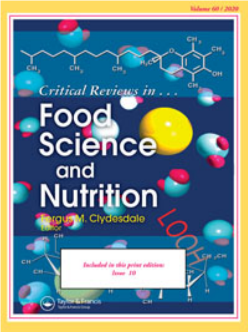

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REVIEW

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An overview of the physical and biochemical transformation of cocoa seeds to beans and to chocolate: Flavor formation

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ABSTRACT
Chocolate is a widely consumed product worldwide due to its exquisite flavor, which comes from the unique and fascinating cocoa flavor. This flavor depends on little controllable variables such as the genotype and the agroecological niche, and on the other side, on postharvest operations: (1) cocoa transformation from seeds to beans that comprises cocoa seeds preconditioning, fermentation, and drying, and (2) the production of chocolate from the bean in which roasting is highlighted. Postharvest transformation operations are critically important because during these, cocoa flavor is formed, allowing the differentiation of two categories: bulk and specialty cocoa. In this sense, this article presents an overview of cocoa postharvest operations, the variables and phenomena that influence and control the physical and biochemical transformation from seeds to cocoa beans, and their relation to the formation of chocolate flavor. Moreover, research perspectives in terms of control and management of postharvest practices in order to obtain cocoa with differentiated and specialty characteristics "from bean to bar" are discussed.

KEYWORDS
Cocoa postharvest operations; flavor precursors; flavor formation; specialty cocoa

Introduction

Nowadays, within the cocoa market, in addition to the bulk cocoa, a new category known as specialty cocoa has been established, which includes fine or flavor cocoa (FFC) and also those that are characterized by differentiating factors such as origin, certifications, and uniqueness (crops in restricted areas) (Ríos et al. 2017).

Specialty cocoa market has experienced the highest percentage of growth compared to other cocoa segments. This demand has been driven by consumer trends in search for healthier chocolate, of single origin and with differentiated organoleptic properties (floral, fruity, caramel, and nutty notes). This type of market offers huge opportunities as well as monetary and non-monetary benefits compared to the bulk cocoa market. The prices in this niche market are independent of the international price of cocoa and are not influenced by the behavior of the London and New York stock exchange markets. For example, premiums of more than US $1000 per ton can be offered for premium and origin cocoa (Ríos et al. 2017; ICCO 2017).

For the reasons explained above, we emphasize that cocoa postharvest transformation should be directed towards the production of specialty cocoa, and controlling the processing conditions according to the quality characteristics demanded by the market. In this sense, several factors influence significantly the attainment of homogeneous and desired quality cocoa.

Chocolate flavor is known to be influenced by the cocoa genotype potential, but also by the postharvest operations to transform the seeds into cocoa beans (which includes stages as seeds preconditioning after harvesting, fermentation, and drying), as well as by the industrial processing of the beans until chocolate is obtained where roasting is underlined (Lima et al. 2011). However, even though fermentation is considered as the "core stage" of the cocoa transformation process from seed to chocolate, it is currently produced mostly by small third-world producers in an empirical way, with little or no technification, without control in processing conditions, originating cocoa batches of low and heterogeneous quality.

It must be highlighted that the cocoa postharvest transformation from seed to bean has not been industrialized in any producing country. The value added in this raw material is retained in the industrial link of the cocoa–chocolate value chain. In fact, processors manufacture premium dark chocolate with high cocoa content and seek to innovate, generating products with a distinctive flavor and that come from specialty cocoa (in which the fine cocoa category is considered), due to new consumer preferences. Indeed, chocolate lovers are willing to pay better prices for a unique flavor in a product (Caligiani, Marseglia, and Palla 2016).

The traditional cocoa postharvest processing is mediated by a dynamic of reactions catalyzed by a succession of

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microorganisms (yeasts, lactic acid bacteria, and acetic acid bacteria) that inoculate cocoa pulp spontaneously, and produce metabolites such as ethanol, and lactic and acetic acids. When these diffuse into the cocoa seed, they generate a decrease in pH (from 7 to 4.0–5.5) and an increase in temperature (up to 45 °C or more). This, in turn causes the death of the seed embryo, the destruction of subcellular structures and the liberation of endogenous enzymes that catalyze the production of peptides and amino acids from seed storage proteins (Hansen, Del Olmo, and Burri 1998). Additionally, the inversion of sugars such as sucrose and the subsequent formation of reducing sugars occurs (De Vuyst and Weckx 2016). These products resulting from enzymatic reactions of proteolysis and hydrolysis of the seed components are considered as flavor precursors. Then, during roasting, these interact through nonenzymatic browning Maillard reactions leading to the formation of molecules such as pyrazines, alcohols, ketones, Strecker aldehydes, pyrroles, furans, terpenes, and terpene alcohols. These are in turn responsible for generating sensory notes, e.g. flowery, fruity, caramel, nutty, among others, that comprise the chocolate flavor (Owusu, Petersen, and Heimdal 2012). On the other hand, the action of polyphenoloxidases reduces the amount of polyphenols that contribute to the astringency and bitterness to the cocoa bean, by converting them into quinones or high molecular mass and water-insoluble tannins (Damoradan, Parkin, and Fennema 2008).

Although, what happens during cocoa postharvest transformation from seeds to cocoa beans have been widely studied in prior published works, the process phenomena responsible for physical and biochemical changes have not been studied in terms of their optimization in a controlled manner. Studies focused on solving this research gap will contribute to define the more favorable physical conditions of the system so the enzymes are able to generate the flavor precursors according to the desired characteristics in chocolate. These phenomena, both of a physical nature (transport phenomena such as mass transfer by acidification and heat transfer) and chemical phenomena (simple reaction networks catalyzed by endogenous enzymes) induce changes in the physical conditions of the transformation systems, especially pH, temperature, and oxygen concentration, which finally are the variables that govern the biochemical changes inside cocoa seeds.

In this sense, different researches have generated some technological bases that propose alternatives to achieve a better control and manage the physical and biochemical cocoa transformation processes from seeds to beans. Some results obtained with the use of starter cultures can be highlighted (Crafack et al. 2014, 2013; Pereira et al. 2012; Lefeber et al. 2012), in which an increase in process speed and the reduction of the variability of the chocolate quality was observed, obtaining a consistent flavor independent of the region and the fermentation method used.

Likewise, in several works postharvest transformation processes under laboratory conditions has been explored (John et al. 2016; Eyamo Evina et al. 2016; Kadow et al. 2015; Yaw 2014), using chemical catalysts during the process such as lactic, citric, and acetic acids, as well as under controlled atmospheric conditions (oxygen and nitrogen) and temperature. The results showed that flavor formation precursors from the proteolysis and the inversion of nonreducing sugars, as well as the decrease of polyphenols was possible with the use of chemical catalysts and controlling physicochemical variables. Additionally, conclusions stated that obtaining cocoa beans with reproducible quality was viable, from which the potential of this methodology for the standardization and mechanization of the cocoa bioprocessing was observed. However, it should be considered that scaling up the bioprocess implies that results are usually different, because the relative speed of the different phenomena changes significantly, i.e. these must be studied in isolation and properly coupled to describe the evolution of the system as a whole (Garcia-Ochoa et al. 2010).

The current review discusses the operations, phenomena, and variables that significantly influence the flavor formation during cocoa transformation from seed to bean and then to chocolate. In addition, we show the current state of the new postharvest processing methodologies that allow obtaining high quality and uniform cocoa beans, as well as research perspectives with focus on the control and management of postharvest processing practices in order to obtain cocoa beans with differentiated and specialty characteristics.

Cocoa genotype

At present, the most widespread cocoa classification is based on the flavor, generating a commercial categorization that includes four main *Theobroma cacao* genotypes. (1) *Forastero*, considered by its quality as bulk. (2) *Criollo*, cataloged as fine and flavor cocoa. (3) *Trinitario*, that is a hybrid between *Forastero* and *Criollo*, which can be considered as bulk or fine cocoa; and (4) *Nacional* that is a native genotype from Ecuador defined as fine cocoa (Badrie et al. 2015).

The importance of the cocoa genotype on quality led to the development of studies that analyze the genetic diversity of cocoa materials in regions or countries in order to optimize the use of genetic resources and propose core collections for the development of breeding programs (Osorio-Guarín et al. 2017).

Table 1 shows some sensory attributes of genotypes evaluated in different countries (Kongor et al. 2016; Afoakwa et al. 2008). Differences in flavor between different genotypes of the same country are observed. Cocoa genotype determines the type and amount of storage proteins, carbohydrates, polyphenols, and the activity of the enzymes within the seeds. The chemical composition of each cocoa genotype, conditions the production of flavor precursors, which are generated during spontaneous fermentation. These precursors are of considerable interest due to their direct role in the final organoleptic quality of chocolate. The typical cocoa flavor is generated once the chemical precursors are transformed during the roasting of the cocoa beans, into key compounds that, depending on their presence, establish the sensorial attributes of cocoa, i.e. flowery, green, chocolate, caramel, sweaty, nutty, and fruity notes (Caligiani, Marseglia, and Palla 2016).

On the other hand, it is noteworthy that flavor formation is influenced by other processes such as enzymatic biochemical reactions that develop inside the seed during

Table 1. Cocoa flavor profile from different origins.

Origin	Cocoa genotype	Flavor characteristics
Côte d'Ivoire	Forastero	Good cocoa impact, low bitterness, low acidity, fruity, nutty
Ghana	Forastero hybrids	Strong basic cocoa, fruity notes
Nigeria	Forastero hybrids	Medium cocoa, occasional off-notes
São Tomé & Principe	Forastero	Good cocoa flavor, bitter, spicy, fruity, earthy
Madagascar	Criollo	Winey, putrid, citrus
Venezuela	Criollo 'Porcelana'	Mild chocolate, slightly bitter, distinct fruity notes (plum and cherry), fruity, nutty
Venezuela	Trinitario	Low cocoa, acidic
Venezuela	Forastero	Fruity, raisin, caramel
Brazil	Forastero	Cocoa impact, bitter, acid, astringent (sometimes rubber, hammy, smoky), some fruitiness, no nutty notes
Colombia	Trinitario and Criollo	Fruity, bitter, cocoa
Peru	Forastero	Slightly bitter and fruity
Ecuador (*Arriba*)	Forastero (Nacional)	Balanced profile, low chocolate, floral, fruity, grass, earthy notes, spicy
Ecuador	Forastero (CCN 51)	Acidic, harsh, low cocoa
Mexico (Tabasco)	Criollo/Forastero hybrids	Low chocolate, strong acid, low fruitiness
Panamá	Forastero	Moderate chocolate, acidic, fruit and nut notes
Jamaica	Forastero	Fruity
Dominican Republic (Sanchez)	Criollo/Forastero hybrids	Low cocoa, flavorless, bitter
Dominican Republic (Hispaniola)	Criollo/Forastero hybrids	Winey, earthy, can have tobacco notes
Costa Rica	Forastero	Fruity, balanced cocoa flavor
Trinidad & Tobago	Trinitario	High cocoa, nutty, raisin, molasses, and winey notes, aromatic
Indonesia	Criollo/Forastero hybrids	Low chocolate, acidic, fruity
Sulawesi	Criollo/Forastero hybrids	High bitter, low sour, low cocoa, astringent
Java	Criollo/Forastero hybrids	Mild, bland profile, acid, low cocoa, light color
Papua New Guinea	Hybrids/pure Criollo and Forastero	Variable strong acid, floral, mild, nutty
Papua New Guinea	Trinitario	Fruity, acidic
Malaysia	Forastero hybrids	Low to medium cocoa, medium to high acidity, astringent (due to fermentation level), phenolic.

Source: The data in this table is being reused with permission from Elsevier (Kongor et al. 2016) and was adapted using data from Afoakwa et al. (2008).

fermentation such as proteolysis, which varies depending on the transformation method considering variables such as time, native microflora, aeration, use of starter cultures, or chemical and/or enzymatic catalysts. Moreover, also results in variability in the peptide pattern in fermented cocoa beans according to their origin (De Vuyst and Weckx 2016).

Apart from that, according to the known common classification, the *Forastero* cocoa variety represents about 90% of the cocoa production worldwide and is cultivated in West Africa, particularly in Côte d'Ivoire, Ghana, Nigeria, and Cameroon. It is generally used for the production of cocoa paste, powder, cocoa butter, and dark and white chocolate. This cocoa variety produces seeds with lower aromatic potential and therefore, it has a lower quality than the *Criollo* variety, and needs a longer fermentation period to generate the flavor precursors that generates the flavor characterized by a strong basic cocoa flavor, without differentiated flavor notes typical of fine chocolate (Aprotosoaie, Luca, and Miron 2016). However, the *Nacional* variety cultivated only in Ecuador, despite being classified as an Amazonian *Forastero*, is an exception within that group, because it produces fine cocoa (*Arriba* flavor) highlighted by spices and floral aromatic notes (ICCO 2017).

In contrast, from the *Criollo* cocoa variety, outstanding products with an excellent flavor quality using shorter fermentation times are obtained. In this case, flavor is defined as soft and highly aromatic (with floral, fruity, nutty, tea, caramel, and molasses notes). However, in order to develop this profile, adequate management is required in the postharvest transformation process (Seguine et al. 2014). This type of cocoa represents 5–10% of the volume commercialized worldwide and is used for manufacturing dark gourmet chocolate (Aprotosoaie, Luca, and Miron 2016). It is estimated that 53.42% comes from Ecuador, followed in decreasing order, by Peru (16.06%), Papua New Guinea (10.42%), Dominican Republic (9.61%), Colombia (3.94%), Madagascar (2.91%), and Venezuela (2.57%) (Ríos et al. 2017).

When comparing chocolates produced with *Forastero* and *Criollo* cocoa varieties, some authors (Kongor et al. 2016) reported finding higher levels of aromatic compounds in chocolates produced with the *Criollo* variety, which may be due to the high concentration of flavor precursors (amino acids, peptides, and reducing sugars) before roasting. In addition, these studies concluded that *Criollo* cocoa has lower concentrations of polyphenols, especially procyanidins, which are attributed to cause astringency and bitterness in chocolate. Furthermore, chocolate derived from this cocoa genotype can also show acid notes (Saltini, Akkerman, and Frosch 2013). Although on the other hand it is reported that due to the short postharvest transformation period given to *Criollo* cocoa, it exhibits lower acidity compared to other genotypes (Ascrizzi et al. 2017).

The *Trinitario* hybrid is cultivated especially in Western India, and Central and South America. This variety shows a flavor with a basic and strong character of chocolate and wine notes (Counet et al. 2004). Nonetheless, there is a particular case of cocoa from Cameroon produced by trinitarian trees, whose flavor has been classified as bulk.

However, the classification of the genetic diversity of cocoa according to its quality potential, taking into account classically the four most known commercial materials already mentioned, is considered as a limited vision. This highlights the fact that there are missing research results that explain the reasons that cause the differences in flavor potential between genetic materials. Moreover, it has been observed that there are significant variances between the cocoa genotypes, but no relationship has been established among the flavor potential and the key enzymatic activities in raw cocoa seeds, and the type of flavor precursors generated. Therefore, the regulation of enzymatic processes, the substrates and products that are related to desirable flavor, and the limiting factors for the enzymatic contribution to the fermentation processes are not yet clear (Afoakwa et al. 2008).

Hence, It is necessary to investigate the postharvest transformation process with the largest number of varieties reported as different due to their genetic fingerprint identity, in order to find the reasons that cause dissimilarities between materials related to flavor precursors which are responsible for the formation of the molecules that generate the flavor profile in chocolate (Caligiani, Marseglia, and Palla 2016). Namely, it must be demonstrated that, genetically, the *Criollo* variety, unlike the *Trinitario* or another variety, can code to produce certain compounds. Among these, we find proteins, carbohydrates, and on the other hand enzymes, which as has been demonstrated by different studies (Voigt, Biehl, et al. 1994; Biehl and Passern 1982; Biehl, Wewetzer, and Passern 1982), generate the chemical compounds during the postharvest transformation responsible for the formation of outstanding and desirable flavor attributes typical of fine cocoa.

In addition, research is still needed on how to develop an outstanding flavor profile, despite some cocoa materials being classified as *Forasteros*, and be valued for their differentiated quality, as in the case of the *Nacional* cocoa variety. In contrast, also the reasons why some trinitarian materials, considering the theory of a high potential to develop a special flavor, are categorized as bulk cocoa (Cameroon cocoa case).

Recently, Kumari et al. (2018) through the study of the peptidomic and proteomic cocoa fingerprints, found that the protein content serves to classify cocoa according to its geographical origin and genotype. Thus, the quantification of the degradation of the albumin subunit of 17-kDa could facilitate the distinction of African cocoa, meanwhile the quantification of the degradation of the vicilin subunit could serve to classify the South American cocoa samples with different genetic codes. In addition, authors showed that the peptide profile is an indicator that allows evaluating the cocoa quality since it defines the degree of fermentation of cocoa and is independent of its origin.

Studies aimed at showing the relationship between genetic diversity and its quality potential to generate flavor precursors have transformed the materials using different protocols, without considering that the transformation dynamics of the cocoa varieties can vary. This means that it is possible that during fermentation, each variety reaches an optimum concentration point of flavor precursors at a specific time. Based on the above, always one cocoa sample has been analyzed in terms of quality from a single final transformation process time, through the identification and quantification of flavor precursors, sensory profile and the determination of volatile organic compounds in chocolate. In this way, it is of great interest to evaluate the complete transformation dynamics of cocoa materials that will be compared, so that independently for each material, the completion of the postharvest transformation should be determined, depending on the time in which the maximum point of flavor precursor concentrations is reached.

Thus, on the other hand it is confirmed under the same essay conditions, the differentiation between the initial genetic compositions of different materials, i.e. content of proteins, carbohydrates, and phenols; and the potential of these to generate typical fine cocoa sensory profiles. Likewise, although different studies mention that *Criollo, Forastero,* or *Trinitario* materials are being used in experiments, this statement is not supported by molecular studies that certify the relationship between genetic diversity and quality; this is even more relevant, taking into account that high hybridization can occur in this crop.

Postharvest operations for cocoa transformation from seeds to beans: generation of cocoa flavor precursors

The main interest of the physical and biochemical transformation of cocoa seeds to beans is to generate flavor precursor compounds: free amino acids, peptides, and reducing sugars; furthermore, from specific components of the seed: proteins and carbohydrates through reactions catalyzed by enzymes that take place inside the seed.

The microorganisms and their metabolites that act in spontaneous fermentation, the use of chemical catalysts and/or starter cultures have been reported as inducers of different biocatalytic processes that occur inside the seeds. Figure 1 shows schematically the changes occurred in the seeds during their transformation to cocoa beans, the process phenomena that modulates these changes, as well as the dynamics of chemical compounds generated, considered as flavor precursors.

Knowledge of the phenomena that modulate the biochemical reactions that occur during the transformation of cocoa seeds is necessary to establish a control over this bioprocess, based on its proper management to produce cocoa with desired quality characteristics (Caligiani, Marseglia, and Palla 2016). In this sense, some authors have studied this bioprocess under laboratory conditions to generate a knowledge base as a guide to elucidate the dynamics of flavor formation during cocoa transformation from seeds to beans (John et al. 2016; Eyamo Evina et al. 2016; Kadow et al. 2015; Oracz and Nebesny 2014; Yaw 2014; Amin, Jinap, and Jamilah 1998; Voigt and Biehl 1995; Voigt, Wrann, et al. 1994; Voigt, Biehl, et al. 1994; Voigt, Voigt, et al. 1994; Biehl et al. 1985). These studies are of great relevance for

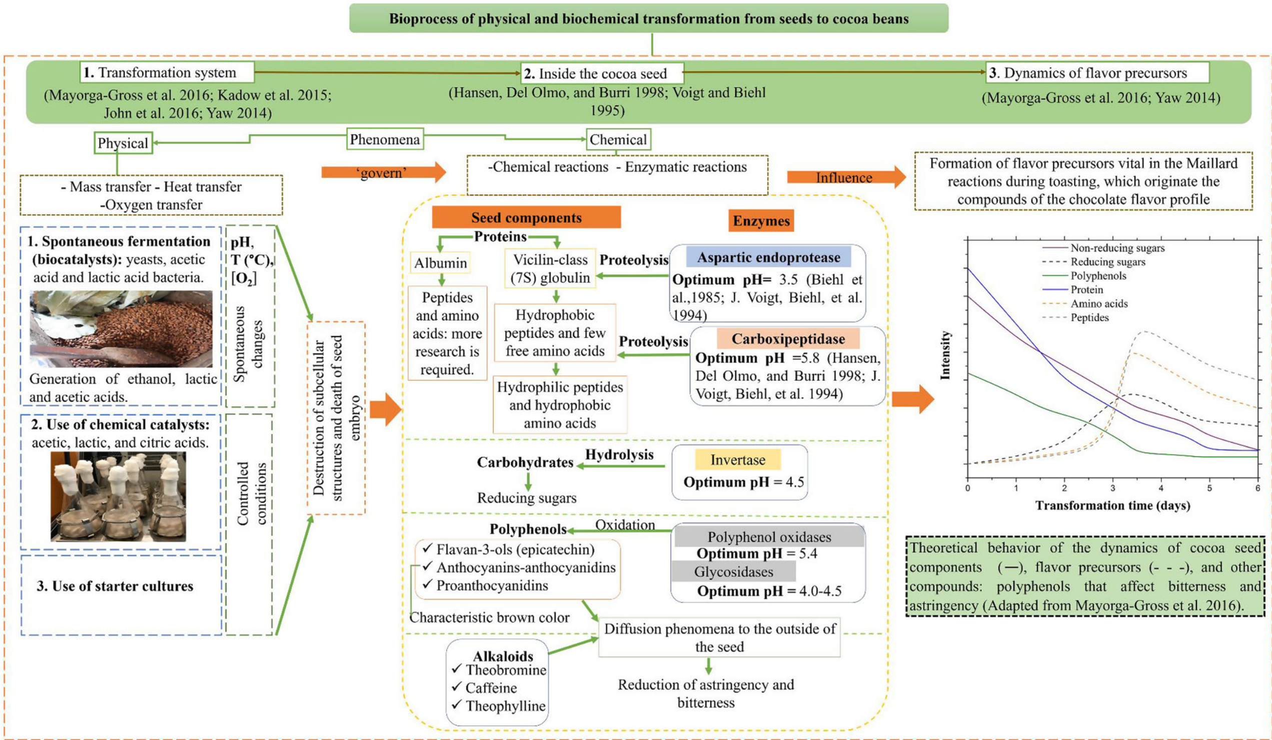

Figure 1. Relationship between the process phenomena that controls and directs physical and biochemical cocoa transformation from seeds to beans, and its effect on flavor precursor generation. Source: elaborated by the authors.

being pioneers in developing methodologies that allow carrying out the cocoa transformation of seeds in a controlled manner. In this way, under the same process scheme, future studies will be able to approach different variables responsible for the generation of flavor precursors, both in isolation and coupled. These include presence/absence of microorganisms, temperature, oxygen concentration, turbulence, type, and concentration of chemical catalysts (acetic, lactic, and citric acids), and pH, with the interest of managing and optimizing cocoa transformation from seeds to beans.

As shown in Fig. 1, seed transformation comprises a set of reactions biocatalyzed by their endogenous enzymes: proteolysis, hydrolysis, and oxidation, from constituent components such as proteins, carbohydrates, and polyphenols, which are specific substrates of biocatalytic activity.

Flavor precursors as biomarkers for process control. Fig. 2 presents in more detail the chemical changes related with these cocoa seed constituents which are the base for the flavor precursors formation.

Proteins. After lipids, proteins are the second most abundant constituent in cocoa beans and represent 10–15% of the dry weight of the seeds. Of the total protein content, albumin constitutes 52%. Moreover, 43% corresponds to the storage protein vicilin (7S)-class globulin (Kumari et al. 2016; Afoakwa et al. 2008).

During the transformation of cocoa seeds, proteins are hydrolyzed. This reaction is mediated by the physicochemical conditions of the process: temperature and pH, and occurs between the vicilin (7S)-class globulin protein and albumin, and the joint action of the enzymes: aspartic endoprotease and carboxypeptidase of the cocoa seeds. Proteolysis is one of the most important biocatalyzed reactions because it generates flavor precursors as peptides and free amino acids (Caligiani, Marseglia, and Palla 2016). Hue et al. (2016) point out that differences in flavor are due to the degree of proteolysis achieved during fermentation, instead of the protein combination of the cocoa genotypes, and each cocoa material has its own protein kinetics degradation.

The formation of cocoa-specific aroma precursors depends on both the structure of the globular storage protein (vicilin 7S), and the particular specificity of cleavage of the cocoa aspartic protease (Janek et al. 2016; Kumari et al. 2016; Voigt and Biehl 1995; Voigt, Wrann, et al. 1994; Voigt, Voigt, et al. 1994).

Furthermore, the degradation rate of different subunits of vicilin-class globulin (7S) varies throughout the fermentation, which suggests that its decomposition is not homogeneous. As shown in Fig. 2a, it was reported that the two largest variants of vicilin can be cleaved extensively during cocoa fermentation, so they could be the main source of oligopeptides. Conversely, the smaller subunits may experience a different type of degradation, which culminates earlier and could contribute to the generation of free amino acids or di and tripeptides (Kumari et al. 2016).

The diversity and dynamics of peptides originated from proteins, contributes to define the most relevant ones (i.e. hydrophilic peptides and hydrophobic amino acids) that can be considered as biochemical markers of the cocoa transformation process, and in that sense, those useful for establishing cocoa quality (Kumari et al. 2016; Marseglia et al.

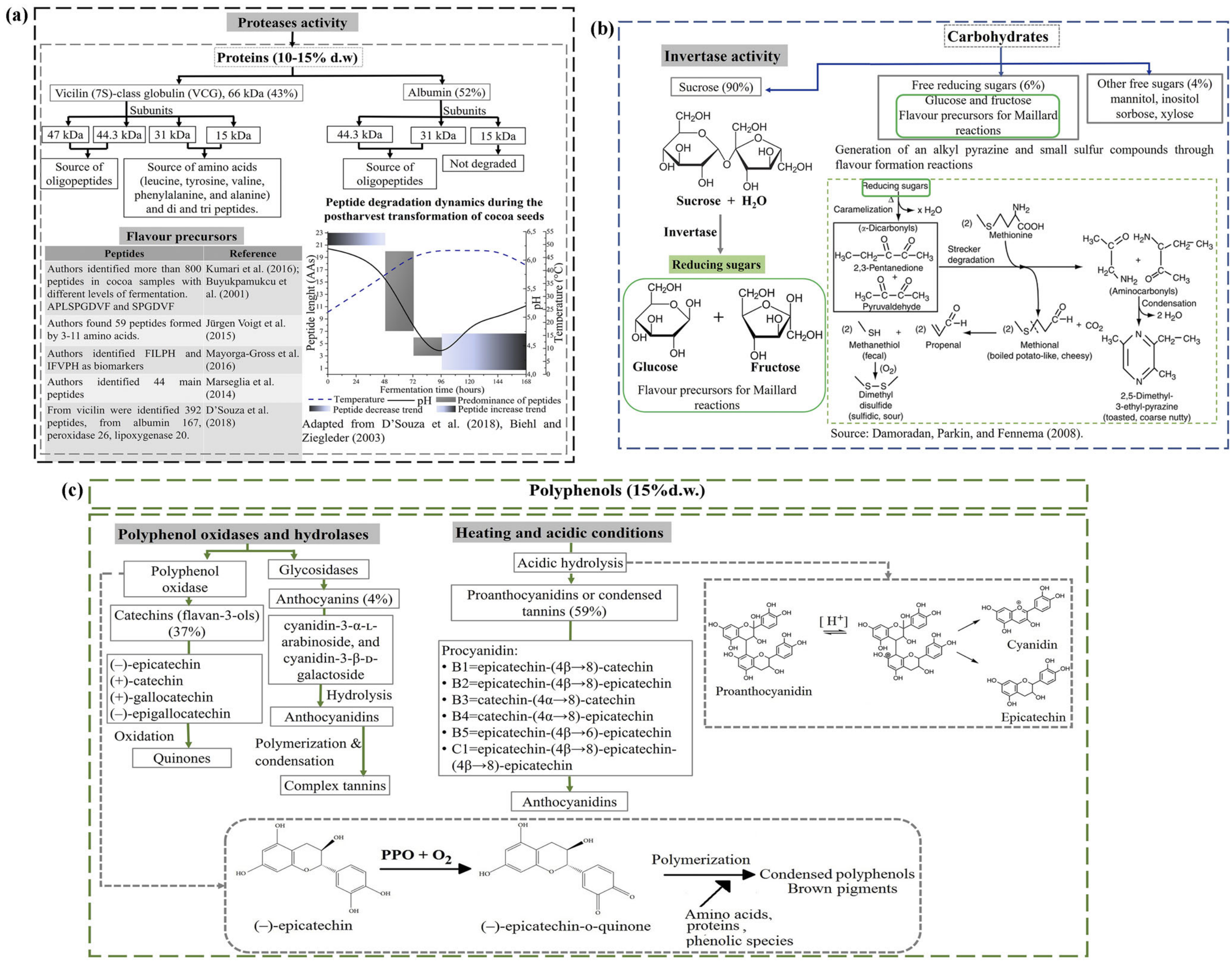

Figure 2. Chemical changes of the cocoa seeds constituents and the generation of flavor precursors compounds during cocoa postharvest transformation.

2014). Additionally, it must be highlighted that the identification and sequencing of relevant peptides is of interest because precursor regions can be determined in the protein (considering that there are different subunits derived from this). Thus, as shown in Fig. 2a, in several studies (Kumari et al. 2018; Buyukpamukcu et al. 2001; Voigt et al. 2016; Mayorga-Gross et al. 2016; Marseglia et al. 2014; D'Souza et al. 2018) some peptides were informed as flavor precursors.

Recently, D'Souza et al. (2018) reported the most robust study of cocoa peptide identification found in the literature. For the first time, the relative quantification of peptides during the bioprocess of spontaneous cocoa fermentation was documented. Thus, a new way of evaluating the cocoa quality, different from the physical methods used, could be considered in terms of the analysis of peptides formed during postharvest transformation. Among the most interesting results of this research is the trend of degradation of larger peptides into smaller peptides as fermentation proceeds, and the degradation pathways can be followed quantitatively. The authors presented the predominance and tendency of certain types of peptides according to their length at specific points in the fermentation (Fig. 2a). Additionally, in the peptide dynamics, we highlight the importance of variables such as pH and temperature during the bioprocess because they determine the enzymatic activity of the proteases. In this sense, future research that correlates the operation variables with the flavor precursors formation is required to determine the optimum processing conditions that maximize the production of these flavor compounds. On the other hand, the study of the C- and N-termini in the peptides is of great interest because it shows the potential of the functional groups that can react in the processes of flavor formation. The amine moieties at the N-termini are important because they participate in the Maillard reactions together with reducing sugars. D'Souza et al. (2018) found peptides that have the C- and N-termini with relative abundances of Ala, Asx, Glu, Phe, Ile, Ser, and Val during fermentation and these showed a maximum tendency at 48 h of fermentation. Interestingly, the above shows the selectivity of proteases for specific amino acids that can participate in flavor reactions by Strecker degradation. Finally, the authors state that the previous studies showed a limited number of peptides as possible flavor precursors and with the findings of their work, i.e. finding hundreds of peptides, new research perspectives are opened to discover their role in flavor

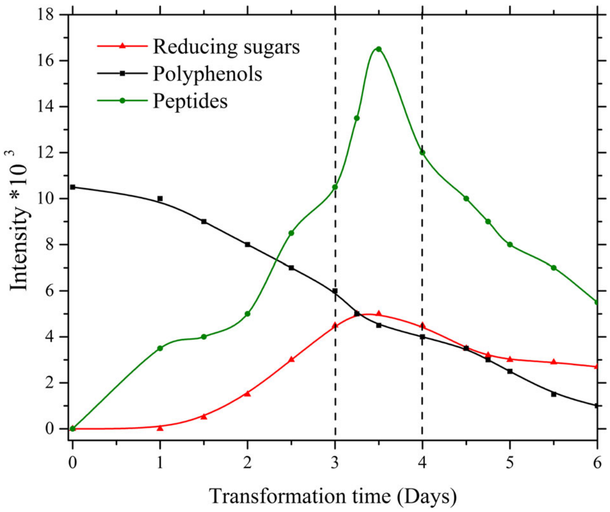

Figure 3. Flavor precursor dynamics in cocoa seeds during spontaneous fermentation. Source: elaborated based on the information reported by Mayorga-Gross et al. (2016).

formation by studying the reactions pathways that develop during roasting.

Generally, in vitro studies in which proteins and proteases from cocoa seeds were isolated, as well as works on the evaluation of enzymatic activity and the generation of peptides and key amino acids in flavor formation, reported that these precursors come only from the degradation of the vicilin-class globulin protein by coordinated action of the aspartic endoprotease and carboxypeptidase. The first enzyme releases hydrophobic peptides but low amounts of free amino acids from cocoa seeds, and the latter, releases hydrophilic peptides and hydrophobic amino acids from cocoa seeds (Voigt and Biehl 1995; Voigt, Heinrichs, et al. 1994). However, recent studies have shown that flavor precursors are also generated from albumin (Caligiani, Marseglia, and Palla 2016; Marseglia et al. 2014). Caligiani, Marseglia, and Palla (2016), finding that *Criollo* genotypes obtained a higher proportion of peptides from the albumin protein. This finding must be studied in more detail, since this protein is not considered as a precursor substrate for the generation of specific cocoa flavor. This is of particular interest as this cocoa variety is considered as fine flavor cocoa. Marseglia et al. (2014) also found a considerable number of oligopeptides derived from albumin in samples of properly fermented cocoa beans.

In this sense, some investigations have focused on analyzing the dynamics of flavor precursors (referred to as metabolomic changes) during the process of physical and biochemical transformation of cocoa seeds through spontaneous fermentation (Figure 3). It was observed that after 3–4 days, most peptides, free amino acids, and reducing sugars (glucose and fructose), increased to maximum levels and decreased in the following days (5–6 days). It seems that in this period, flavor precursors reach the optimum balance, which leads to presume that a short fermentation time (3–4 days), instead of a traditional fermentation period (6 days), is sufficient to obtain cocoa with the potential to develop a quality flavor. This is due to the increase in the content of flavor precursors (Mayorga-Gross et al. 2016; Janek et al. 2016).

Carbohydrates. Fig. 2b shows the hydrolysis enzymatic reaction that sucrose undergoes to produce reducing sugars (glucose and fructose), considered as necessary flavor precursors involved in nonenzymatic Maillard browning reactions. Additionally, cocoa seeds contain free reducing sugars that could also participate in these important flavor reactions.

We present the chemical formation process of a specific chocolate flavor compound: alkyl pyrazine from these chemical precursors. It starts with the interaction of α-dicarbonyl compounds with amino acids (in this case methionine) through the Strecker degradation. The amino acid leads to formation of a volatile organic compound (VOC) (methional) that contains both aldehyde and thioether groups. This VOC decomposes further to generate methanethiol that oxidizes to dimethyl disulfide. In this way, it is obtained a source of reactive, low molecular weight sulfur compounds that contribute to chocolate flavor development. On the other hand, the transfer of the amino group to the dicarbonyl facilitates the integration of amino acid nitrogen into small compounds that then suffer condensation reactions and as a product, 2,5-dimethyl-3-ethyl-pyrazine is generated, which is associated with toasted and nutty flavor notes (Damoradan, Parkin, and Fennema 2008).

Polyphenols. Other components that contribute to define the flavor profile and are responsible for the formation of the brown color of cocoa beans are polyphenols. The main groups are catechins, anthocyanins, and proanthocyanidins. As shown in Figure 2c, catechins are oxidized to quinones through enzymatic reactions catalyzed by the polyphenol oxidase (PPO). Reactive quinones can irreversibly react and polymerizes with nitrogen compounds such as amino acids, phenolic species, peptides, and proteins to form condensed tannins of high molecular mass (molecules formed by more than three subunits that are insoluble, i.e. brown pigments) (Hue et al. 2016;Wollgast and Anklam 2000).

Polyphenols dynamics during the postharvest transformation of cocoa shows that after 3–4 days of fermentation, these decreased during bioprocess. This is because diffusion transport phenomena lead polyphenols spread outside the cocoa seeds, reducing the bitterness, and astringency of the cocoa beans (De Vuyst and Weckx 2016).

In addition, Fig. 2c presents that anthocyanins as 3-β-D-galactosidyl and 3-α-D-arabinosyl-cyanidins, are hydrolyzed by glycosidases enzymes, acting in the sugar moiety and this generates an anthocyanidin (an aglycone: the nonsugar hydrolysis product). This hydrolysis causes changes in coloration and the brown color typical of well-fermented cocoa is generated (Schwan and Fleet 2014).

Proanthocyanidins, other polyphenols known as condensed tannins, that are mostly flavan-3,4-diols, forming 4–8 or 6–8 dimers and trimers, or oligomers with epicatechin as the main extension subunit, under heating and acidic conditions are hydrolyzed to anthocyanidins (into cyanidin and epicatechin). Then the subsequent products in both type of reactions are oxidized and polymerized (Damoradan, Parkin, and Fennema 2008).

Process phenomena. In the bioprocess of physical and biochemical transformation of cocoa seeds, the followings transverse phenomena are found: mass transfer (caused by acidification and oxygen transfer) and heat transfer (generated by the increase in temperature), which are initially necessary for the destruction of the subcellular structures in the cotyledons. This allows the interaction between seed components (substrates: proteins, carbohydrates, and polyphenols) with the enzymes (proteases, hydrolases, and oxidoreductases). Furthermore, acidification and heat transfer are essential for the formation of flavor precursors, because they create inside the seed, an environment with optimal pH and temperature conditions for cocoa biocatalysts (enzymes: endoprotease, carboxypeptidase, invertase, PPO, and glycosidases). In this way, the enzymes can act on the seed components and catalyze the reactions that generates flavor precursors (Voigt and Biehl 1995; Biehl et al. 1985).

It should be noted that the aforementioned phenomena and the composition of the seed could even be affected before the central transformation process of seeds to cocoa beans (fermentation) occurs. This is due to preconditioning treatments such as pre-drying (Biehl et al. 1990; Duncan et al. 1989), depulping of seeds (Meyer et al. 1989), and pod storage (Afoakwa et al. 2013; Nazaruddin et al. 2006). Therefore, the importance of studying their contribution to the formation of cocoa flavor is highlighted, and will be discussed later on.

As we mentioned before, changes in the cellular structures of cocoa seeds depend on the temperature, the acidification degree, and the lipid content. In vitro laboratory studies, which used acetic acid as a chemical catalyst in the cocoa seed transformation process, found that proteins hydrate and become more accessible to proteases in a temperature range from 40 °C to 45 °C, before the fusion of the lipid vacuoles occurs at 50 °C. This phenomenon inhibits the entry of water into protein storage vacuoles and decreases its solubility before the entry of acids. At 50 °C the proteins are insoluble and less accessible to proteolytic enzymes (because they are coated by lipids) when the pH is reduced before the water enters the vacuoles that contain them (Biehl and Passern 1982; Biehl, Passern, and Passern 1977).

The mass and heat transfer phenomena also significantly affect the catalytic activity of cocoa seed enzymes, because they direct the internal conditions of the medium. For this reason, the optimal operation conditions of the biocatalysts of the cocoa processing bioprocess are of vital importance to modulate biocatalytic reactions in order to promote the formation of specific flavor precursors in cocoa. According to Biehl et al. (1985) and Voigt, Biehl, et al. (1994) the optimal pH value of the aspartic endoprotease corresponds to 3.5, meanwhile the optimal activity of carboxypeptidase occurs with a pH of 5.8 (Hansen, Del Olmo, and Burri 1998; Voigt, Biehl, et al. 1994).

Other authors propose that a very strong acidification (pH 4.5–4.0) results in cocoa batches with low flavor potential, because it generates a nonspecific proteolysis of the cocoa seed proteins. As a consequence, specific flavor precursors are not obtained, and by contrast, cocoa with low content of free amino acids and a large amount of hydrophobic peptides is generated. Conversely, high flavor potentials are obtained from fermentations with moderate acidification (pH 5.5–5.0), where the proteins are selectively degraded. Therefore, cocoa results with high levels of hydrophobic amino acids (leucine, valine, alanine, phenylalanine, tyrosine) and hydrophilic peptides compared to acid fermentations (pH 4.5–4.0) (Voigt and Biehl 1995; Voigt, Biehl, et al. 1994).

Moreover, Biehl et al. (1985) found that the biocatalyst activity of the enzymes, the phenomena that occurs inside the seed and the intensity of the flavor cannot be correlated in a simple way. From laboratory essays using acetic acid as a chemical catalyzer, authors concluded that a high flavor potential could be produced with low acidification and low content of amino acids and peptides in the seeds. Furthermore, in spontaneous fermentation trials, the cocoa flavor potential was independent from the amount of proteolytic products of the seeds. Their findings suggest that it is probable that the presence of specific peptides is more important than the total content of peptides or amino acids released, highlighting also that in cocoa seeds, amino acids are not critical flavor precursors.

On the other hand, the postharvest treatment of cocoa seeds also contemplates drying as a stage that receives special attention within the physical and biochemical transformation. This process will be addressed in more detail later.

Operations of cocoa seeds preconditioning

Cocoa seed composition and its relationship with the biochemical transformation in postharvest operations

As shown in Figure 4, the cocoa seed is composed of four main parts: the seed coat (seed cover or shell, which comprises 10–14% of the dry weight), the embryo or germ, two cotyledons (86–90% of the dry weight of the seed) and the mucilaginous pulp that surrounds the seed and constitutes approximately 40% of its fresh weight (Kongor et al. 2016; Lima et al. 2011). Fresh cocoa seeds are composed of sugars (4–6% starch, 4–6% pentosans, 2–3% cellulose, 2–3%

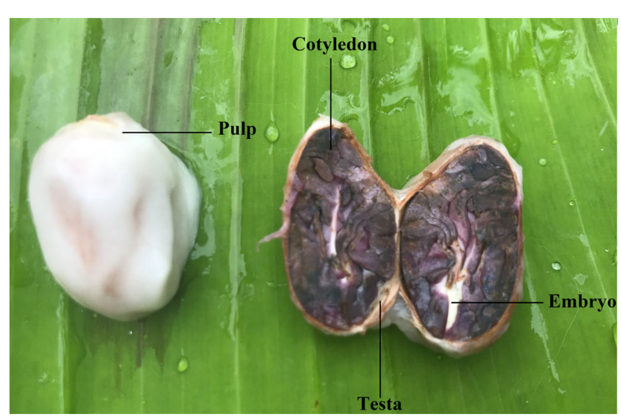

Figure 4. Cocoa seed. Its structure is given by four primary components: (1) the pulp that surrounds the seed, (2) the testa that corresponds to the seed layer or outer membrane, (3) the cotyledon, and (4) the embryo. Source: picture taken by the authors.

sucrose), proteins such as albumin and vicilin-class (7S), globulin (10–15%), and polyphenols (15% in total, but from this percentage, there are 59% proanthocyanidins, 37% catechins or flavan-3-oles, and 4% anthocyanins). Depending on anthocyanins content in polyphenol storage cells, cocoa seeds can oscillate between white and dark purple. The latter is due to the delocalization of π electrons in the structure of the chromophore molecule with eight conjugated double bonds. In addition, alkaloids (1–2% theobromine and 1% caffeine), contribute to the definition of the sensory chocolate profile, specifically with the bitter notes (De Taeye et al. 2016).

Furthermore, cocoa pulp is a substrate that can be metabolized easily and spontaneously by the microorganisms involved in cocoa fermentation such as yeasts and bacteria, due to its composition. This substrate consists of 82–87% of water, 10–15% of sugars (glucose, fructose, and sucrose), 2–3% of pentosans, 1–3% of citric acid and 1–1.5% of pectin (Kongor et al. 2016; Leal et al. 2008). Some authors highlight its great influence on the development of flavor due to its composition. This is how in the *Criollo* genotype, part of the characteristic flavor is attributed to the pulp (Ascrizzi et al. 2017).

The initial pulp content and the aforementioned compounds can be modified by cocoa seed preconditioning and influence the subsequent transformation stages. Due to the above, changes in pulp properties affect the production of metabolites such as ethanol and lactic and acetic acids generated by microorganisms. These are compounds that play a key role in the dynamics of the physical and biochemical transformation of cocoa seeds, because they induce changes in the subcellular structures of these, and encourage the initiation of a network of reactions biocatalyzed by endogenous enzymes that lead to the formation of flavor precursors compounds (Meyer et al. 1989).

Preconditioning is considered as a transformation operation and is linked to different practices carried out by producers before opening the cocoa pods or after carrying out seed extraction, and before fermentation. These practices are determined by logistical aspects of the harvest such as cropping extension and labor availability, or by application of traditional knowledge.

Moreover, preconditioning operations reported in the literature are cocoa pod storage, pre-drying, and cocoa seeds depulping (Kongor et al. 2016; Afoakwa et al. 2008; Schwan and Wheals 2004; Meyer et al. 1989).

Cocoa pod storage. This type of preconditioning consists in collecting the pods after harvesting for a period prior to the physical and biochemical transformation of seeds. It was found that implementing cocoa pods storage, fermentation is faster, but it also increases the possibility of over-fermentation. In addition, the water and sugar contents in the pulp decreased. This influences the microbial dynamics, since the availability of the substrates necessary for yeasts and bacteria (that inoculate spontaneously the pulp of the seeds) decreases; and this affects their metabolic processes, for example the acetic acid production, which in high quantities, generates a cocoa with an elevated level of acidity.

Several authors have evaluated the effect of cocoa pod storage on the physical and chemical characteristics of seeds after being transformed by spontaneous fermentation. The results indicate that cocoa pod storage increases the pH and reduces considerably the acidity in fermented and dried cocoa beans. This factor could be beneficial to improve the acid taste that some types of cocoa tend to develop. Additionally, as the storage time increases, the levels of reducing and non-reducing sugars, fat, proteins, and polyphenols such as (−)-epicatechin and (+)-catechin, also decrease. This leads to a certain improvement in the sensory properties of cocoa, due to the reduction of acidity, bitterness, and astringency, because the content of acids and polyphenols decreased (De Vuyst and Weckx 2016; Schwan and Fleet 2014; Saltini, Akkerman, and Frosch 2013; Afoakwa et al. 2013; Afoakwa et al. 2008; Nazaruddin et al. 2006; Biehl and Ziegleder 2003).

However, it is necessary to study with more detail the effect of the reduction of compounds such as sugars and protein on the dynamics of flavor precursor formation during postharvest transformation and the flavor potential that determines the organoleptic quality of chocolate.

Pre-drying of cocoa seeds. It has been considered as an alternative and the most efficient method to reduce seed acidification during fermentation. The surface of the cocoa seed pulp is subjected to rapid drying by natural sun convection or forced convection with hot air using mechanical dryers (Biehl et al. 1990). Biehl et al. (1990) and Duncan et al. (1989) found that pre-drying led to a decrease in the volume, the water and the sugar contents of the cocoa seed pulp. Moreover, the spontaneous fermentation bioprocess was developed in a similar way when pod storage was carried out as a preconditioning method. We highlight some effects of this preconditioning treatment in the biochemical transformation of the seeds by spontaneous fermentation. Among these, we include a decrease in the time that the anaerobic phase lasts, a temperature increase in a shorter period, and a significant reduction in the contents of acetic acid and polyphenols. Furthermore, fermented and dried cocoa beans show higher pH values, which allows improving the chocolate flavor due to the reduction in acidity, bitterness, and astringency (Afoakwa et al. 2008; Camu et al. 2008).

Depulping cocoa seeds. This operation is carried out using equipment that eliminates up to 10% of the weight of the fresh seed without affecting its physical structure. It allows decreasing slightly the volume of pulp during fermentation, minimizing the production of acid compounds in cocoa beans after the bioprocess, which favors the development of a pleasant flavor profile in chocolate (Camu et al. 2008; Meyer et al. 1989).

The previous preconditioning treatments affect the phenomena of mass transfer (inside the seed) and oxygen transfer (inside and outside the seed), as well as the biochemical reactions that occur during the bioprocess. Mass transfer occurs with the diffusion of acetic acid from the medium to the seed, which involves the destruction of cellular structures and the consequent development of the reactions biocatalyzed by the endogenous enzymes that direct the formation of flavor precursor molecules. Acidification controls biocatalysis as the enzymes involved in the bioprocess have optimal pH values. On the other hand, the oxygen transfer into the fermentation mass is favored by the decrease in the total pulp content. However, the effects of aeration have been poorly studied. Finally, the reduction in compounds such as sugars in the pulp and polyphenols in the seed denotes desirable modifications in cocoa. For example, aeration promotes oxidation and condensation of polyphenols with subsequent decrease of bitterness and astringency from the sensorial viewpoint. Nevertheless, the compositional changes inside the seed compared to the content of protein and sugars require further investigation, in order to explain the influence of its decrease on the dynamics of flavor formation precursors and their relationship with cocoa quality.

Physical and biochemical transformation of cocoa seeds: biocatalytic and/or catalytic processes

Ideally and with a forward-looking approach, the physical and biochemical transformation of cocoa seeds should be managed in a targeted manner. It is necessary to establish the physical and chemical process conditions that promote the occurrence of biochemical reactions to generate optimally, cocoa flavor precursors.

However, currently the cocoa postharvest transformation is traditionally developed as a spontaneous fermentation and under uncontrolled operating conditions, through the activity of a microbial succession of yeasts, lactic acid, and acetic acid bacteria on the pulp that surrounds the seeds (Pereira et al. 2012). These microorganisms are naturally present on the surface of the cocoa pods, the hands of workers who carry out harvesting and postharvesting activities, and the tools used in the fermentation process (Crafack et al. 2014; Ozturk and Young 2017). Nonetheless, in the bioprocess the enzymatic reactions within the cotyledons also occur, as explained previously. Under this context, research has been developed in order to optimize the postharvest transformation of cocoa seeds. In this field of study, the use of starter cultures and chemical and/or enzymatic catalysts is highlighted to evaluate their influence on the chemical and sensory characteristics of cocoa. This is done by studying the formation of flavor precursors, the composition of VOCs, and the sensory profile of chocolate (Kadow et al. 2015; Crafack et al. 2013, 2014; Lefeber et al. 2012).

In this section, we will discuss three technologies that have been studied for the transformation of cocoa seeds: spontaneous fermentation, use of chemical, and/or enzymatic catalysts, and the use of starter cultures, under the approach that involves the correlation between the isolated and coupled physical and chemical process phenomena that controls the dynamics of flavor precursor generation.

Traditional process by spontaneous fermentation

Under the name of spontaneous fermentation, we include the fermentation traditionally carried out by cocoa producers on their farms, which is developed as an uncontrolled bioprocess and has not been industrialized. There are large variations between the systems and methods used by producers to carry out fermentation both internally within the same country (Table 2: Colombia case), as well as between different cocoa producing countries (Table 3: comparative case between Colombia and Nigeria). Most research has been directed, on the one hand, to generate information that contributes to understand how the physical and biochemical transformation of seeds occurs, and on the other hand, to generate solutions for problems in the traditional process that takes place on the farm.

Transformation of cocoa seeds by spontaneous fermentation comprises two dimensions that can facilitate their study: methodologies and systems/equipment to carry out the process.

Process methodologies. Currently, a methodology designed specifically to optimize the generation of flavor precursor compounds of postharvest transformation of cocoa seeds has yet not been published. Traditionally, the fermentation method carried out by producers involves two transformation phases, an initial anaerobic and then an aerobic. The duration of these phases and the aeration frequency of the mass in the aerobic phase varies depending on the cocoa producer (Escobar et al. 2019).

All the reactions that can occur in both phases inside the seeds are mediated by the action of microorganisms. Their biocatalytic activity on cocoa generates physical and chemical transformations in cocoa seeds. These are significantly influenced by phenomena, i.e. the transfer of oxygen through the aeration of the cocoa seed mass, the mass transfer of acids, and ethanol (metabolites produced by microorganisms) into the seed, and heat transfer caused by the exothermic reactions, which together modulate flavor precursors formation.

- *Anaerobic fermentation phase.* Pectinolytic yeasts (*Saccharomyces cerevisiae, Hanseniaspora* spp., *Pichia* and *Kluyveromyces*) generate ethanol and lactic and are responsible for liquefying the pulp, generating sweatings that comes out the system and allows the entry of air into the fermentation mass (Pereira et al. 2013). During this phase, the pH value in the pulp is initially below 4 due to the content of citric acid. Later, when the activity of the microorganisms begins, it increases slowly along with the temperature, thus, creating the ideal growth conditions for the development of other ones that follow the presence of yeasts i.e. lactic acid (Eyamo Evina et al. 2016).
  The LAB (*Lactobacillus plantarum* and *Lactobacillus fermentum*) convert citric acid and residual carbohydrates of the pulp, mainly in lactic acid, acetic acid, and/or mannitol (Pereira et al. 2013).
- *Aerobic fermentation phase.* The predominant microorganisms are acetic acid bacteria (AAB) (species as *Acetobacter pasteurianus* and *Gluconobacter frateurii*) which are involved in ethanol oxidation produced by yeasts, and lactic acid generated by LAB to generate acetic acid (Lefeber et al. 2011).

Table 2. Postharvest processing practices carried out by Colombian cocoa farmers clusters.

Cluster or postharvest practice	Number of farmers using the practice	Operations and postharvest variables: Seed preconditioning (storage days)	Fermentation: Fermentation time (days)	Fermentation: Duration of anaerobic phase (hours)	Fermentation: Duration of aerobic phase (hours)	Fermentation: Aeration frequency (intervals in hours)	Drying: Initial solar exposure time (hours)
1	49	3	6	34	109	24	9
2	40	0	6	37	107	24	9
3	29	2	4	30	60	24	9
4	45	2.5	6	42	101	30	4
5	49	2	5	57	72	43	9
6	8	8	6	52	92	32	9

Source: Escobar et al. (2019). It was the cite that we used in the original version.

AAB growth is facilitated by the liquefaction process suffered by the pulp, which allows the oxygen transfer in cocoa seeds. During this phase, the acetic acid content increases, with the consequent diffusion of ethanol and acetic acid into cocoa seeds, causing a pH decrease of 7 to 4–4.5 inside the seeds. In addition, exothermic reactions occur reaching temperatures between 40 °C and 50 °C that favors the heat transfer to the fermentation mass, essential for the development of flavor. This range corresponds to the optimum temperature value that promotes the destruction of subcellular structures and the biocatalytic activity of endogenous enzymes involved in flavor formation precursors (Aprotosoaie, Luca, and Miron 2016). The combination of acidification and the effect of heat leads to the interaction between enzymes (proteases: endoproteases and carboxypeptidases, invertases, PPO, and glycosidases) and substrates (storage proteins, carbohydrates, flavanols and anthocyanins), initially separated in cells or specialized compartments, due to the loss of selectivity in the membrane permeability of the storage vacuoles. In this way, a rapid degradation of proteins and carbohydrates occurs, carried out by seed enzymes, generating peptides, free amino acids and reducing sugars, which are considered flavor precursors. The anthocyanins degradation and the oxidation and condensation of the phenolic compounds, result in browning reactions, which produce the brown color that is characteristic of properly fermented cocoa (Leal et al. 2008).

In terms of oxygen transfer, previous works evaluated the effect of aeration on fermentation by increasing or decreasing the frequency or duration of the mixing (Bole Biehl et al. 1985; Senanayake, Jansz, and Buckle 1997). However, enough information has not been reported that allows observing the complete dynamics of flavor precursors according to aeration during the time the bioprocess lasts.

Lefeber et al. (2012) studied the effect of aeration on the profile dynamics of hydrophobic amino acids and methylxanthines during the transformation of cocoa, using a transformation bioprocess with starter cultures by means of a heap fermentation system. In this way, as shown in Fig. 5, it is evident that the oxygen transfer to the fermentation system promotes the generation of hydrophobic amino acids (important flavor precursors) in a shorter postharvest transformation time.The effect of oxygen as a substrate for AAB or to contribute to catalyze enzymatic reactions in cocoa seeds must be directly studied as a key parameter (oxygen consumption) for scaling up in terms of its optimization for the design of the bioprocess and the production of flavor precursors (Escobar et al. 2016).

Table 3. Comparison of postharvest processing practices carried out between two producer countries, Colombia and Nigeria.

Variable	Percentage of producers (%): Nigeria	Percentage of producers (%): Colombia
Sorting before breaking pods		
Sorted	27.2	83.7
Unsorted	72.8	16.3
Duration before breaking		
Less than 1 week	93.8	98
More than 1 week	6.2	2
Fermentation duration		
4 days	N.R.	9
5 days	51.1	22
6–9 days	48.9	69
Fermentation methods		
Heap	73.3	5
Boxes and other methods	26.7	95
Aeration of fermentation mass		
Turning	2	90
No turning	98	10
Surface material of the drying system		
Tarpaulin	48.7	15
Rock	15.7	0
Wooden platform	10.7	41
Concrete	10.3	18
Others	12.9	13
Other drying methods	1.7	13
Total number of farmers surveyed	232	220

N.R: not reported. Source: Dongo et al. (2009) and Escobar et al. (2019).

The dynamics of fermentation are influenced by the genetics of the cocoa variety (Caligiani, Marseglia, and Palla 2016) and also by the maturity state, the harvest time and the preconditioning of pods (De Vuyst and Weckx 2016; Janek et al. 2016). Additionally, different authors have mentioned the effect of other variables on quality as the agronomic conditions of the crop, the agroecological niche, the age of the trees and the chemical composition of the soil (Kongor et al. 2016). However, no information has been generated about their influence on cocoa quality.

All the factors mentioned above generate changes in the phenomenology of seed transformation, and when it is considered as an overall and heterogeneous effect, it prevents the standardization of the fermentation bioprocess (Seguine et al. 2014). Nevertheless, some of these factors certainly represent benefits, due to the possibility of taking advantage

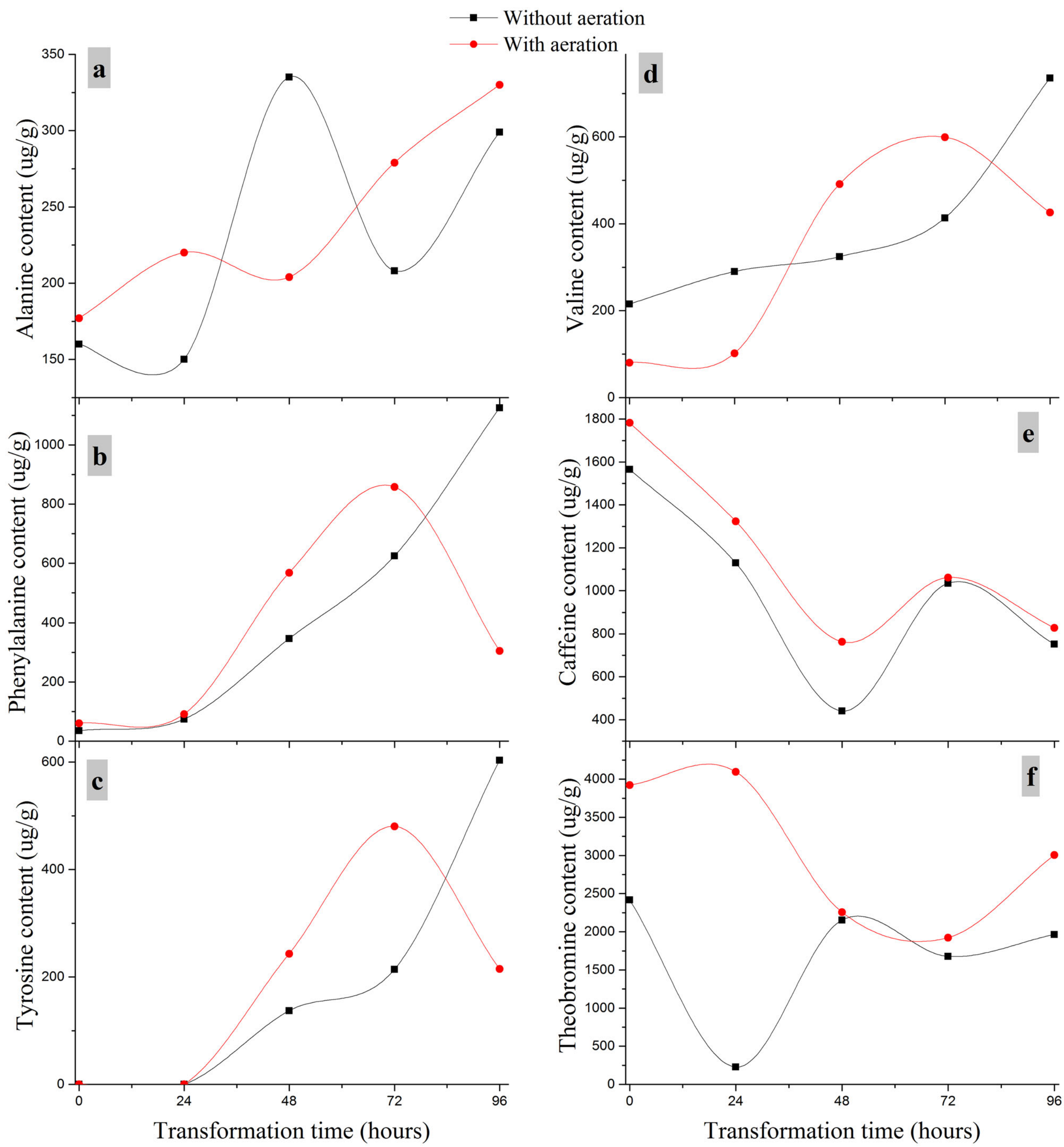

Figure 5. Dynamics of flavor precursor behavior: hydrophobic amino acids (a) alanine, (b) phenylalanine, (c) tyrosine, (d) valine and other compounds that influence flavor quality (e) caffeine, and (f) theobromine, during the postharvest transformation under fermentation with starter cultures, varying the aeration in a heap system. Source: elaborated based on data from Lefeber et al. (2012).

of the high diversity of genetic cocoa materials and agroclimatic niches, in order to expand the supply of specialty cocoa.

However, studies are needed to evaluate the phenomena that controls the speed of all the reactions that occur during fermentation, in terms of defining which of these, in an articulated manner, are responsible for the changes that are required to occur in cocoa seeds in order to optimize the production of flavor precursors. Under this context, and as was explained previously, flavor precursors have been widely studied, and they must be considered as the control parameters (process biomarkers) that allow fermentation management. The knowledge of the relationship between process phenomena and control biomarkers will lead to the design of transformation systems and process methodologies, depending on the variety or mixture of cocoa varieties, the agroclimatic niche, and market demands.

Spontaneous fermentation systems. The design of systems reported (Kadow et al. 2015; Hashim et al. 1998) for the transformation of cocoa seeds by spontaneous fermentation (as piles on Musaceae leaves on the ground, wooden trays, wooden boxes, and a rotary drum) is generally not based on ergonomics or considering cocoa quality. In view of this, it is of interest the design of transformation systems that guarantee safety and systematization. This, in order to control bioprocess variables and obtain a more homogeneous cocoa, taking into account the variations in the cocoa quantity collected in each harvesting.

Thus, Pereira et al. (2013) studied spontaneous fermentation using a stainless steel tank system as a new option compared to fermentation systems in wooden boxes. The results showed that in the tank fermentation system, yeast and bacteria species with important roles and the metabolites in cocoa bean after the bioprocess were similar to those found in wooden boxes. This could be an alternative transformation system to control the operation variables and optimize the process.

Moreover, one of the parameters that affects the transformation bioprocess of seeds is the ratio between the quantity of cocoa seed mass versus the capacity of the fermentation system.

In studies conducted by Hashim et al. (1998), in a rotary drum system using a small quantity of cocoa seed mass for fermentation, they found a low flavor precursors content (free amino acids, peptides, fructose, glucose, total sugars, and pyrazines). Meanwhile, increasing the amount of the seed mass, the concentration of these compounds increases until a maximum point is reached (with a capacity of 55–60 kg). Interestingly, when the quantity of the cocoa seed mass was further increased, the concentrations of these compounds decreased again. As an explanation to the above, the authors established that the fermentation of a very high cocoa seed mass leads to a decrease in the oxygen transfer during the fermentation process, which results in a reduced microbiological activity, and therefore, a lower temperature and proteolytic activity, which leads to low contents of flavor precursor compounds. On the other hand, the fermentation of a very small mass of seeds in the same conditions generates a high oxygen transfer, which probably causes a loss of heat reducing the temperature, and therefore, the metabolic rates of microorganisms, decreases.

Because fermentation is a bioprocess that is developed in a traditional way and is not very technically advanced in cocoa producing farms, a great variation is found in both the fermentation methodologies and the systems used. As an alternative to the above, what would be ideal to develop in the near future is a cocoa postharvest transformation bioprocess in a controlled manner by modulating variables such as aeration (oxygen transfer), temperature (heat transfer), and even applying starter cultures, with the aim of scaling up the process to an industrial level and obtain cocoa with desired quality characteristics. It is also important that these technologies can be transferred to producers and contributes to rural development.

Use of chemical catalysts under controlled conditions

This is a type of physical and biochemical transformation of the seeds developed on a laboratory scale that has been called "fermentation-like incubation", in which chemical catalysts are used as inducers of phenomena that occur inside the cocoa seeds. In this process, variables such as temperature, pH of the medium and atmospheric conditions (presence of oxygen or nitrogen) and the concentration of chemical catalysts are controlled, in order to simulate the spontaneous fermentation of cocoa.

This process develops in absence of microorganisms, as the key factors that guide the formation of flavor precursors during fermentation are the physical conditions of the system that limits heat transfer and acidification of seed tissues. In order for these precursors to be produced, the presence of a microorganisms sequence is not necessary, but the interaction between the metabolites produced by these and the seeds is required (Kadow et al. 2015).

Research has been conducted to control parameters such as temperature (with variation between 30 °C and 45 °C), the pH of the medium (3.0–5.5), the atmospheric conditions (oxygen or nitrogen concentration), and metabolites (ethanol, citric acid, lactic acid, and acetic acid). Additionally, even experimentation with the addition of enzymes such as pectinase to accelerate the bioprocess has been implemented (Eyamo Evina et al. 2016; John et al. 2016; Kadow et al. 2015; Yaw 2014).

As we previously mentioned, several authors studied the set of physical and biochemical changes that take place inside the seeds in order to explain the formation processes of flavor precursor compounds, using a cocoa transformation bioprocess under laboratory conditions with chemical catalysts and under controlled conditions. In this sense, the changes of the subcellular structures (storage vacuoles) as a function of temperature were elucidated, establishing the optimum values that promote the enzymatic reactions (Biehl, Wewetzer, and Passern 1982). The authors explained the behavior of the endogenous enzymes involved in the degradation of the compositional components of seeds (substrates) and the reactions that biocatalyze, among these, mainly proteolysis. For this, the isolation of proteins and proteases was used to carry out in vitro evaluations, to establish their optimal conditions and to study the flavor precursor compounds resulting from the catalysis.

There is a great interest in finding those specific compounds responsible for cocoa flavor. Studies usually refer to a group of compounds that share chemical characteristics (hydrophobic amino acids, hydrophilic peptides, and reducing sugars), but still lack clarity and definition in the compounds that could be considered as biomarkers of bioprocess quality. This is due to their potential to develop desirable sensorial attributes because of their key role in Maillard reactions during roasting. In this sense, results of some works carried out using mixtures of amino acids and peptides in vitro, show the essential nature of the latter for the formation of the characteristic cocoa flavor (Voigt, Textoris-Taube, and Wöstemeyer 2018). In addition, studies have focused on the identification of the peptides and sequences of the vicilin-class (7S) protein involved in flavor formation (D'Souza et al. 2018; Kumari et al. 2018).

Eyamo Evina et al. (2016), John et al. (2016), Kadow et al. (2015) and Yaw (2014), evaluated the formation of flavor precursors (peptides, amino acids, and reducing sugars) and changes in compounds such as polyphenols, which together, have a noteworthy influence on the definition of cocoa flavor.

Kadow et al. (2015) found that the cocoa transformation in the laboratory using chemical catalysts developed in a comparable way to the spontaneous fermentation, due to the content of free amino acids, peptides, reducing sugars, and phenolic compounds (epicatechin and catechin), showed concentrations within the ranges reported in commercial cocoa (fermented and dried spontaneously and traditionally). The fermentation index showed effective postharvest seed transformation and the cutting test revealed the characteristic brown color in well-fermented beans.

In addition, the sensory analysis of the chocolate made with processed cocoa beans using chemical catalysts carried out by trained panel judges described that it had a pleasant flavor, that it also had the typical chocolate flavor, and was slightly astringent and acid. The authors concluded that the application of these types of treatments has the potential to allow standardization and mechanization of the cocoa postharvest transformation (Kadow et al. 2015).

In Fig. 6 information has been compiled to show the dynamics of the behavior of flavor precursors during the transformation time of cocoa seeds using a chemical catalyst (Yaw 2014). This same author evaluated the behavior of flavor precursors under a transformation process using acetic acid and under controlled pH and temperature conditions. Fig. 6 shows the dynamics of these compounds by varying the pH of the transformation medium. Moreover, the relative polypeptides content increased as the pH of the medium increased. After the second day of the process, peptides content obtained at a pH of 3.5 was relatively higher than at a pH 3. Nevertheless, the highest peptides content was observed at a pH 5.5. Additionally, the accumulation of peptides was evident between the second and the third day of the process. After the third day, the quantity of these precursors decreased quickly until being insignificant after the sixth day.

Yaw (2014) also found that the highest concentration of free amino acids occurred during days 3 and 4 in all pH values evaluated. In addition, when the treatments were compared, the seeds transformed at a pH 3 had the highest free amino acids content, followed by those processed through the treatment at a pH 3.5. On day 4, free amino acids content is four times higher when compared to the start of the bioprocess. After the fourth day, the content of free amino acids begins to decrease gradually until the end of the transformation. On the other hand, a higher quantity of hydrophobic amino acids was found at less acidic pH values. Its concentration reached the maximum value at the beginning of the process and decreased rapidly throughout the transformation time. The hydrophobic amino acids found in higher proportions were alanine, phenylalanine, leucine, valine, tyrosine, and isoleucine.

In summary, the concentration of these precursors generally increases from the beginning of the transformation process, until reaching a maximum value between days 3 and 4,

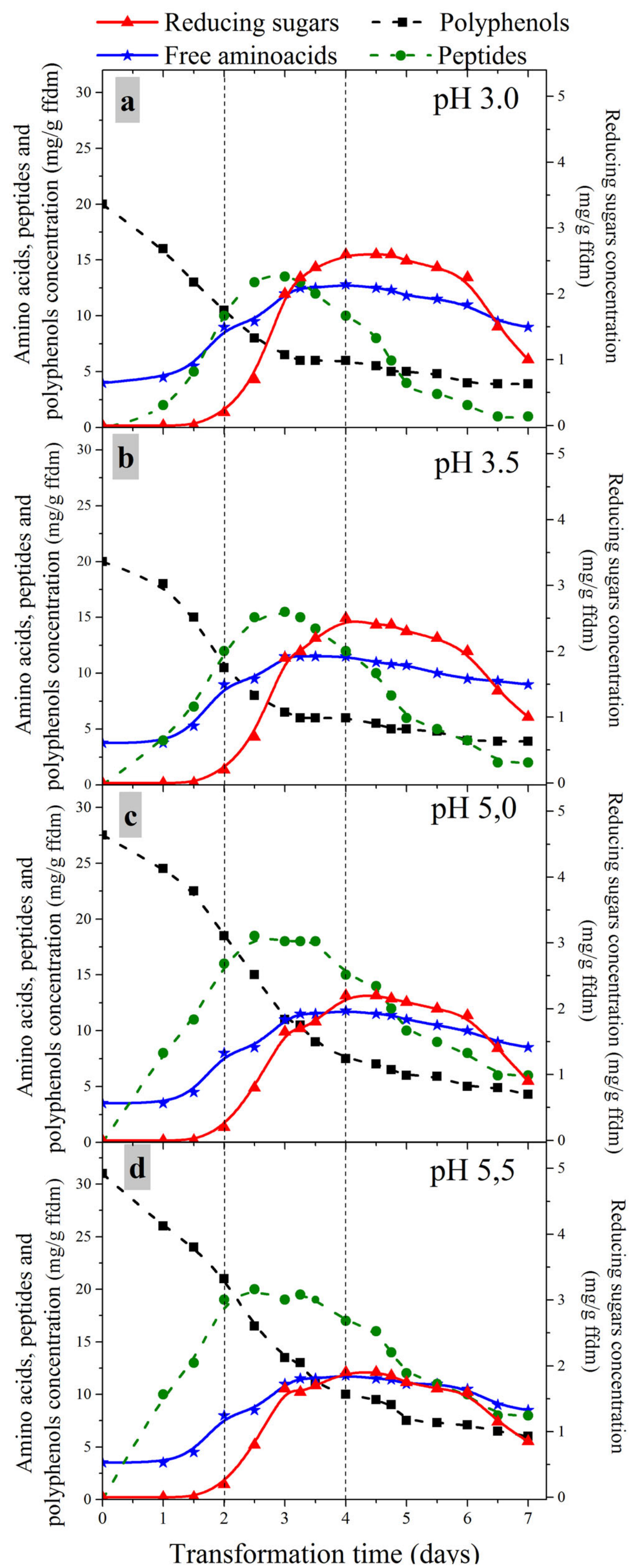

Figure 6. Flavor precursor behavior in cocoa seeds during physical and biochemical transformation in the laboratory using acetic acid as a chemical catalyst under various controlled pH conditions: (a) 3.0, (b) 3.5, (c) 5.0 and (d) 5.5. Source: elaborated based on the information reported by Yaw (2014).

and then the concentration decreases until reaching a low content on day 7.

Moreover, the study published by Yaw (2014) showed that fructose and glucose were found from the second day and their concentrations increased until the fourth day (maximum peak) in all the pH values assessed. The generation speed of the reducing sugars was faster at a pH 3, followed by 3.5, then 5 and finally a pH of 5.5. The above shows that an increase in fructose and glucose concentrations is faster at a low pH. In all the pH evaluated, the maximum reducing sugars content was found on the fourth day of incubation. However, it should be noted that the fructose content was slightly higher and its accumulation faster than for glucose.

With regard to the content of polyphenols in the biochemical transformation process with the use of acetic acid developed by Yaw (2014), a high content of epicatechin and catechin was found at the beginning of the process, which decreased rapidly until reaching a minimum value on the seventh day. At high pH values, higher polyphenols content was found compared to the incubation media with low pH.

Thereby, the concept to take into account based on the information shown in Figs. 3 and 5 is to make objective decisions to develop and scale a process method according to the key objective of the postharvest transformation phase, i.e. producing flavor precursors. Thus, establishing a 3–4 days period as a final transformation point will not respond to subjective factors of a physical nature such as color, or to recommendations by ancestral traditions, but rather to objective information associated to response variables related to flavor formation aspects.

Use of biocatalysts: starter cultures

As an alternative to manage the physical and biochemical transformation of cocoa seeds, several investigations have been developed with the use of starter cultures that have shown promising results in cocoa fermentation. This, because they influence the process speed and the dynamics of the physical and biochemical changes of the seeds, i.e. degraded substrates are generated, metabolites are produced, and consequently, chocolate flavor is generated. Properly selected starter culture can produce uniformly fermented cocoa beans (without variations in the fermentation degree and without deviations in flavor) in less time, as well as a standard quality product. However, until now, starter cultures have not been used for the commercial production of large-scale cocoa beans, due to the complexity of the methodology in terms of implementation logistics, production costs, maintenance of lyophilized starter cultures, and lack of inoculation procedures for small-scale fermentation processes. The aforementioned makes its application in the field difficult (De Vuyst and Weckx 2016; Moens, Lefeber, and De Vuyst 2014; Lefeber et al. 2011).

In a starter culture for cocoa fermentation, the species of microorganisms are chosen according to a specific and desired contribution to the fermentation process. For this purpose, thermo-tolerant and tolerant strains to acidic environments and ethanol have been selected, which are efficient for their pectinolytic activity, and they allow developing a faster and more controlled postharvest processing of cocoa seeds (Pereira et al. 2012).

Crafack et al. (2013) suggests carrying out future trials to study the possibility of using mixed yeast cultures combining the aromatic and functional properties of several strains. In this sense, it was found that *Kluyveromyces marxianus,* a hybrid strain with high pectinolytic activity increases the output speed of sweating in the fermentation process. With the use of this yeast, the degradation of proteins inside the seed improved, and the acidity was reduced. In addition, from the sensory analysis, authors found that the chocolate obtained from the fermented beans with the hybrid strain of *K. marxianus* had greater acceptability in comparison with the chocolate derived from the seeds fermented spontaneously (Leal et al. 2008).

Furthermore, it was found that starter cultures influence positively the flavor profile of chocolate in the sensory evaluation (Crafack et al. 2013). However, it was reported that although the application of starter cultures affects the profile of VOCs of chocolate aroma, the differences with respect to a control chocolate from spontaneously fermented cocoa seeds were minimal, and in the sensory evaluation, the panel judges did not perceive statistically significant differences. Faced with these results, these authors proposed to use a higher inoculation density to accentuate the impact of starter cultures on the sensory quality of chocolate (Crafack et al. 2014).

Drying

During drying, the heat transfer into the beans causes that the water and the acetic acid evaporates to the exterior of the seeds. This operation is carried out until the beans reach a moisture content $\leq$7% (on a humid basis) to avoid over-fermentation, the development of undesirable microorganisms (especially fungi) during storage, increase their shelf life, and facilitate their transport. During this stage, the flavor development continues and browning reactions occur (Zahouli et al. 2010).

The drying speed is a critical factor that significantly affects the final bean quality, as it helps to modulate the flavor and brown characteristic color of properly fermented beans and reduce acidity and bitterness. When the initial drying temperature is very high, the seed testa hardens and adheres to the cotyledon, limiting the transport of oxygen to the interior of the bean. This significantly reduces the enzymatic activity of the PPO, preventing the oxidation of anthocyanins and catechins and its transformation into quinones, and giving rise to violet beans of higher astringency and bitterness (Lasisi 2014). Conversely, if drying is adequate, this enzyme oxidizes polyphenols, transforms them into quinones and these undergoes condensation with free amino acids and sulfhydryl groups, to give brown polymerization products. Aside from this, the drying conditions also affect outward migration of acids from the beans (Biehl and Ziegleder 2003).

Sun drying has been considered as the best method to obtain the maximum flavor development and is often carried out by small cocoa farmers (Hii, Law, and Cloke 2009). However, this drying system has disadvantages because it is an uncontrolled process, so it depends on environmental conditions (considered a critical stage during the rainy season), and sometimes it requires long processing times. Therefore, cocoa with heterogeneous quality is produced throughout the year.

Additionally, alternative drying methods have been studied, such as artificial drying by forced convection with hot air, which is usually used in productive units with higher technical capacities (Páramo et al. 2010; Hii, Law, and Cloke 2009). The results of some investigations indicate that artificially dried cocoa beans have a higher acidity and a lower content of fatty acids than sun-dried cocoa beans (García-Alamilla et al. 2007). However, other authors reported that the content of fatty acids and the pH of the sun-dried cocoa beans were not significantly different compared to artificially dried cocoa (Guehi et al. 2010). Furthermore, Faborode, Favier, and Ajayi (1995) found that the cocoa quality evaluation showed the best results when a low drying temperature is applied or when the drying is intermittent (using resting periods).

For artificial drying, different temperatures have been evaluated. The most commonly used vary between 40 °C and 60 °C under laboratory conditions (Rodriguez-Campos et al. 2012; Schwan and Wheals 2004). Hii, Law, and Cloke (2009) reported that high drying temperatures have negative effects on cocoa flavor. However, the number of researches in the literature that report the relationship between fermentation and drying with flavor quality, that includes the profile of VOCs and the sensory profile in chocolate, is reduced. Rodriguez-Campos et al. (2012) found that cocoa drying at 70 °C for 8 h by forced convection with hot air produced a profile of VOCs including alcohols, aldehydes, ketones, esters, acids, and pyrazines, similar to the profile obtained with sun drying. For this reason, to promote the reactions that are responsible for cocoa flavor, the drying process must be carried out slowly at a controlled temperature (Faborode, Favier, and Ajayi 1995; Kyi et al. 2005; Alean, Chejne, and Rojano 2016).

Transformation of cocoa beans into chocolate: volatile organic compounds and flavor

Chocolate is a unique and widely consumed food in the world due to its exquisite flavor. Worldwide chocolate sales were estimated at more than 101,000 million US dollars in 2015. From these, only Europe represents 45% of the world consumption (Konstantas et al. 2018). Chocolate flavor is influenced by a sum of factors, including the cocoa genotype, growing conditions of cocoa trees, stages of postharvest seed transformation processes (fermentation and drying), and finally, the agro-industrial processing operations such as roasting and conching. These two stages allow converting fermented and dried cocoa beans into a microbiologically stable product, with desirable flavor, color, and texture, attributes that are considered highly dependent on the operating parameters, i.e. time and temperature (Acierno et al. 2016; Ascrizzi et al. 2017).

The sensory attributes of chocolate flavor are a combination of two fractions, a volatile and a nonvolatile. The nonvolatile flavor fraction is formed by polyphenols, methylxanthines, and organic acids (lactic acid) (Lima et al. 2011). Meanwhile the volatile flavor fraction is much more diverse and complex. In this sense, more than 600 volatile compounds are reported that intervene in the matrix that characterizes the chocolate aroma, which includes aldehydes, pyrazines, acids, alcohols, esters, ketones, furans, pyrroles, phenols, terpenes, and terpene alcohols (Ioannone et al. 2015).

In the literature, there is a general consensus that fermentation is the most important factor which influences chocolate flavor quality (De Vuyst and Weckx 2016; Pereira et al. 2012; Tran et al. 2015). Proper fermentation ensures the release of crucial flavor precursors for the formation of aroma compounds, but it also imparts a “fermentative flavor” derived from acids, alcohols, esters, and ketones produced by yeasts, LAB and AAB (Crafack et al. 2014).

However, at the level of cocoa industrial processing, we highlighted roasting as one of the basic technological operations that affects the chocolate quality because the moisture content is reduced (from 7% to 1–5%), transformation reactions of bioactive compounds such as phenols (by reactions of oxidation, condensation, and complexation) occur, microorganisms and their vegetative forms are destroyed, and roasting also facilitates the evaporation of undesirable compounds such as volatile acids, especially acetic acid, which in large quantities deteriorate the chocolate flavor quality. But beyond these points, is considered a fundamental operation in the formation of the chocolate flavor because during this stage, the Maillard reactions take place due to the interaction of the chemical precursors formed during the fermentation (reducing sugars and amino acids-peptides), which have special interest and are of high importance, because these nonenzymatic browning reactions allow the formation of compounds related to the flavor and the color of chocolate (Van Durme, Ingels, and De Winne 2016).

In the Maillard reaction, the reducing sugar reacts generally and reversibly with an amino group of a protein to form a Schiff base (an imine, $RHC = NHR'$), which can be cyclized to form a glycosylamine or fructosilamine. A Schiff base undergoes the Amadori rearrangement reaction to generate an Amadori compound, for example in the case of D-glucose, a derivative of 1-amino-1-deoxy-D-fructose. Amadori compounds are intermediates in the nonenzymatic browning reaction sequence and undergo transformation through known pathways; these compounds generate a complex mix of products. One of the intermediate compounds formed by this mechanism are 1-, 3-, and 4-deoxydicarbonyl compounds, commonly known as 1-, 3-, and 4-deoxyosones. The most prevalent intermediate is usually 3-deoxiosone. The osones are also cleaved, either between the two carbonyl groups or at the site of an enediol forming shorter chain products, mainly aldehydes that may undergo several reactions (Damoradan, Parkin, and Fennema 2008).

Strecker degradation is also a relevant reaction of the dicarbonyl compounds (osones and deoxyosones). The reaction of these compounds with α-amino acid, results first in the formation of a Schiff base, then in decarboxylation (CO_2 release), dehydration and elimination to produce an aldehyde. Among the most important VOCs from the Strecker degradation we find 3-methylthiotpropanal (methional, from L-methionine), phenylacetaldehyde (from L-phenylalanine), methylpropanal (from L-valine), 3-methylbutanal (from L-leucine) and 2-methylbutanol (from L-isoleucine) (Damoradan, Parkin, and Fennema 2008).

The amino acid largely determines the flavor potential in chocolate, since both the derived aldehydes and the following reactions that these suffer, in which heterocyclic compounds are generated, contribute significantly to flavor formation. For example, the reaction between leucine and glucose generates aroma described as "sweet chocolate", threonine and glutamine with glucose produce "chocolate" aroma, and valine and glucose produce sensory notes of "penetrating chocolate" (Afoakwa et al. 2008).

The product mixture formed is a function of temperature and time of roasting, pH, the nature of the reducing sugar and the nature of the amino compound. Different sugars undergo nonenzymatic browning at different speeds. For example, D-glucose suffers the browning reaction faster than D-fructose. Different reaction products are obtained from the nature of the amines depending on whether they are primary or secondary. Because the reaction has a relatively high activation energy, heat application is usually required. The speed of the Maillard reaction is also a function of water activity (a_w) and pH, reaching a maximum in a_w values of 0.6–0.7 and pH 4–7. Therefore, for some foods, this nonenzymatic reaction can be controlled by modulating the water activity, reagent concentrations, time, temperature, and pH (Damoradan, Parkin, and Fennema 2008).

During toasting, cocoa beans are exposed to temperatures ranging from 110 °C to 160 °C, in a time interval between 5 and 120 min. In general, the establishment of roasting conditions is based on the cocoa genotype that will be transformed, the cocoa material that is used (beans, nibs, or liquor), the final product to obtain (dark or milk chocolate) and in the desired flavor attributes. It is considered that the fine or flavor varieties require lower temperatures than bulk cocoa varieties (Żyżelewicz et al. 2016).

While many Maillard products generate aroma notes described as roasted, baked, and nutty, alcohols and esters formed mainly by yeasts during the initial fermentation phase, are associated with fruity, floral and sweaty flavor descriptors, and these are very desirable for flavor balance (Crafack et al. 2014).

The compounds involved in chocolate aroma differ according to the cocoa variety. For example, volatile acids, terpene, and non-terpene alcohols, aldehydes (especially nonanals) and esters are detected in larger quantities in the *Criollo* genotypes, that show a more diverse and peculiar aroma profile (Ascrizzi et al. 2017). In the case of linalool, it is highlighted that it contributes to the profile of the *Arriba* cocoa flavor from a *Nacional* variety (De Vuyst and Weckx 2016). Owusu, Petersen, and Heimdal (2012), indicated that aldehydes and pyrazines are the main compounds formed during roasting. The pyrazines can be the key objects to study and characterize both the process and the chocolate flavor, in particular methylpyrazines, due to the special sensory attributes that they confer (Perego et al. 2004).

One way to approach the study of the chocolate flavor would be to relate the sensory perception with its volatile organic components (Owusu, Petersen, and Heimdal 2012). However, there are a limited number of studies that correlate the profile of volatile compounds with the sensory analyses of chocolate. Frequently, the authors are limited to analyze only certain important parameters within the stages of the postharvest or processing. Some works are focused on studying for example, in the fermentation stage, only the behavior of flavor precursors (reducing sugars, amino acids, and peptides), the phenolic compounds or physicochemical properties (pH, acidity, minerals, fermentation index), as well as the dynamics of microorganisms during the process. However, most ignore their effect on the chocolate flavor, which could be studied at a sensory or chemical level. Therefore, the number of investigations in which the cocoa by-product quality is evaluated with a broader scope is quite reduced. It is necessary to analyze the correlation between flavor precursors, sensory analysis and the profile of volatile aroma compounds, taking into account the effects caused by the application of a set of postharvest operations and agro-industrial processing on cocoa beans.

In this sense Crafack et al. (2013) and Lefeber et al. (2012) evaluated the sensory quality and studied the profile of volatile aroma compounds of chocolate after using starter cultures in fermentation. The authors found that the microorganisms inoculated could positively influence the flavor profile. In this study, the chemical identification of the volatile compounds was correlated with the results obtained from the sensory evaluation. They associated the presence of a high content of an aroma compound identified with a high intensity of a flavor attribute with which it relates, and concluded that the volatile compounds serve as indicators of particular flavor characteristics.

Additionally, Owusu, Petersen, and Heimdal (2012) analyzed volatile aroma compounds in chocolate aiming to evaluate the effect of fermentation, roasting and conching methods. Among the most relevant results, we highlight that roasting at a temperature between 120 °C and 150 °C produces higher content of volatile aroma compounds, but it is reduced when long conching periods (10 h) are used. In addition, the fermentation method (using tray or heap systems) significantly influences the volatile compound profile, since in all the treatments, high differences were found, which are accentuated by varying the conditions of roasting and conching. Owusu, Petersen, and Heimdal (2012) and Afoakwa et al. (2008) consider that the key VOCs identified in dark chocolate are those referenced to in Table 4.

Additionally, it should be noted that with the information obtained from different studies on the relationship between flavor precursors on the profile of VOCs and the sensory profile, with the variables of cocoa postharvest and chocolate processing, a data platform for the development of a prediction model could be built. It would allow obtaining cocoa

Table 4. Key VOCs identified in dark chocolate.

Volatile organic compound	Aroma descriptor
Aldehydes	
2-Methylbutanal	Chocolate
2-Methylpropanal	Chocolate
3-Methylbutanal	Chocolate
Benzaldehyde	Earthy, nutty
5-Methyl-2-phenyl-2-hexenal	Sweet, cocoa, roasted
Ketones	
2,3-Butanedione	Buttery, sweet
Pirazines	
3 (or 2), 5-Dimethyl-2 (or 3)-ethylpyrazine	Chocolate
3,5-(or 6)-Diethyl-2- methylpyrazine	Chocolate
2,5-Dimethylpyrazine	Popcorn, chocolate, roasted nuts and earthy flavor
2,3-Dimethylpyrazine	Cocoa, chocolate, nutty, coffee, caramel, sweaty, malty
2,3,4,5-Tetramethylpyrazine	Cocoa, chocolate, nutty, mocha, roasted, coffee
2-Ethyl-5-methylpyrazine	Roasted, coffee
2,3,5-Trimethylpyrazine	Potato chip-like, grass, musty, nutty and roasted peanuts
Sulfur compounds	
Dimethyltrisulfide	Rubbery
Acids	
Acetic acid	Acid, sour
3-Methylbutanoic acid	Sweaty
Furans	
Linalool oxide	Sweet, flowery
2(3H)-Furanone, dihydro-	Sweet, caramel
Alcohols	
2,3-butanodiol	Sweet, flowery
Esters	
Benzyl acetate	Fruity, Flowery
2-Pentyl acetate	Green, cucumber
Ethyl-3-methylbutanoate	Fruity, flower
Pyrroles	
Acetylpyrrole	Chocolate
Furfurylpyrrole	Chocolate, cocoa, chocolate, nutty, coffee

Source: adapted from Owusu, Petersen, and Heimdal (2012) and Afoakwa et al. (2008).

with defined quality characteristics according to the specifications of the chocolate demanded by the market.

Conclusions and forthcoming research

The control of the phenomena and the variables that condition this bioprocess is of interest because it allows managing cocoa bean production and obtain homogeneous quality batches, with specific and desired characteristics according to market demands (the differentiated segment of specialty cocoa market highlights, it offers premiums for cocoa quality). From this point of view, we consider that more research and advances related to the evaluation and selection of biomarkers (flavor precursors) are required for the quality control of cocoa postharvest transformation to produce specialty cocoa.

Recent technologies have been developed focused on transforming seeds to cocoa beans under controlled process conditions in order to manage the variables that significantly affect the physical and chemical phenomena of the bioprocess depending on the desired quality in chocolate. However, it is necessary to implement studies for industrial scaling to analyze their potential to produce specialty cocoa. In addition, research should continue to elucidate the relationship of the physical (mass, oxygen, and heat transfer) and chemical (networks of reactions catalyzed by enzymes) phenomena involved in the bioprocess and their effect on the generation of flavor precursors. In this way, the physical and biochemical transformation from seeds to cocoa beans can be designed in a controlled way, under the limits of quantitative process variables (temperature, pH, oxygen concentration, biocatalysts/catalysts), that allow optimization depending on the key objective of this stage, i.e. producing flavor precursors. To this end, it has been proposed to initiate studies aimed at designing and developing appropriate equipment as bioreactors that can be adapted to rural conditions, which allows transforming cocoa on a large scale under controlled process parameters.

For the development and management of the cocoa postharvest transformation as a function of obtaining chocolate with previously defined quality characteristics, more research is needed on the relationship between the dynamics of cocoa flavor precursors, the profile of VOCs and the sensory profile of chocolate. This, in order to collect enough information to establish simulation models that allow establishing process methodologies and predict chocolate attributes. In the opposite direction, determining the type of cocoa that should be used depending on the kind of chocolate that is required to be produced.

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References

Acierno, V., S. Yener, M. Alewijn, F. Biasioli, and S. Van Ruth. 2016. Factors contributing to the variation in the volatile composition of chocolate: Botanical and geographical origins of the cocoa beans, and brand-related formulation and processing. *Food Research International* 84:86–95. doi: 10.1016/j.foodres.2016.03.022.

Afoakwa, E. O., J. E. Kongor, J. Takrama, and A. S. Budu. 2013. Changes in nib acidification and biochemical composition during fermentation of pulp pre-conditioned cocoa (*Theobroma cacao*) beans. *International Food Research Journal* 20 (4):1843–53.

Afoakwa, E. O., A. Paterson, M. Fowler, and A. Ryan. 2008. Flavor formation and character in cocoa and chocolate: A critical review. *Critical Reviews in Food Science and Nutrition* 48 (9):840–57. doi: 10.1080/10408390701719272.

Alean, J., F. Chejne, and B. Rojano. 2016. Degradation of polyphenols during the cocoa drying process. *Journal of Food Engineering* 189: 99–105. doi: 10.1016/j.jfoodeng.2016.05.026.

Amin, I., S. Jinap, and B. Jamilah. 1998. Proteolytic activity (aspartic endoproteinase and carboxypeptidase) of cocoa bean during fermentation. *Journal of the Science of Food and Agriculture* 76 (1):123–8. doi: 10.1002/(SICI)1097-0010(199801)76:1 < 123::AID-JSFA917 > 3.0.CO;2-N.

Aprotosoaie, A. C., S. V. Luca, and A. Miron. 2016. Flavor chemistry of cocoa and cocoa products-An overview. *Comprehensive Reviews in Food Science and Food Safety* 15 (1):73–91. doi: 10.1111/1541-4337.12180.

Ascrizzi, R., G. Flamini, C. Tessieri, and L. Pistelli. 2017. From the raw seed to chocolate: Volatile profile of Blanco de Criollo in different phases of the processing chain. *Microchemical Journal* 133:474–9. doi: 10.1016/j.microc.2017.04.024.

Badrie, N., F. Bekele, E. Sikora, and M. Sikora. 2015. Cocoa agronomy, quality, nutritional, and health aspects. *Critical Reviews in Food Science and Nutrition* 55 (5):620–59. doi: 10.1080/10408398.2012.669428.

Biehl, B., B. Meyer, M. B. Said, and R. J. Samarakoddy. 1990. Bean spreading: A method for pulp preconditioning to impair strong nib acidification during cocoa fermentation in Malaysia. *Journal of the Science of Food and Agriculture* 51 (1):35–45. doi: 10.1002/jsfa.2740510105.

Biehl, B., and D. Passern. 1982. Proteolysis during fermentation-like incubation of cocoa seeds. *Journal of the Science of Food and Agriculture* 33 (12):1280–90. doi: 10.1002/jsfa.2740331215.

Biehl, B., U. Passern, and D. Passern. 1977. Subcellular structures in fermenting cocoa beans. Effect of aeration and temperature during seed and fragment incubation. *Journal of the Science of Food and Agriculture* 28 (1):41–52. doi: 10.1002/jsfa.2740280107.

Biehl, B., V. C. Quesnel, D. Passern, and W. Sagemann. 1982. Water uptake by cocoa seeds during fermentation-like incubation. *Journal of the Science of Food and Agriculture* 33 (11):1110–6. doi: 10.1002/jsfa.2740331108.

Biehl, B., C. Wewetzer, and D. Passern. 1982. Vacuolar (storage) proteins of cocoa seeds and their degradation during germination and fermentation. *Journal of the Science of Food and Agriculture* 33 (12): 1291–304. doi: 10.1002/jsfa.2740331216.

Biehl, B., and G. Ziegleder. 2003. *Cocoa. Encyclopedia of food sciences and nutrition*. Cambridge, MA: Academic Press.

Biehl, B., E. Brunner, D. Passern, V. Quesnel, and D. Adomako. 1985. Acidification, proteolysis and flavour potential in fermenting cocoa beans. *Journal of the Science of Food and Agriculture* 36 (7):583–98. doi: 10.1002/jsfa.2740360710.

Buyukpamukcu, E., D. M. Goodall, C. E. Hansen, B. J. Keely, S. Kochhar, and H. Wille, 2001. Characterization of peptides formed during fermentation of cocoa bean. *Journal of Agricultural and Food Chemistry* 49 (12):5822–7. doi: 10.1021/jf0104127.

Caligiani, A., A. Marseglia, and G. Palla. 2016. *Cocoa: Production, chemistry, and use. Encyclopaedia of food and health*. Oxford: Academic Press.

Caligiani, A., A. Marseglia, B. Prandi, G. Palla, and S. Sforza, 2016. Influence of fermentation level and geographical origin on cocoa bean oligopeptide pattern. *Food Chemistry* 211:431–9. doi: 10.1016/j.foodchem.2016.05.072.

Camu, N., T. De Winter, S. K. Addo, J. S. Takrama, H. Bernaert, and L. De Vuyst, 2008. Fermentation of cocoa beans: Influence of microbial activities and polyphenol concentrations on the flavour of chocolate. *Journal of the Science of Food and Agriculture* 88 (13): 2288–97. doi: 10.1002/jsfa.

Counet, C., C. Ouwerx, D. Rosoux, and S. Collin, 2004. Relationship between procyanidin and flavor contents of cocoa liquors from different origins. *Journal of Agricultural and Food Chemistry* 52 (20): 6243–9. doi: 10.1021/jf040105b.

Crafack, M., H. Keul, C. E. Eskildsen, M. A. Petersen, S. Saerens, A. Blennow, M. Skovmand-Larsen, J. H. Swiegers, G. B. Petersen, H. Heimdal, and D. S. Nielsen, 2014. Impact of starter cultures and fermentation techniques on the volatile aroma and sensory profile of chocolate. *Food Research International* 63:306–16. doi: 10.1016/j.foodres.2014.04.032.

Crafack, M., M. B. Mikkelsen, S. Saerens, M. Knudsen, A. Blennow, S. Lowor, J. Takrama, J. H. Swiegers, G. B. Petersen, H. Heimdal, and D. S. Nielsen, 2013. Influencing cocoa flavour using *Pichia kluyveri* and *Kluyveromyces marxianus* in a defined mixed starter culture for cocoa fermentation. *International Journal of Food Microbiology* 167 (1):103–16. doi: 10.1016/j.ijfoodmicro.2013.06.024.

D'Souza, R. N., A. Grimbs, S. Grimbs, B. Behrends, M. Corno, M. S. Ullrich, and N. Kuhnert, 2018. Degradation of cocoa proteins into oligopeptides during spontaneous fermentation of cocoa beans. *Food Research International* 109:506–16. doi: 10.1016/j.foodres.2018.04.068.

Damoradan, S., K.L. Parkin, and O. R. Fennema. 2008. *Fennema's – Food chemistry*. 4th ed. London: CRC Press.

De Vuyst, L., and S. Weckx, 2016. The cocoa bean fermentation process: From ecosystem analysis to starter culture development. *Journal of Applied Microbiology* 121 (1):5–17. doi: 10.1111/jam.13045.

De Taeye, C., V. J. Eyamo Evina, G. Caullet, N. Niemenak, and S. Collin, 2016. Fate of anthocyanins through cocoa fermentation. Emergence of new polyphenolic dimers. *Journal of Agricultural and Food Chemistry* 64 (46):8876–85. doi: 10.1021/acs.jafc.6b03892.

Dongo, L., E. Aigbekaen, C. Jayeola, L. Emaku, and S. Orisajo. 2009. Influence of farmers practices on cocoa bean quality: Nigeria field experience. African Crop Science Conference Proceedings, 9: 299–302.

Duncan, R. J. E., G. Godfrey, T. N. Yap, G. L. Pettipher, and T. Tharumarajah, 1989. Improvement of Malaysian cocoa bean flavour by modification of harvesting, fermentation and drying methods – The Sime-Cadbury process. *Cocoa Grower's Bulletin* 42:42–57.

Escobar, S., A. Rodriguez, E. Gomez, A. Alcon, V. E. Santos, and F. Garcia-Ochoa, 2016. Influence of oxygen transfer on *Pseudomonas putida* effects on growth rate and biodesulfurization capacity. *Bioprocess and Biosystems Engineering* 39 (4):545–54. doi: 10.1007/s00449-016-1536-6.

Escobar, S., M. Santander, C. Contreras, P. Useche, and J. Rodríguez. 2019. Aligning strategic objectives with R&D proposals: A technological plan to impact the well-being and competitiveness of Colombian cocoa farmers, (Forthcoming).

Eyamo Evina, V. J., C. De Taeye, N. Niemenak, E. Youmbi, and S. Collin, 2016. Influence of acetic and lactic acids on cocoa flavan-3-Ol degradation through fermentation-like incubations. *LWT – Food Science and Technology* 68:514–22. doi: 10.1016/j.lwt.2015.12.047.

Faborode, M., J. F. Favier, and O. Ajayi, 1995. On the effects of forced air drying on cocoa quality. *Journal of Food Engineering* 25 (4): 455–72. doi: 10.1016/0260-8774(94)00018-5.

García-Alamilla, P., M. Salgado-Cervantes, M. Barel, G. Berthomieu, G. Rodríguez-Jimenez, and M. García, 2007. Moisture, acidity and temperature evolution during cacao drying. *Journal of Food Engineering* 79 (4):1159–65. doi: 10.1016/j.jfoodeng.2006.04.005.

Garcia-Ochoa, F., E. Gomez, V. E. Santos, and J. C. Merchuk, 2010. Oxygen uptake rate in microbial processes: An overview. *Biochemical Engineering Journal* 49 (3):289–307. doi: 10.1016/j.bej.2010.01.011.

Guehi, S. T., S. Dabonne, L. Ban-Koffi, D. K. Kedjebo, and G. I. B. Zahouli, 2010. Effect of turning beans and fermentation method on the acidity and physical quality of raw cocoa beans. *Advance Journal of Food Science and Technology* 2 (3):163–71.

Hansen, C. E., M. Del Olmo, and C. Burri, 1998. Enzyme activities in cocoa beans during fermentation. *Journal of the Science of Food and Agriculture* 77 (2):273–81. doi: 10.1002/(SICI)1097-0010(199806)77:2 < 273::AID-JSFA40 > 3.0.CO;2-M.

Hashim, P., J. Selamat, S. K. Syed Muhammad, and A. Ali, 1998. Effect of mass and turning time on free amino acid, peptide-N, sugar and pyrazine concentration during cocoa fermentation. *Journal of the Science of Food and Agriculture* 78 (4):543–50. doi: 10.1002/(SICI)1097-0010(199812)78:4<543::AID-JSFA152>3.3.CO;2-U.

Hii, C. L., C. L. Law, and M. Cloke, 2009. Modeling using a new thin layer drying model and product quality of cocoa. *Journal of Food Engineering* 90 (2):191–8. doi: 10.1016/j.jfoodeng.2008.06.022.

Hue, C., Z. Gunata, A. Breysse, F. Davrieux, R. Boulanger, and F. X. Sauvage, 2016. Impact of fermentation on nitrogenous compounds of cocoa beans (*Theobroma cacao* L.) from various origins. *Food Chemistry* 192:958–64. doi: 10.1016/j.foodchem.2015.07.115.

ICCO. 2017. Fine or Flavour Cocoa. Accessed January 15, 2018. http://www.icco.org/about-cocoa/fine-or-flavour-cocoa.html.

Ioannone, F., C. D. Di Mattia, M. De Gregorio, M. Sergi, M. Serafini, and G. Sacchetti, 2015. Flavanols, proanthocyanidins and antioxidant activity changes during cocoa (*Theobroma cacao* L.) roasting as affected by temperature and time of processing. *Food Chemistry* 174:256–62. doi: 10.1016/j.foodchem.2014.11.019.

Janek, K., A. Niewienda, J. Wöstemeyer, and J. Voigt, 2016. The cleavage specificity of the aspartic protease of cocoa beans involved in the generation of the cocoa-specific aroma precursors. *Food Chemistry* 211:320–8. doi: 10.1016/j.foodchem.2016.05.033.

John, W. A., N. Kumari, N. L. Böttcher, K. J. Koffi, S. Grimbs, G. Vrancken, R. N. D'Souza, N. Kuhnert, and M. S. Ullrich, 2016. Aseptic artificial fermentation of cocoa beans can be fashioned to replicate the peptide profile of commercial cocoa bean fermentations. *Food Research International* 89:764–72. doi: 10.1016/j.foodres.2016.10.011.

Kadow, D., N. Niemenak, S. Rohn, and R. Lieberei, 2015. Fermentation-like incubation of cocoa seeds (*Theobroma cacao* L.) – Reconstruction and guidance of the fermentation process. *LWT – Food Science and Technology* 62 (1):357–61. http://10.0.3.248/j.lwt.2015.01.015. doi: 10.1016/j.lwt.2015.01.015.

Kongor, J. E., M. Hinneh, D. V. de Walle, E. O. Afoakwa, P. Boeckx, and K. Dewettinck, 2016. Factors influencing quality variation in cocoa (*Theobroma cacao*) bean flavour profile – A review. *Food Research International* 82:44–52. doi: 10.1016/j.foodres.2016.01.012.

Konstantas, A., H. K. Jeswani, L. Stamford, and A. Azapagic, 2018. Environmental impacts of chocolate production and consumption in the UK. *Food Research International* 106:1012–25. doi: 10.1016/j.foodres.2018.02.042.

Kumari, N., A. Grimbs, R. N. D'Souza, S. K. Verma, M. Corno, N. Kuhnert, and M. S. Ullrich, 2018. Origin and varietal based proteomic and peptidomic fingerprinting of *Theobroma cacao* in non-fermented and fermented cocoa beans. *Food Research International* 111:137–47. doi: 10.1016/j.foodres.2018.05.010.

Kumari, N., K. J. Kofi, S. Grimbs, R. N. D'Souza, N. Kuhnert, G. Vrancken, and M. S. Ullrich, 2016. Biochemical fate of vicilin storage protein during fermentation and drying of cocoa beans. *Food Research International* 90:53–65. doi: 10.1016/j.foodres.2016.10.033.

Kyi, T. M., W. R. W. Daud, A. B. Mohammad, M. Wahid Samsudin, A. A. H. Kadhum, and M. Z. M. Talib, 2005. The kinetics of polyphenol degradation during the drying of Malaysian cocoa beans. *International Journal of Food Science and Technology* 40 (3):323–31. doi: 10.1111/j.1365-2621.2005.00959.x.

Lasisi, D. 2014. A comparative study of effects of drying methods on quality of cocoa beans. *IJERT, International Journal of Engineering Research and Technology* 3 (1):991–6.

Leal, G. A., L. H. Gomes, P. Efraim, F. C. De Almeida Tavares, and A. Figueira, 2008. Fermentation of cacao (*Theobroma cacao* L.) seeds with a hybrid *Kluyveromyces marxianus* strain improved product quality attributes. *FEMS Yeast Research* 8 (5):788–98. doi: 10.1111/j.1567-1364.2008.00405.x.

Lefeber, T., W. Gobert, G. Vrancken, N. Camu, and L. De Vuyst, 2011. Dynamics and species diversity of communities of lactic acid bacteria and acetic acid bacteria during spontaneous cocoa bean fermentation in vessels. *Food Microbiology* 28 (3):457–64. doi: 10.1016/j.fm.2010.10.010.

Lefeber, T., Z. Papalexandratou, W. Gobert, N. Camu, and L. De Vuyst, 2012. On-farm implementation of a starter culture for improved cocoa bean fermentation and its influence on the flavour of chocolates produced thereof. *Food Microbiology* 30 (2):379–92. doi: 10.1016/j.fm.2011.12.021.

Lima, L. J. R., M. H. Almeida, M. J. Rob Nout, and M. H. Zwietering, 2011. *Theobroma cacao* L., 'the food of the gods': Quality determinants of commercial cocoa beans, with particular reference to the impact of fermentation. *Critical Reviews in Food Science and Nutrition* 51 (8):731–61. doi: 10.1080/10408391003799913.

Marseglia, A., S. Sforza, A. Faccini, M. Bencivenni, G. Palla, and A. Caligiani, 2014. Extraction, identification and semi-quantification of oligopeptides in cocoa beans. *Food Research International* 63:382–9. doi: 10.1016/j.foodres.2014.03.046.

Mayorga-Gross, A. L., L. M. Quirós-Guerrero, G. Fourny, and F. Vaillant, 2016. An untargeted metabolomic assessment of cocoa beans during fermentation. *Food Research International* 89:901–9. doi: 10.1016/j.foodres.2016.04.017.

Meyer, B., B. Biehl, M. B. Said, and R. J. Samarakoddy, 1989. Postharvest pod storage: A method for pulp preconditioning to impair strong nib acidification during cocoa fermentation in Malaysia. *Journal of the Science of Food and Agriculture* 48 (3):285–304. doi: 10.1002/jsfa.2740480305.

Moens, F., T. Lefeber, and L. De Vuyst, 2014. Oxidation of metabolites highlights the microbial interactions and role of *Acetobacter pasteurianus* during cocoa bean fermentation. *Applied and Environmental Microbiology* 80 (6):1848–57. doi: 10.1128/AEM.03344-13.

Nazaruddin, R. L., K. Seng, O. Hassan, and M. Said. 2006. Effect of pulp preconditioning on the content of polyphenols in cocoa beans (*Theobroma cacao*) during fermentation. *Industrial Crops and Products* 24 (1):87–94. doi: 10.1016/j.indcrop.2006.03.013.

Oracz, J., and E. Nebesny, 2014. Influence of roasting conditions on the biogenic amine content in cocoa beans of different *Theobroma cacao* cultivars. *Food Research International* 55:1–10. doi: 10.1016/j.foodres.2013.10.032.

Osorio-Guarín, J. A., J. Berdugo-Cely, R. A. Coronado, Y. P. Zapata, C. Quintero, G. Gallego-Sánchez, and R. Yockteng. 2017. Colombia a source of cacao genetic diversity as revealed by the population structure analysis of germplasm bank of *Theobroma cacao* L. *Frontiers in Plant Science* 8:1994. doi: doi:10.3389/fpls.2017.01994.

Owusu, M., M. A. Petersen, and H. Heimdal. 2012. Effect of fermentation method, roasting and conching conditions on the aroma volatiles of dark chocolate. *Journal of Food Processing and Preservation* 36 (5):446–56. doi: 10.1111/j.1745-4549.2011.00602.x.

Ozturk, G., and G. M. Young. 2017. Food evolution: The impact of society and science on the fermentation of cocoa beans. *Comprehensive Reviews in Food Science and Food Safety* 16 (3):431–55. doi: 10.1111/1541-4337.12264.

Páramo, D., P. García-Alamilla, M. A. Salgado-Cervantes, V. J. Robles-Olvera, G. C. Rodríguez-Jimenes, and M. A. García-Alvarado. 2010. Mass transfer of water and volatile fatty acids in cocoa beans during drying. *Journal of Food Engineering* 99 (3):276–83. doi: 10.1016/j.jfoodeng.2010.02.028.

Perego, P., B. Fabiano, M. Cavicchioli, and M. Del Borghi. 2004. Cocoa quality and processing: A study by solid-phase microextraction and gas chromatography analysis of methylpyrazines. *Food and Bioproducts Processing* 82 (4):291–7. doi: 10.1205/fbio.82.4.291.56402.

Pereira, G. V. M., K. T. Magalhães, E. G. de Almeida, I. da Silva Coelho, and R. F. Schwan. 2013. Spontaneous cocoa bean fermentation carried out in a novel-design stainless steel tank: Influence on the dynamics of microbial populations and physical-chemical properties. *International Journal of Food Microbiology* 161 (2):121–33. doi: 10.1016/j.ijfoodmicro.2012.11.018.

Pereira, G. V. M., M. G. C. Miguel, C. L. Ramos, and R. F. Schwan. 2012. Microbiological and physicochemical characterization of small-scale cocoa fermentations and screening of yeast and bacterial strains to develop a defined starter culture. *Applied and Environmental Microbiology* 78 (15):5395–405. doi: 10.1128/AEM.01144-12.

Ríos, F., C. Rehpani, A. Ruiz, and J. Lecaro. 2017. Estrategias país Para La oferta de cacaos Especiales - Políticas e iniciativas privadas exitosas En El perú, Ecuador, Colombia y república dominicana. Bogotá, Colombia.

Rodriguez-Campos, J., H. B. Escalona-Buendía, S. M. Contreras-Ramos, I. Orozco-Avila, E. Jaramillo-Flores, and E. Lugo-Cervantes, 2012. Effect of fermentation time and drying temperature on volatile compounds in cocoa. *Food Chemistry* 132 (1):277–88. doi: 10.1016/j.foodchem.2011.10.078.

Saltini, R., R. Akkerman, and S. Frosch. 2013. Optimizing chocolate production through traceability: A review of the influence of farming practices on cocoa bean quality. *Food Control* 29 (1):167–87. doi: 10.1016/j.foodcont.2012.05.054.

Schwan, R. F., and A. E. Wheals. 2004. The microbiology of cocoa fermentation and its role in chocolate quality. *Critical Reviews in Food Science and Nutrition* 44 (4):205–21. doi: 10.1080/10408690490464104.

Schwan, R., and G. Fleet. 2014. *Cocoa and coffee fermentation*. New York: CRC Press.

Seguine, E., D. Mills, J. Marelli, J. Motamayor, and I. Da Silva. 2014. Micro-fermentation of cocoa Patent. US Patent 20140199437A1, filed August 12, 2011, and issued 2014.

Senanayake, M., E. R. Jansz, and K. A. Buckle. 1997. Effect of different mixing intervals on the fermentation of cocoa beans. *Journal of the Science of Food and Agriculture* 74 (1):42–8. doi: 10.1002/(SICI)1097-0010(199705)74:1 < 42::AID-JSFA768 > 3.0.CO;2-U.

Tran, P. D., D. Van de Walle, N. De Clercq, A. De Winne, D. Kadow, R. Lieberei, K. Messens, D. N. Tran, K. Dewettinck, and J. Van Durme. 2015. Assessing cocoa aroma quality by multiple analytical approaches. *Food Research International* 77:657–69. doi: 10.1016/j.foodres.2015.09.019.

Van Durme, J., I. Ingels, and A. De Winne. 2016. Inline roasting hyphenated with gas chromatography-mass spectrometry as an innovative approach for assessment of cocoa fermentation quality and aroma formation potential. *Food Chemistry* 205:66–72. doi: 10.1016/j.foodchem.2016.03.004.

Voigt, J., K. Textoris-Taube, and J. Wöstemeyer. 2018. PH-dependency of the proteolytic formation of cocoa- and nutty-specific aroma precursors. *Food Chemistry* 255:209–15. doi: 10.1016/j.foodchem.2018.02.045.

Voigt, J., K. Janek, K. Textoris-Taube, A. Niewienda, and J. Wöstemeyer. 2016. Partial purification and characterisation of the peptide precursors of the cocoa-specific aroma components. *Food Chemistry* 192:706–13. doi: 10.1016/j.foodchem.2015.07.068.

Voigt, J., and B. Biehl. 1995. Precursors of the cocoa-specific aroma components are derived from the vicilin-class. *Botanica Acta* 108 (4):283–9. doi: 10.1111/j.1438-8677.1995.tb00496.x.

Voigt, J., B. Biehl, H. Heinrichs, S. Kamaruddin, G. G. Marsoner, and A. Hugi, 1994. *In-vitro* formation of cocoa-specific aroma precursors: Aroma-related peptides generated from cocoa-seed protein by co-operation of an aspartic endoprotease and a carboxypeptidase. *Food Chemistry* 49 (2):173–80. doi: 10.1016/0308-8146(94)90155-4.

Voigt, J., H. Heinrichs, G. Voigt, and B. Biehl, 1994. Cocoa-specific aroma precursors are generated by proteolytic digestion of the vicilin-like globulin of cocoa seeds. *Food Chemistry* 50 (2):177–84. doi: 10.1016/0308-8146(94)90117-1.

Voigt, J., G. Voigt, H. Heinrichs, D. Wrann, and B. Biehl, 1994. In vitro studies on the proteolytic formation of the characteristic aroma precursors of fermented cocoa seeds: The significance of endoprotease specificity. *Food Chemistry* 51 (1):7–14. doi: 10.1016/0308-8146(94)90040-X.

Voigt, J., D. Wrann, H. Heinrichs, and B. Biehl, 1994. The proteolytic formation of essential cocoa specific aroma precursors depends on particular structures of the vicillin class globulin of the cocoa seeds lacking in the globular storage proteins of coconuts, hazelnuts and sunflower seeds. *Food Chemistry* 51 (2):197–205. doi: 10.1016/0308-8146(94)90257-7.

Wollgast, J., and E. Anklam, 2000. Review on polyphenols in *Theobroma cacao*: Changes in composition during the manufacture of chocolate and methodology for identification and quantification. *Food Research International* 33 (6):423–47. doi: 10.1016/S0963-9969(00)00068-5.

Yaw, I. 2014. Raw cocoa (*Theobroma cacao* L.) quality parameters – With special reference to West Africa. PhD diss., University of Hamburg.

Zahouli, B., S. T. Guehi, G. Irie, A. M. Fae, L. Ban-Koffi, and J. G. Nemlin, 2010. Effect of drying methods on the chemical quality traits of cocoa raw material. *Advance Journal of Food Science and Technology* 2 (4):184–90.

Żyżelewicz, D., W. Krysiak, J. Oracz, D. Sosnowska, G. Budryn, and E. Nebesny, 2016. The influence of the roasting process conditions on the polyphenol content in cocoa beans, nibs and chocolates. *Food Research International* 89:918–29. doi: 10.1016/j.foodres.2016.03.026.