DFA, Principal Component Analysis Discriminant Function Analysis; SVM, Support Vector Machine.