Review Article

Formation of aromatic compounds precursors during fermentation of Criollo and Forastero cocoa

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Abstract

There are three main genetic varieties of cocoa (Theobroma cacao L) used in chocolate making: Forastero, Trinitario and Criollo, which are distinguished by their aroma, an attribute that determines their quality. Criollo cocoa is of the highest quality and is used in the manufacture of fine chocolates because of its fruity aroma. The aroma of Criollo cocoa is defined by volatile compounds such as pyrazines and aldehydes, which are formed during roasting of the bean, from aroma precursors (reducing sugars and free amino acids) that are generated inside the bean via enzymatic reactions during fermentation; for this reason, fermentation is the most important process in the value chain. This review discusses the production of aroma precursors of Criollo and Forastero cocoa by studying the kinetics of spontaneous fermentation and the role of starter cultures.
to produce aroma precursors. Fine aroma precursors produced in the pulp during the fermentation phase will migrate into the bean when its permeability is improved and then retained during the drying phase. Diffusion of aroma precursors into the cocoa bean may be possible, this process is mathematically characterized by the coefficient of molecular diffusion D, which describe the process of mass transfer via Fick’s Second Law. The current state of knowledge is analyzed based on existing research and reports some gaps in the literature, suggesting future research that will be necessary for a better understanding of cocoa fermentation.

Keywords: Food technology, Food science

1. Introduction

Cocoa (*Theobroma cacao* L) and its most important products, such as chocolate, are known as luxury foods that provide an astringent taste and typical aroma (Pedan et al., 2017). The fruit of this tree is a pod that contains seeds, commonly known as cocoa beans (Kongor et al., 2016); when harvested, they are surrounded by a sweet, acidic, aromatic mucilage that is pleasant to the palate and is known as pulp; this material is difficult to extract by mechanical means. There are three commonly recognized genetic varieties: Forastero, Criollo, and Trinitario; a fourth variety that grows in Ecuador is called Nacional (Afoakwa et al., 2011). Criollo is the finest variety of cocoa. Cocoa varieties classification is largely based on the aspect of the fruit, so the terms associated to Criollo are descriptive of the fruit characters (Ascrizzi et al., 2017), however it is the least studied. The production of cocoa is led by Forastero, and Criollo contributes a smaller amount to world production, approximately 5% (Afoakwa, 2010). In Latin American production, Peruvian cocoa is of interest for its quality; however, its production is insufficient for international demand. In Amazonas, Peru, dry fermented Criollo cocoa bean is produced for the Italian market, and this product has received the designation of the origin “Cacao Amazonas Peru” by the Peruvian state.

The most important attribute for which the cocoa bean is commercially accepted is its aroma, which also determines its quality. The aroma is formed by volatile compounds that are perceived by the smell receptors of the olfactory tissue of the nasal cavity (Belitz et al., 2009). The aroma of the genetic varieties mentioned above is used for commercial classification into divided into two types of cocoa: bulk/ordinary cocoa (Forastero) and cocoa with a fine aroma (Criollo and Trinitario) (Kongor et al., 2016; Chetschik et al., 2017). The volatile compounds of the cocoa aroma and chocolate are formed during the roasting of the bean, transforming the molecules known as aroma precursors, which are generated during fermentation by proteolysis of the proteins stored inside this bean (Janek et al., 2016). Adequate quantities and proportions of the formed precursors are essential for the optimal
production of aromatic volatiles compounds (Afokwa, 2010). In Criollo cocoa (Frauendorfer and Schieberle, 2008) revealed concentrations above the odor threshold for 22 compounds in the unroasted and 27 compounds in the roasted cocoa beans, respectively. It is also assumed that cocoa pulp has an impact on the development of aroma during fermentation, due to the possible migration of aroma compounds from the pulp to the bean tissue, and is considered a deposit for the fineness of cocoa (Chetschik et al., 2017).

To improve the quality of cocoa, the effects of the use of starter cultures composed of different types of microorganisms that can lead a controlled fermentation is investigated. Although experimental applications in clone cocoa fermentations of these cultures have given good results (Schwan, 1998; Sandhya et al., 2016), the process is still performed spontaneously, at least among small producers (Váquez-Ovando et al., 2016). There are no studies on the use of starter culture in fermentation of Criollo cocoa, all studies on the subject mentioned in this document refer to cocoa of other varieties. Therefore, this review to discuss about the production of cocoa aroma precursors during spontaneous and controlled fermentation. The role of the microorganisms in the production of aroma precursors, that influence in the final content of aromatics compounds, and its production kinetics in cocoa beans are analyzed. The current state of knowledge is analyzed based on existing research and reports about some gaps in knowledge, suggesting future research that will be necessary for a better understanding of Criollo cocoa fermentation.

2. Main text

2.1. Formation of the aromatic compounds in cocoa beans

2.1.1. Volatile aromatic compounds in Criollo cocoa

The aroma and taste of chocolate depends on the origin and genotype of the cocoa bean (Menezes et al., 2016), which has been used by the International Cocoa Organization in numerous studies to conclude that, in general, cocoas from different origins have different aroma profiles, thus eliminating market competition among them (ICCO, 2017a). Volatile aromatic compounds are present in traces, mainly at levels of a few μg/kg or no more than a few mg/kg, with approximately 100 different pyrazines present in the predominant aroma fraction (Beckett, 2009).

Criollo cocoa is of high value and is a fine cocoa used to produce high-quality chocolates. The fine aromas include fruit notes (fresh and ripe), floral, herbal, wood, nuts and caramel notes; monoterpenes such as linalool are also part of the compounds responsible for the fine aroma in cocoa; therefore, fine cocoas contain higher amounts of linalool than bulk cocoa (Ziegleder, 1990). Frauendorfer and
Schieberle (2008) analyzed unroasted and roasted Criollo cocoa, they concluded that various compounds contributing to the aroma of roasted cocoa beans, such as 3-methylbutanoic acid, ethyl 2-methylbutanoate and 2-phenylethanol, were already present in unroasted, fermented cocoa beans and were not increased during roasting. Table 1 shows the compounds of the fine aroma identified in Criollo cocoa of different origins and processing stages (fresh, fermented and roasted bean) (Ascrizzi et al., 2017; Tran et al., 2015). In the Peruvian Criollo cocoa, linalool was not found as the main component, the presence of this component will depend on the geographical origin where the cocoa is cultivated; however, other components responsible for the aroma of floral and caramel were founded.

The basic aromas of cocoa beans include pleasant and balanced chocolate notes (ICCO, 2017b), and the aromas of cocoa and chocolate are attributed to 2,3,5,6-tetramethylpyrazine (TMP) and 2,3,5-trimethylpyrazine (TrMP), which form the so-called basic notes (Sukha et al., 2013). In addition to pyrazines, aldehydes also contribute to the chocolate aroma (Diab et al., 2014). Chocolate manufacturers

### Table 1. Components of fine aroma identified in samples of Criollo cocoa of different origins.

| Aromatic compound                  | Description                      | Provided by Amedei’s factory (country not identified)* | Peru** Raw cocoa beans, fermented and dried, husked, % | Roasted cocoa beans with husk, % | Roasted cocoa beans, % |
|------------------------------------|----------------------------------|-------------------------------------------------------|-------------------------------------------------------|---------------------------------|-------------------------|
| 2-Heptanol                         | Citrus, fresh, lemon grass-like  | 0.7                                                   | 0.2                                                   |                                 |                         |
| Phenylethyl alcohol                | Flowery, spicy, honey-like, rose | 1.6                                                   | 0.8                                                   |                                 |                         |
| Ethyl octanoate                    | Fruity, floral, pineapple        | 0.5                                                   | 0.3                                                   |                                 |                         |
| Ethyl phenylacetate                | Fruit, sweet, honey-like         | 0.5                                                   | 0.4                                                   |                                 |                         |
| Ethyl decanoate                    | Pear, grape, brandy              | 0                                                     | 0.1                                                   |                                 |                         |
| Acetophenone                       | Sweet, almond, flowery, must-like| 0.2                                                   | 0                                                     |                                 |                         |
| cis-Linalool oxide (furanoid)      | Sweet, nutty                     | 0.2                                                   | 0                                                     |                                 |                         |
| Linalool                           | Flowery                          | 1.2                                                   | 0.5                                                   |                                 |                         |
| trans-Linalool oxide (pyranoid)    | Floral                            | 0.2                                                   | 0.1                                                   |                                 |                         |
| 2-Phenylethylacetate               | Fruity, sweet, flowery           | 2.5                                                   | 1.5                                                   | 0.73                            |                         |
| 1,3-Butanediol                     | Sweet, flowery, caramel          |                                                       |                                                       |                                 | 16.21                   |
| 2,3-Butanediol                     | Sweet, flowery                   |                                                       |                                                       |                                 | 5.12                    |

* Ascrizzi et al. (2017).
** Tran et al. (2015).
separate cocoa beans into fines and bulk; fines are characterize their peculiar notes and are used to produce fine chocolates (Ascrizzi et al., 2017), while bulk beans are used to produce low-quality chocolates and products such as cocoa powder (Vargas Jentzsch et al., 2016). Table 2 shows a comparison between the roasted dry fermented beans of Criollo cocoa obtained from Tumbes (Peru), Grenada and Venezuela, and Forastero cocoa from Ghana (Africa); the named sensory description is accepted by U. S flavor industry (FEMA, 2018). In fact, there is a clear quantitative difference between the volatile aroma compounds of fine cocoa and bulk cocoa (Tran et al., 2015), fines (Peru) have higher levels of pyrazine than bulk beans (Ghana) and a higher percentage of 1,3-butanediol, which gives notes of sweet, floral and caramel (Tran et al., 2015), to mention just one example. In Criollo cocoa beans from Venezuela, 1,3-butanediol was identified (Álvarez, 2016). 3-methylbutanal (malty) is a Strecker aldehyde (Frauendorfer and Schieberle, 2008) and it is crucial aroma compound contributing to the chocolate aroma intensity (Van Durme et al., 2016; Saputro et al., 2018). Excluding the basic aromas of cocoa, the majority of fine aroma features depend on the sensory quality of ripe pod: for Criollo beans, caramel and walnut notes are due to the very sweet nature of their pulp (Ascrizzi et al., 2017). The Criollo cacao from Granada has a greater amount of 3-methylbutanal than those from the other countries, also this cacao and the one from Venezuela contain linalool as one of its components; this corroborates the fact that the geographical origin influences the aromatic profile of Criollo cocoa; however, research is still scarce.

Table 2. Comparison of aromatic notes of fine and bulk cocoa in dry and roasted fermented bean.

| Aroma notes | Identified component | Sensory description | Percentage contribution to aroma |
|-------------|----------------------|---------------------|---------------------------------|
|             |                      |                     | Fine (Criollo - Peru)<sup>2</sup> | Fine (Criollo - Grenada)<sup>3</sup> | Fine (Criollo - Venezuela)<sup>4</sup> | Bulk (Forastero - Ghana)<sup>2</sup> |
| Fine notes  | 3-methylbutanal      | Sweet, floral and   | 0.73                            | 8.08                          | 0.5                              | 0.41                     |
|             | 1,3-butanediol       | caramel<sup>2</sup> | 13.18                           | NI                            | I                                | 11.11                    |
|             | Linalool             | Coriander, Floral,  | NI                              | 0.03                          | I                                | NI                      |
|             |                      | Lavender, Lemon, Rose<sup>1</sup> |                     |                               |                                  |                         |
|             | 2-phenylethylacetate | Frutal, sweet, floral<sup>1</sup> | 0.73                            | 0.22                          | I                                | 0.27                    |
| Basic notes | TrMP                 | Cocoa, Earth, Must, Potato, Roast<sup>1</sup> | 4.4                             | 0.22                          | I                                | 3.65                    |
|             | TMP                  | Cocoa, Coffee, Green, Mocha, Roast<sup>1</sup> | 17.28                           | NI                            | 0.5                              | 5.49                    |

1. FEMA, 2018; 2. Tran et al. (2015); 3. Frauendorfer and Schieberle (2008); 4. Álvarez (2016).
NI: not identified, I: identified, but no value.
2.1.2. Development of volatile aromatic compounds from aroma precursors

Typical aromatic compounds are developed during the roasting stage (Ascrizzi et al., 2017) from aroma precursors by the Maillard reaction (Giacometti et al., 2015). The fermentation of cocoa pulp is crucial for the generation of aroma precursors, and this process is performed by microorganisms such as yeast, lactic acid bacteria (LAB) and acetic acid bacteria (AAB) (John et al., 2016). Fresh beans with low precursor content will have limited commercial use, and fermentation will not be able to rectify this shortfall; therefore, an appropriate amount and ratio of aroma precursors are essential for the optimal production of volatile aromatic compounds in roasting (Afoakwa et al., 2008; Aprotosoaie et al., 2016). Tran et al. (2015) related the degree of fermentation of fermented cocoa beans from different origins with the presence of aroma precursors, establishing as well fermented when there is a high concentration of free amino acids and absence of sucrose (Table 3). In Criollo from Peru, a higher concentration of amino acids was found than in Vietnam and Ghana varieties, so it could be said that it has a better potential to generate aromatic compounds during roasting, provided that the fermentation has been carried out efficiently.

The results of previous studies have shown that peptides and free hydrophobic amino acids, such as leucine, alanine, phenylalanine and tyrosine, are precursors that contribute to the aroma formation of cocoa and chocolate (Voigt, 2009; Sukha et al., 2017) and develop during fermentation (Hashim et al., 1998) through the proteolysis of vicilin-class globulin (VCG) (Janek et al., 2016; Kumari et al., 2016), which is induced by acetic and lactic acids (Voigt et al., 2016) and cooperative action of the aspartic endoprotease and carboxypeptidase that are present in.

Table 3. Aroma precursors founds in different varieties and origin of cocoa beans.

| Clone/variety | Criollo | Trinitario x Forastero | Forastero |
|---------------|---------|------------------------|-----------|
| Hybrid name   | Tumbes  | TD10                   | PA7xIFC5  |
| Origin        | Peru    | Vietnam                | Ghana     |
| Sucrose, mg/g | 0       | 0                      | 2.42 ± 0.18 |
| Glucose, mg/g | 0       | 0                      | 0         |
| Fructose, mg/g| 4.61 ± 0.98 | 10.01 ± 0.13 | 7.18 ± 1.15 |
| Leucine, mg/g | 3.14 ± 0.01 | 2.75 ± 0.02 | 2.00 ± 0.07 |
| Alanine, mg/g | 2.27 ± 0.00 | 2.05 ± 0.01 | 1.38 ± 0.06 |
| Phenylalanine, mg/g | 2.45 ± 0.00 | 2.14 ± 0.03 | 1.58 ± 0.10 |
| Tyrosine, mg/g | 1.55 ± 0.01 | 1.33 ± 0.01 | 1.09 ± 0.05 |

Data extracted from Table 2 published by Tran et al. (2015).
mature cocoa beans and those that are not germinated. Hydrophilic peptides and free hydrophobic amino acids contribute to the aroma by their reaction with fructose and glucose (Afoakwa, 2010) during roasting. In the pulp, sucrose is hydrolyzed to glucose and fructose by the invertase activity of the yeast, as well as in the bean by diffusion of acetic acid, lactic acid and ethanol, together with the production of heat (de Melo Pereira et al., 2013) (Fig. 1). Approximately 25% of free amino acids and 70% of glucose and fructose are used (Beckett, 2009). Leucine and glucose produce aromatic notes described as “sweet chocolate” (Afoakwa, 2010). Recent work concludes that there is no clear quantitative correlation between the amounts of precursors and the aromatic compounds formed (Tran et al., 2015; Frauendorfer and Schieberle, 2008), while others claim that Criollo cocoa beans contain high levels of precursors that can produce high levels of pyrazines (Giacometti et al., 2015), however, pyrazines are not responsible for the fine aroma of Criollo cocoa. The compounds 3-methylbutanal and phenylacetaldehyde (honey, green, floral (Crafack et al., 2014)) correspond to the so-called aldehydes of Strecker degradation, which are the products derived from valine, leucine, isoleucine and phenylalanine. The compounds 3-methylbutanal, 2-methylbutanal and 2-methylpropanal provide a fruity aroma and contribute to the sugary taste that is characteristic of Criollo cocoa (Álvarez et al., 2012). In a previous analysis of dry fermented beans of Criollo cocoa

**Fig. 1.** Aroma precursors produced during cocoa bean fermentation by microbial action and its molecular diffusion (adapted from Beckett, 2009). (*) In addition to the internal production of phenylalanine from the VCG, this amino acid can also be produced by *Bacillus* in the pulp of the bean, then called external precursor, and later enter the interior of the bean by molecular diffusion to be part of the internal precursors.
produced by the Asociación de Productores Cafetaleros y Cacaoteros de Amazonas (APROCAM)-Peru, 28.08% of 3-methylbutanal and 9.17% of 2-methylbutanal were detected (data not yet published). In addition to the endogenous formation of the precursors, there is growing evidence of the exogenous influence of the pulp directly on the development of the aroma (Sukha et al., 2017). It is believed that aromatic compounds directly penetrate from the pulp into the bean tissue during fermentation and can be retained during the drying process (Eskes et al., 2012).

There are few studies on volatiles compounds of Criollo cocoa, (Rodriguez-Campos et al., 2011, 2012; Ramos et al., 2014) characterized the dynamics of volatile compounds during fermentation of cacao varieties, including the bulk Forastero and various cacao hybrids, but the fine-flavor cultivars Criollo were not assessed. The volatile aromatic compounds present in Criollo, Forastero, and Trinitario cocoa beans from the same origin have been characterized (Qin et al., 2016; Tran et al., 2015; Counet et al., 2004) but changes in aroma precursors occurring during fermentation Criollo cocoa were not reported.

2.2. Action of the microorganisms in the production of aroma precursors and aromatics compounds

In spontaneous fermentation of cocoa beans Forastero hybrid from Ivory Coast (Visintin et al., 2016), isolated 106 yeasts, 105 LAB and 82 AAB which were identified by means of rep-PCR grouping and sequencing of the rRNA genes. Oxygen is one of the factors that determines the microbial succession. Yeasts and LAB are the predominating microbial species at the initial phase of the fermentation process (Romanens et al., 2018). Jamili (2016); Ardhana (2003) states that the yeast at most the number and the type found in 24–36 hours after fermentation of cocoa because of their role in the process of degradation the various types of sugar that contained in the cocoa bean pulp. Visintin et al. (2017) found that in fermentation of two different cocoa hybrids (PS1319 and SJ02), the yeast (Saccharomyces cerevisiae and Torulaspora delbrueckii) metabolism results in ethanol production. In region called Agneby-Tiassa, species of Pichia kudriazevii and Candida nitrativorans was isolated; they are resistant to high temperatures (40 °C) and high ethanol concentrations (20%). Moreover, they exhibited pectin-hydrolysing enzymatic activities, suggesting its key role in the degradation of cocoa bean pulp during fermentation (Samagaci et al., 2016).

Spontaneous Malaysian cocoa bean box fermentations were carried out with high-quality raw material, resulting in successfully fermented dry cocoa beans and good chocolates produced thereof, it is likely that the prevailing species Hanseniaspora opuntiae, S. cerevisiae, Lactobacillus fermentum and Acetobacter pasteurianus were responsible for it (Papalexandratou et al., 2013). In cocoa beans fermentation performed in Abidjan (South East of Ivory Coast) (Koné et al., 2016), identified
yeasts that produced a total of 33 aroma compounds, among all yeasts involved *P. kudriavzevii, S. cerevisiae, Galactomyces geotrichum* and *Wickerhamomyces anomalous* could be considered as the most important contributors to the formation of cocoa specific aromatic compounds. Yeasts produced ethanol from sugars, and LAB produced lactic acid, acetic acid, ethanol, and mannitol from sugars and/or citrate (Camu et al., 2007; Papalexandratou et al., 2013). The experiment conducted by (Magalhães da Veiga Moreira et al., 2016) in Porto Híbrido 9, Porto Híbrido 15 and Porto Híbrido 16 cocoa beans at the Vale do Juliana farm in Igrapiúna, Bahia, Brazil, *Hanseniaspora uvarum, P. kluveri, P. caribbica* and *S. cerevisiae* were the predominant yeasts in the three fermentations. *L. plantarum, A. pasteurianus, Bacillus cereus* and *Lysinibacillus fusiformis* were the common bacterial species isolated from the fermentations. Twenty-seven volatile compounds were identified during the cocoa fermentation of all hybrids (Koffi et al., 2018). investigated 743 yeast strains, two strains *S. cerevisiae YB14* and *P. kudriavzevii YP13* were able on one hand to resist to most parameters such as temperature, pH, ethanol, organics acid, and on the other hand they were able to produce specific enzymes like pectinase, β-glucosidase, protease necessary to have a good cocoa fermentation process, and finally to produce acetoin which is desirable for flavor development in the fermentation process. These two strains could therefore be used as starter cultures which may contribute to the control of cocoa fermentation in Ivory Coast. Other results shown that *P. kudriazevii* and *Candida nitrativorans* are key players in cocoa bean fermentation in the Agneby-Tiassa region and they are promising candidates for developing starter cocktails that could be used to improve the overall efficiency of cocoa fermentation in Ivory Coast (Samagaci et al., 2016). Furthermore some specific yeast isolates could be used as biological markers to predict the determining of chocolate sensorial characteristics and to indicate the geographical origin or processing story of cocoa bean batches (Koné et al., 2016). Actually, it was concluded that the growth of LAB and AAB may not be essential for the fermentation of cocoa beans (Ho et al., 2018).

The experiment conducted by (de Melo Pereira et al., 2013) at a cocoa farm located in the city of Itajuípe, Bahia State, Brazil, the dominant species of major physiological roles were *S. cerevisiae* and *Hanseniaspora sp.* in the yeast group; *L. fermentum* and *L. plantarum* the LAB group; *A. tropicalis* belonging to the AAB group; and *Bacillus subtilis* in the *Bacillaceae* family. It demonstrated that at least three species of *Bacillus* are involved in cocoa fermentation, namely *B. subtilis, B. flexus* and *B. megaterium* (de Melo Pereira et al., 2013). Aerobic spore-forming bacteria, such as *Bacillus* strains, produce a variety of chemical compounds, including 2,3-butanediol, pyrazines, acetic acid and lactic acid, under fermentative conditions, which may contribute to the acidity and perhaps, at times, to off-flavors of fermented cocoa beans (Schwan, 1998). Increased aeration, increased pH value (3.5-5.0) of cocoa pulp, and a rise in temperature to about 45 °C in the cocoa mass in the later stages
of fermentation are associated with the development of bacteria of the genus *Bacillus* (Schwan and Wheals, 2004).

In Forastero and Trinitario cultivars at commercial fermentations in East Java, Indonesia was investigated. The later stages of fermentation were dominated by the presence of *Bacillus* species, mostly, *B. pumilus* and *B. licheniformis* (Ardhana, 2003; Nielsen et al., 2007). With regard to the fate of *Bacillus* spp. towards the end of the fermentation, this constitutes a microbial group that persists until the end (Lima et al., 2011). *Bacillus* spp. has also been classified as responsible for the production of TMP (de Melo Pereira et al., 2013) and 2,3-butanediol (sweet, floral (Crafack et al., 2014; Ardhana, 2003)) during cocoa fermentation; a temperature of 50–55 °C assists in this formation (Selamat et al., 1994).

The role of bacilli in cocoa fermentation is not well known (Nielsen et al., 2007). However, Ouattara et al. (2008) suggest that *Bacillus* sp. is liable to produce at least one enzyme during cocoa fermentation, so the degradation of the pulp during cocoa fermentation might not be only due to pectinolytic enzymes produced by yeasts but rather by a combined action of enzymes produced by both yeast and *Bacillus* strains. Ouattara et al. (2017) assumed that, during the latter stage of cocoa fermentation when simple sugars are depleted, production of pectate lyases (Pel) in *Bacillus* is stimulated by DegU gene to supply microbial cells with carbon source from polymeric pectic compounds. It is possible that *Bacillus* spp. play a bigger role in box fermentations than in heap fermentations, but more fermentations need to be investigated before firm conclusions can be drawn (Nielsen et al., 2007).

In other raw materials, the result showed that *Bacillus* sp. KR-8104 growth and the production of α-amylase on a wheat bran substrate could successfully (Hashemi et al., 2011). The production of L-phenylalanine (phenylalanine) is related to strains of *B. subtilis* (De Boer and Dijkhuizen, 1990). In brewery wastes, *B. subtilis* UO-01 was studied. Protease production delay seemed to be related to the consumption of non-protein and protein nitrogen, the maximum protease level (9.77 EU/mL) was obtained at pH 7.1 and 37.8 °C (Sánchez Blanco et al., 2016). In soya roasted cotyledons fermented for 18 h and 36 h at 35 °C, growth of *B. subtilis* led to the formation of volatile compounds: acetoin, 2,5-dimethylpyrazine (2,5-DMP) and TrMP (Owens et al., 1997). 2,5-DMP and TMP were produced using *B. subtilis* IFO 3013 grown on soybeans (Besson et al., 1997).

In a previous analysis of dry fermented beans of Criollo cocoa from APROCAM, considerable amounts of TMP, TrMP, 2,3-butanediol and acetoin (butter, creamy, green pepper (FEMA, 2018)) were founds (data not yet published). The presence of these compounds would lead us to think that in addition to the yeasts, LAB and AAB there may be certain *Bacillus* strains during the spontaneous fermentation of Criollo cocoa, however there is no information to corroborate this hypothesis, since the data so far found only they refer to other varieties or clones of cocoa.
Likewise, in previous studies (not in cocoa) the production of phenylalanine by *B. subtilis* has been reported, which gives the possibility of finding this amino acid in spontaneous cocoa fermentation and we would be facing a process of production of “external aroma precursors” by microbial action on the outside of the cocoa bean (Fig. 1).

### 2.3. Solid-state fermentation (SSF) of cocoa beans

#### 2.3.1. Spontaneous fermentation

Fresh cocoa beans are very astringent and do not develop the delicious and typical chocolate aromas alone (Papalexandratou and Nielsen, 2016) because they do not contain specific aroma precursors (Kadow et al., 2015); thus, the reason for the fermentation of cocoa is to reach biochemical reactions inside the bean that lead to the formation of aroma precursors, flavor and color, reduction of bitter and astringent tastes, and improvement of the physical appearance of cocoa (Apriyanto et al., 2017). Well-conducted fermentation is a prerequisite for the production of high-quality chocolates; if performed improperly, the production of specific aroma compounds will fail during the post-processing stages (Crafack et al., 2013). A 5-day fermentation period is common for the Forastero cultivar while shorter periods of about 2–3 days are required for Criollo and Nacional cultivars (Cevallos-Cevallos et al., 2018). In Peru, fermentation systems are used in wooden fermentation boxes, forming a batch process. These boxes act as bioreactors; the process begins at room temperature (27 °C) and ends at approximately 50 °C in seven days, with the transfer of the beans from one box to another from the second day to incorporate air. Cocoa beans are fermented by small producers in a traditional and uncontrolled process known as spontaneous fermentation (Magalhães da Veiga Moreira et al., 2017). During fermentation, small amounts of free water are present, and this process is characterized as SSF (Nielsen et al., 2014).

Until the pods open, the beans are sterile microbiologically. Once the pod is opened, the beans are exposed to numerous sources of microorganisms, including the hands of farmers and utensils. The immediate effect is the initiation of microbiological attack on the pulp sugars by the yeasts, and this action is followed by the LAB and AAB (Afoakwa, 2010). This succession is directed by yeasts, which dominate the microbial population during the first hours, followed by the LAB, which decrease after 48 hours of fermentation, and finally the AAB increase in number (Ardhana, 2003). The fermentation of cocoa beans occurs in two phases. The first involves reactions that occur in the pulp, and the second involves several hydrolytic reactions that occur within the bean (Pereira et al., 2012). While the first phase is performed at 25–45 °C, the second phase is at 42–52 °C (Kadow et al., 2015). Changes in pH, temperature, sugar content and fermentation products or metabolites exert a selection pressure on existing natural biotypes, favoring the strains that are better adapted to...
this environment (Pereira et al., 2012). These metabolites kill the bean and trigger a series of biochemical reactions in their interior (Pereira et al., 2016), generating aroma precursors (Fernández Maura et al., 2016); the pulp is solubilized, giving rise to a liquid material (exudate) that drains through the holes of the fermenter boxes (Ardhana, 2003), and this material serves as a way of transport for the metabolites of fermentation.

The pulp is the base substrate for microorganisms during fermentation. It is important to define the best state of the pulp for a successful fermentation according to the best results of quality determining components (Voigt and Lieberei, 2014). In fine cocoas, the level of the basic aromatic components (basic notes) of cocoa (and chocolate) is usually low due to the short duration of the fermentation (2–4 days), compared to bulk cocoa (5–7 days) (Torres-Moreno et al., 2012). The findings of previous studies show that linalool is a constituent of the pulp that is transferred to the beans during fermentation (Voigt and Lieberei, 2014). Linalool can be produced by S. cerevisiae from the catabolism of leucine (Carrau et al., 2005). It is possible that glycosidase enzymes (α-arabinosidase, β-galactosidase, α-mannosidase) play an important role in the release of this compounds (Voigt and Lieberei, 2014). For example, 1,020 ppb of linalool was found in dry fermented Criollo cocoa beans (Chetschik et al., 2017), or, in another study, 130 μg/kg (Frauendorfer and Schieberle, 2008).

The quality of beans is strongly dependent on the degree and time of acidification of the cotyledon during the fermentation process (Voigt and Lieberei, 2014). Cocoa fermentation at a pH of approximately 5 shows a cocoa with the highest potential for specific aromas (Janek et al., 2016). It is known that strong acidification of the cotyledon (pH < 5) during the fermentation produces a poor potential of aroma in the cocoa beans, because the activity of carboxypeptidase is optimal at approximately pH 5.5 and is reduced to pH < 5. At pH values >5.5, the aspartic endopeptidase is inactivated (Voigt et al., 1994).

Spontaneous fermentation is an empirical procedure, which may not yield beans of consistent quality, which requires the chocolate industry to continuously modify its formulations (de Melo Pereira et al., 2013). Common problems involve acidity levels and incomplete fermentation, resulting in deficient or unpleasant aromas (Schwan, 1998). The studies carried out in a simulated medium of cocoa pulp and indicate that there could be competition among specific strains called L. plantarum, L. fermentum and A. pasteurianus during the spontaneous fermentation process, improving the aroma of the bean (Lefeber et al., 2010); however, there is no further information on how this has improved the fermentation process of the cocoa bean under field conditions (Ghosh, 2016), and thus producers still use spontaneous fermentation for their commercial cocoa. As a alternative (de Melo Pereira et al., 2013), described the first experimental validation of the stainless steel tank method
for cocoa bean fermentation. They concluded that the use of stainless steel tanks may be of great interest for those who seek improved control over the cocoa fermentation process and/or to optimize cocoa fermentation through the use of starter cultures. In accord (Mota-Gutierrez et al., 2018), found that the microbial dynamics and associations between the bacteria, yeast and metabolites were found to depend on the type of fermentation. With the growth of niche markets for chocolate manufacturers, a better understanding of the factors that contribute to flavor variations will have significant commercial implications (Afoakwa et al., 2008). As a consequence of the increasing implementation of traceability systems in the food industry, detailed information about how products are handled could be transferred to the producers and could be used to optimize the processes (Saltini et al., 2013).

Although it is true that cocoa fermentation has been widely studied, there are few works on spontaneous fermentation of Criollo cocoa, in the work made by (Cevallos-Cevallos et al., 2018), spontaneous fermentation was carried out in a greenhouse at ambient temperature (about 35 °C) for Forastero, Criollo and Nacional cultivars; fine-flavor cultivars were characterized by the presence of significant amounts of hydrocarbons such as valencene, aromadendrene, germacrene, 1,3 cyclohexadiene and octacosane while fermented beans of the Forastero bulk cocoa showed negligible levels of these volatiles. In Amazonas, the fermentation process is the same for both Criollo cocoa and CCN 51 clone (bulk cocoa); the same boxes are used and a product is obtained in 7 days, which leads to obtaining cocoa beans of different qualities. This is a problem that has not yet been solved due to the lack of research.

2.3.2. Fermentation controlled by starter culture

As far as we know, the use of starter cultures in Criollo cocoa fermentation has not been studied; therefore, the studies analyzed here generally correspond to cocoa clones. Studies of fermentation and chocolate using PS1319 hybrid cocoa and starter cultures can provide important results for improving the fermentation of cocoa and the quality of chocolate (Batista et al., 2016). The use of LAB starter cultures could lead to a controlled fermentation process and thus the possibility of controlling the aroma (Saltini et al., 2013). SSF was used to ferment Forastero cocoa with a starter culture; it was found that a 10% culture composed of S. cerevisiae, L. plantarum and A. aceti was adequate to produce chocolate with 7.5 out of 10 points on a hedonic scale for acceptability of the aroma of chocolates produced from cocoa beans fermented with starter culture (Sandhya et al., 2016). Other researchers have inoculated starter culture composed of S. cerevisiae, L. plantarum and A. pasteurianus in the fermentation of cocoa hybrids; the fermentation process was accelerated, and the chocolates made with inoculated beans were bitter, sweet and cocoa-flavored, with considerable amounts of 3-methylbutanal, 2-phenylethylacetate (Magalhães
da Veiga Moreira et al., 2017). In the roasted liquor of cocoa produced from fermentations of hybrids inoculated with *L. fermentum* L18, *A. pasteurianus* A149, *P. kluyveri* and *Kluyveromyces marxianus*, 1,3-butanediol was detected (Crafack et al., 2014). Thus, inoculation affects the profiles of volatile compounds and their relative concentrations, which influence the sensory characteristics of chocolate (Batista et al., 2016).

Results reported by (Pereira et al., 2017) indicated that inoculated fermentations with *P. kudriavzevii* LPB06 and *P. kudriavzevii* LPB07 generated cocoa beans with better color development and richer aroma composition, suggesting that cocoa-associated yeast diversity at strain level can be exploited for flavor modulation of cocoa beans. In fermentation inoculated with *S. cerevisiae* FNCC 3056, *Lactococcus lactis* FNC 0086 and *A. aceti* FNCC 0016, an increase of hydrophobic amino acids was obtained, demonstrating that the addition of inoculum can gradually degrade protein to produce more hydrophobic amino acids as aroma precursors (Apriyanto et al., 2017). LAB can also metabolize sugars and organic acids to produce various aldehydes, ketones and other volatile components that can impact the sensory quality of the bean (Ardhana, 2003). It is known that some compounds come from the metabolism of yeasts, such as phenylethylacetate (fruit, sweet, honey (Magalhães da Veiga Moreira et al., 2016)), 2-phenylethanol (honey, floral (Magalhães da Veiga Moreira et al., 2016)), 2-phenylacetic acid and acetoin (butter, cream (Batista et al., 2016; Visintin et al., 2017)). Table 4 shows that in the fermentation of cocoa hybrids controlled by a starter culture, it is possible to obtain aromatic compounds identified in Criollo cocoa (Crafack et al., 2014; Batista et al., 2016; Visintin et al., 2017; Magalhães da Veiga Moreira et al., 2017). It may not be necessary to use starter cultures to improve the aromatic quality of Criollo cacao, which is why this type of research has not been carried out with this variety, since its own genetics and origin would determine its fine aroma. However, we consider it important to study the process of fermentation of Criollo cocoa to have a standard process based on the amount of aroma precursors produced. Then, so that the Forastero cocoa fermentation reaches this standard, a type and concentration of the starter culture would be necessary. From the isolation of the best strains found in the Criollo cocoa fermentation, this culture would be developed and used in Forastero cocoa fermentation.

### 2.4. SSF kinetics through mathematical modeling and its application in cocoa

A mathematical model serve as tools for engineers and scientists to develop an understanding of important systems and processes using mathematical equations (Ribas-García et al., 2011; Rasmuson, 2014), is an important instrument which allows understanding the behavior of food, predict results (Pinheiro et al., 2017),

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generalize the processes evaluating the optimal conditions that lead them (Kulov and Gordeev, 2014) and generate insurance control mechanisms for process quality (Balbinoti et al., 2018). A realistic kinetic model is required for the design, optimization and control of processes (Ccopa Rivera et al., 2017). The SSF is driven by a complex interaction between various microorganisms and their metabolites. To control this process, and consequently the quality of the final product, a deep understanding of the metabolic pathways and their regulation is essential. Therefore, studying the fine aroma compounds produced by different starter cultures is crucial for improving the chocolate industry. Table 4 presents the fine aroma compounds produced by various starter cultures.

**Table 4. Fine aroma compounds produced by starter culture.**

| Starter culture | Sample | Raw material | Fine aroma components identified | Source |
|-----------------|--------|--------------|----------------------------------|--------|
| *S. cerevisiae*  | Chocolate and dry fermented beans | Hybrid PS1319, Brazil | 2-Heptanol, 2-pentanol, Phenylethyl alcohol, 2-Methylbutanal, 2-Phenyl-2-butenal, Ethyl octanoate, Ethyl phenylacetate, 2-Heptanone, 2-Phenylethyl acetate, 2-pentanone, 2-nonenone, 2-undecanone, Acetophenone | Batista et al. (2016) |
| *P. kluyveri*    | Chocolate | PH15 cocoa hybrid, Brazil | 2-Heptanol, 2-pentanol, 2,3-Butanediol, Phenylethyl alcohol, 2-Phenyl-2-butenal, 2-Heptanone, Acetophenone, Linalool, trans-Linalool oxide (pyranoid), cis-Linalool oxide (furanoid), 2-nonenone | Magalhães da Veiga Moreira et al. (2017) |
| *Hanseniaspora uvarum* | Chocolate | hybrids of cocoa: PS1319 and SJ02 de Brazil | 2,3-Butanediol | Visintin et al. (2017) |
| *P. kluyveri*    | Chocolate | Forastero from Ghana | 2-pentanol, 1,3-Butanediol, 2,3-Butaanidol, 2-Methylbutanal, 2-Phenyl-2-butenal, Ethyl phenylacetate, Ethyl decanoate, Ethyl phenylacetate, 2-Heptanone, 2-nonenone, Linalool, trans-Linalool oxide (pyranoid), β-myrcene | Craack et al. (2014) |
understanding of this interaction is necessary (Nielsen et al., 2014). Then, the optimal parameters to control the process can be determined through the development of kinetic models for the process (Liu et al., 2016). For example, progress has already been made in describing the process of SSF conducted by S. cerevisiae in the production of rice wine, for which a first-order kinetic model based on the analysis of the main metabolites of fermentation has been developed (Liu et al., 2015). In the case of cocoa, there have been some attempts to study the transition of the microbial population in heap fermentation, but there are no detailed studies of its kinetics (Ghosh, 2016). Recently (Moreno-Zambrano et al., 2018), has constructed a mathematical model using ordinary differential equations with two distinct types of state variables: (i) metabolite concentrations of glucose, fructose, ethanol, lactic acid and acetic acid and (ii) population sizes of yeast, LAB and AAB. However, it is worth mentioning that the spontaneous fermentation process will be specific for each batch of cocoa, therefore it will be necessary to develop a controlled fermentation that is a standard reference for the batches and thus be able to produce cocoa of the most uniform quality possible.

The study of SSF involves the determination of fermentation kinetics (e.g., biomass production, substrate consumption and production of metabolites). The data collection comprises the measurement of microbial growth quantified by standard methods of isolation and metabolic activity with high-performance liquid chromatography (HPLC) (Nielsen et al., 2014). Kinetic models use parameters that describe how quickly a microorganism can grow, such as the duration of the lag phase and generation time, and those models are used as the response variable for various conditions of pH, temperature, and water activity (Adams and Moss, 2008). Studies have demonstrated the utility of kinetic models that can predict cell growth and ethanol production from sugarcane fermentations (Ccopa Rivera et al., 2017). The influence of fermentation practices on the dynamics of the microbial population over 3 days in National cocoa (a type of fine cocoa) has been studied; analyzing the pulp and bean metabolites and sensory analysis of the chocolates produced from both fermentations (Papalexandratou et al., 2011); however, they did not develop a kinetic model that allows predicting the behavior of the process. In SSF the water content of the solid substrate is usually maintained between 12 and 80% (Chen, 2013). The most important consideration for kinetics is the formation of biomass, either linear (equation 1), exponential (equation 2), logarithmic (equation 3) or two-phase formation (equation 4) (Ghosh, 2016). Where $K$ is the linear growth rate, $\mu$ is the specific growth rate, $x$ is the biomass at a time $t$, $x_m$ is the maximum biomass, $t_a$ is the time when the deceleration phase begins, $k$ is the first order constant and $L$ is a ratio between the growth rate in the deceleration phase and the specific growth rate during the exponential phase. It is still unknown to extent these calculations are valid in food processing SSF (Ghosh, 2016).
\[dx/dt = K\] (1)

\[dx/dt = \mu x\] (2)

\[dx/dt = \mu x\left[1 - (x/x_m)\right]\] (3)

\[dx/dt = \left[\mu L e^{-k(t-t_0)}\right], t > t_a\] (4)

Previous studies suggest that the cotyledon of cocoa absorbs the aromatic components of the pulp during the fermentation process (Eskes et al., 2012). This cotyledon is considered a reservoir for fine notes (Chetschik et al., 2017); however, the exact mechanism by which this situation is reached and the role of the fermentation microflora in this process has not been investigated (Ali et al., 2013). The fine aroma precursors produced in the pulp during the fermentation phase will migrate into the bean when the permeability of the bean is improved and are then retained during the drying phase (Ascrizzi et al., 2017); Then, the diffusion of aroma precursors into the Criollo and Forastero cocoa bean is possible; for example, during the process of fermentation of cocoa, the amino acid phenylalanine (external precursor) produced by bacillus in the pulp of cocoa can enter the bean by molecular diffusion and accumulate inside to join the amino acids (internal precursors) already produced during the internal reaction of the VCG, this way increase the amount of aroma precursors (Fig. 1). The bean shell would be the barrier for this diffusion and its structure would determine the speed of this phenomenon and therefore the speed of the process to obtain an optimal value. Theoretically, this process is characterized by the coefficient of molecular diffusion \(D\). The experimental determination of this coefficient is indispensable to describe the process of mass transfer via Fick’s Second Law (Koukouch et al., 2017) in a non-stationary state.

In other studies, it has been demonstrated that fermentations with added passion fruit pulp showed more pronounced aromas of interest, namely, fruit, acid and floral notes in cocoa beans (Ali et al., 2013). In the cucumber fermentation process, the exchange of malic acid, lactic acid, NaCl and sugar between the cucumber and its brine was monitored; an exponential equation that described the movement of the solutes during the fermentation was described, and the diffusion coefficient of the sugar was estimated between \(1.80 \times 10^{-9}\) and \(9.18 \times 10^{-9}\) m\(^2\)/s, which was used to determine the optimal concentration of sugar within the cucumber at any time; to determine processing parameters in manufacturing, this process was modeled mathematically (Fasina et al., 2002). If this phenomenon occurs in the aforementioned food systems, it will also happen in the process of fermentation of Criollo and Forastero cocoa, where it will be necessary to characterize the phenomenon of transfer of aroma precursors to the interior of the bean to establish an optimal process time. In the case of Criollo de Amazonas cocoa, it is possible that the current fermentation time of 7 days is excessive and it would be risking the aromatic quality of the cocoa to obtain an acceptable percentage of fermentation.
During the last decade, the analysis and the modeling of the kinetics of the convective drying of cocoa beans were the scope of several papers (Hii et al., 2008; Hii et al., 2009b; Hii et al., 2009a; Clement et al., 2009; MacManus Chinanye et al., 2010; Teh et al., 2016; Nwakuba et al., 2017; Olabinjo et al., 2017; Herman et al., 2018) leaving a side the study of the kinetic of formation and diffusion of aroma precursors through mathematical models that allow to improve processes, even more so in Criollo cacao there have been no advances.

3. Conclusions

The cocoa market has divided this product into fine aroma and bulk. Because bulk beans are more productive and resistant to diseases, studies are being carried out with the aim of improving their quality. The technology developed for this purpose is the use of starter cultures that help the fermentation process making it more efficient, this culture will be mainly composed of yeasts, since they would be the most important microorganisms. However, this technology has not been widely adopted by cocoa farmers, at least in Peru the traditional process of spontaneous fermentation has been carried out for all varieties of cocoa, including Criollo and Forastero. To achieve the expected quality results, it is necessary to study the production of external aroma precursors by the microorganisms and the diffusion of these precursors towards the interior of the bean and how they come to be added to the internally generated precursors until reaching optimum concentrations. The study of this phenomenon of molecular diffusion must be carried out through mathematical modeling in order to predict these concentrations in a determined time. We consider it necessary to know in depth the process of fermentation of Criollo cocoa, the process of diffusion of external precursors and isolate the microorganisms founded in the process, from this, develop starter cultures that can be applied to the process of fermentation of cocoa in Forastero (bulk). Undoubtedly, the both origin and genetic factors will play an important role that should not be left aside.

Declarations

Author contribution statement

All authors listed have significantly contributed to the development and the writing of this article.

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**Competing interest statement**

The authors declare no conflict of interest.

**Additional information**

No additional information is available for this paper.

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