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REVIEW ARTICLE

Advances in bean-to-bar chocolate production: Microbiology, biochemistry, processing, and sensorial aspects

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Abstract

To meet the market demand for fine, artisanal, and agroecological chocolates, companies called bean-to-bar have emerged, with full control over the production chain. They establish strict criteria for cocoa bean sourcing and chocolate production. One of the key stages in flavor development is fermentation, which occurs spontaneously through the microorganisms in the environment, fruit, fermentation boxes, and utensils. In the cocoa market, cocoa beans are classified into two types: bulk and flavor. Beans categorized as bulk are derived from commodity cocoa, a result of incorrect or incomplete fermentation, which does not lead to the development of aroma and flavor. Bulk cocoa is widely used in industrial chocolate production because, besides being cost-effective, it does not require fine flavors since other ingredients will be added. On the other hand, 'flavor' cocoa beans are well-fermented, thus developing their flavor and aroma compounds, and these are used in bean-to-bar processing. This present article is a review concerning the general aspect of cocoa microbiology, biochemistry, processing, and their effects on bean-to-bar chocolate production.

Keywords: Cocoa beans; Fermentation; Bean-to-bar; Microbiota.

Highlights

- Cocoa fermentation is the most critical step in the process
- Fermentation is the process of microbial succession
- Bean-to-bar chocolate comes from selected fine cocoa beans

1 Introduction

1.1 The cocoa tree and species varieties

Cocoa beans, the primary ingredient in chocolate production, are naturally inhabited by a diverse microbial community. The composition of this microbiota can vary depending on several factors, including geographic location, farming practices, and post-harvest handling. Understanding the dynamics of these microbial populations is crucial to ensure the quality and safety of bean-to-bar chocolate. The cacao tree (*Theobroma cacao* L.) is a perennial tree that gives rise to the fruit called cocoa. It is native to the humid forests of Tropical America and grows in the shade of other trees. Cocoa, the fruit of the cacao tree, measures from 15 to 30 centimeters. It has an oval shape and it develops on the trunks of the cacao tree. Each fruit contains an average of 25 to 40 seeds or cocoa beans, covered by a white pulp of mucilage rich in glucose, fructose, and sucrose, with a high content of citric acid, as well as organic acids such as acetic acid and lactic acid. These seeds will give rise to cocoa beans, and their pulp will be fermented by microorganisms. As the fermentation process progresses, the pulp degrades and loses its liquid, and the cotyledon begins to turn brown due to biochemical processes. From this point on, the essential reactions for the formation of almond flavor and aroma begin. After the death of the germ, they will be dried and transformed into almonds that will give rise to chocolate (Copetti et al., 2014; Delgado-Ospina et al., 2021).

There are three main varieties of cocoa: Criollo, Forastero, and Trinitario (a hybrid of the previous two), which differ in shape, productivity, and physicochemical characteristics. Criollo is rare and has low productivity, its seeds lack anthocyanins, so they have a white color. However, this variety is highly aromatic and undergoes rapid fermentation (48-72 hours), making it suitable for the production of fine chocolates. On the other hand, Forastero is the most productive, accounting for about 70% of the world's production. However, it is less aromatic and requires approximately 168 hours for fermentation. Trinitario, being a hybrid, is more resistant to pests and also has floral and fruity aromas (Castro-Alayo et al., 2019).

1.2 Production of cocoa

Currently, Africa is the largest producer of cocoa in the world, accounting for 76.3% of the production, followed by the Americas with 17.4%, Oceania and Asia with 6.3% each. In terms of productivity by country, Brazil ranks 7th, in the world with approximately 255,184 tons (4.6%), behind Ivory Coast (39%), Ghana (14.5%), Indonesia (14%), Nigeria (6.3%), Ecuador (5.1%), and Cameroon (5%) (FAOSTAT, 2020).

Cocoa is economically significant as its beans are used to produce chocolate, a globally consumed product. In 2019, Brazil produced a total of 5.6 million tons, according to data from FAOSTAT (2020). Brazil once held the position of the world's largest cocoa producer. However, cocoa production in Bahia was decimated in 1989 by a disease called "witch's broom," caused by the fungus *Moniliophthora perniciosa*. This resulted in a loss of 90% of cocoa production, as infected fruits were rotted, deformed, and hardened.

Nowadays, only three states in Brazil have significant cocoa production. The main cocoa producing state is Pará, followed by Bahia and Espírito Santo. Brazilian cocoa production is mainly concentrated in Bahia and Pará, representing 90% of the national production (AIPC, 2022). Brazilian cocoa has a strong sustainable and historical appeal since part of the production is agroforestry-based, preserving biomes such as the Atlantic and the Amazon Rainforests. According to the Brazilian Association of Chocolate, Peanut, and Candy Industry (ABICAB), there are various types of cocoa beans in Brazil, differentiated not only by species but also by the region of production and the quality employed in the process. This results in two types of cocoa: industrial commodity cocoa, known as "bulk," and fine cocoa, typically used for artisanal production.

1.3 Cocoa distinction

The main distinction between bulk cocoa and fine cocoa is not due to the species or the cultivation but rather the process employed in the primary production phase, which takes place on the farms. Bulk or ordinary cocoa does not undergo selective harvesting, so green, deteriorated, or fungus-infected fruits are used in production. Additionally, fermentation, which is often the key step for flavor and aroma development, is not adequately carried out. As a result, bulk cocoa loses its special sensory characteristics and retains only off-flavors. In this case, cocoa needs to undergo a more intense roasting process to mask the defects and high levels of sugar and flavorings are used to correct and standardize these imperfections (Ferreira, 2017a).

On the other hand, fine cocoa comes from selected beans from the beginning of the harvest, discarding infected fruits and harvesting only fruits at the appropriate stage of ripeness. These fruits undergo the main step for flavor development, which is fermentation. It is during fermentation that the full potential of cocoa's genetic variation and terroir is highlighted in unique sensory characteristics (Ferreira, 2017b).

1.4 Composition of cocoa fruits and its products

To make chocolate, specific components of the cocoa fruit are used, a definition of each of the cocoa compositions is given by Copetti (2009):

- Cocoa: It is the fruit of the cacao tree, which grows on the trunks and has an elongated shape. Its color varies from green to purple to yellow and orange, depending on the variety. It contains 25 to 40 seeds surrounded by a white mucilage called pulp;
- Cocoa pulp: It is the white mucilage that surrounds the seeds and cocoa beans. It is rich in carbohydrates and sugars and serves as a substrate for microorganisms crucial to the fermentation process;
- Cocoa seed: It has an oval shape, measuring 3 to 5 centimeters, varying according to the variety. It consists of two cotyledons, the germ, and the kernel, which is a thin film that covers the seed;
- Cocoa bean: After fermentation, the seed loses its ability to germinate and is then called a cocoa bean;
- Cocoa nibs: These are fragmented cocoa beans without the kernel. They are the primary ingredient used in chocolate production;
- Cocoa butter: It is the fatty portion of the cocoa bean, representing 50% of the total composition. It is obtained through hydraulic pressing, a process carried out industrially. Cocoa butter can be deodorized and clarified;
- Cocoa cake: It is the dough of pressed cocoa and it has 10-20% cocoa butter;
- Cocoa powder: Cocoa cake is finely ground to produce cocoa powder. It is not consumed as such but is used to prepare chocolate drinks, cocoa with sugar, or as an ingredient for the preparation of pies, cookies, and puddings. Due to its strong flavor, the cocoa powder content in the final product is low, normally less than 5%.

2 Primary processing

Primary processing involves the steps carried out in the field (Figure 1). Therefore, it is the responsibility of the farmer to harvest and process the cocoa until it becomes fermented and dried beans.

Figure 1. (a) Fruit Harvest; (b) Opening of almonds; (c) Fermentation boxes; (d) Cocoa stirring; (e) Fermentation trough with almonds; (f) cut test (source: own author).

2.1 Harvesting and opening of fruits

Harvesting is done using tools called pod cutters, when the fruits are ripe and have a sufficient amount of fermentable sugar in their pulp (Figure 1a). The degree of ripeness depends on each variety and season, and it is up to the producer to determine the standard degree of ripeness for harvesting (Copetti et al., 2014).

Fine cocoa harvest is carried out manually to collect only ripe fruits, avoiding the inclusion of immature fruits that cause astringency and undesirable bitterness in chocolate, which hampers fermentation. The fruits are manually opened with the help of machetes on the plantation itself (Figure 1b). The placenta containing the pulp and seeds is removed by hand and often placed on banana leaves on the ground. After harvesting, the opening of the fruits should not exceed 24 hours because they start to deteriorate, which hampers fermentation (Silva, 2018).

2.2 Fermentation

Beans classified as bulk are derived from commodity cocoa, usually from the Forastero variety or the result of incorrect or incomplete fermentation, which does not develop aroma and flavor. Bulk cocoa is widely used in industrial chocolate production because it is cheap and does not require fine flavors since other ingredients will be added. On the other hand, flavor cocoa usually comes from the Criollo and Trinitario varieties or any variety, as long as the beans are well-fermented, developing their aromatic compounds and flavor. Fine cocoa has high added value as it can exhibit floral compounds, citrus notes, tobacco notes, and others, making it suitable for the production of fine or gourmet chocolate (Silva, 2013).

Following this classification, fermentation is the most important step in obtaining fine cocoa as it is where the precursor compounds for aroma are formed, making cocoa special. Fermentation is a spontaneous microbiological succession process involving fungi, yeasts, lactic acid bacteria, and acetic acid bacteria. These microorganisms come not only from the fruit's skin but also from utensils, collectors' hands, fermentation boxes, soil, and air (Ardhana & Fleet, 2003).

After harvesting, the pulp of the fruits is taken to wooden fermentation boxes as shown in Figure 1c, or can be fermented in piles on banana leaves, although the latter method is not very desirable as fermentation occurs unevenly. The beans are stirred and covered with banana leaves or jute sacks, which prevent the surface layer from drying out and retain heat within the cocoa mass.

Spontaneous fermentation lasts for 4 to 7 days, depending on the cocoa variety and processing conditions. The fermentation boxes have holes through which the degraded pulp juice drains, which can be used for making jellies, sweets, juices, and other products. Fermentation is divided into two phases: biological and chemical. The biological phase involves the degradation of the pulp through microbial activity, and the second phase involves transformations of acids and alcohols, resulting in off-flavors (Ferreira, 2017a).

Due to the high sugar content in the pulp, low pH (around 3.5 to 3.6) due to citric acid, and the lack of oxygen due to the overlapping of the seeds, the initial fermentation of the beans is anaerobic. Yeasts are the first microorganisms to appear during fermentation, converting carbohydrates into ethanol and reducing fermentable sugars. This is followed by the fermentation of lactic acid bacteria, which also ferment these sugars, producing lactic acid and slightly raising the pH and temperature (Copetti et al., 2014).

Yeasts and lactic acid bacteria play an important role in the degradation of the pulp through the secretion of pectinolytic enzymes, and during this stage, filamentous fungi can also be found. The next step involves the stirring of the almonds in the fermentation vat, where the material is aerated (Figure 1d). Aerobic fermentation begins, favoring the succession of acetic acid bacteria, promoting the oxidation of ethanol and subsequently producing an exothermic reaction, raising the temperature to 45-50 °C (Nielsen et al. 2013).

As the temperature increases, organic acids (such as oxalic, phosphoric, succinic, and malic acids) are produced, migrating to the inner part of the seeds. Secondary metabolites that give rise to the flavor and aroma of chocolate are also produced (Almeida, 2018). With the production of organic acids, ethanol, and the high temperature, the embryo dies, and the seed is now referred to as an almond (Figure 1f). After the inactivation of vegetative cells, spore-forming bacteria emerge, exhibiting high enzymatic activity and contributing to the formation of off-flavors (Nielsen et al., 2013).

Studies indicate sensory differences according to the terroir of each almond, influenced not only by genetics or variety but mainly by the different microorganisms present in each region (Moreira et al., 2013).

Several microorganisms are involved in cocoa fermentation. In the following sections, the role of each group is described and discussed to obtain the final product.

2.2.1 Yeasts

Yeasts are unicellular fungi, divided into ascomycetes and basidiomycetes. They are spherical, oval, or cylindrical and usually reproduce through budding. Yeasts are present in almost all environments due to their ability to utilize various types of substrates for their growth. They primarily grow in the presence of sugars and also require sources of carbon and hydrogen for their development. They are predominantly aerobic and facultative anaerobes, with an optimal growth temperature of 25 to 30 °C and pH range of 4 to 7. The yeast species most associated with and described in cocoa fermentation are: *Candida krusei, C. pelliculosa, C. rugopelliculosa, C. rugosa, C. humicola, Kluveromyces marxianus, Kloechera apiculata, K. thermotolerans, Lodderomyces elongisporus, Pichia fermentans, Saccharomyces cerevisiae, Torulaspora pretoriensis* (Schwan et al., 2014).

2.2.2 Lactic Acid Bacteria

Lactic acid bacteria (LAB) are characterized by their strictly fermentative metabolism, with lactic acid being the product of sugar fermentation. They are the main source of probiotics in foods. They are Gram-positive, non-spore-forming, acid-tolerant, fastidious, and generally non-motile. They are mesophilic, generally growing in a temperature range from 5 °C to 45 °C, and require amino acids, pyrimidine, and purine bases, as well as B-complex vitamins for their growth. They are facultative anaerobes, able to ferment in both anaerobic and aerobic environments, albeit more slowly. LAB are encompassed in the order *Lactobacillales* and subdivided into six families: *Aerococcaceae, Carnobacteriaceae, Enterococcaceae, Lactobacillaceae, Leuconostocaceae,* and *Streptococcaceae*, comprising more than 530 species and subspecies (List of Prokaryotic Names with Standing in Nomenclature, 2021).

The classification of LAB is based on their morphology, the way they ferment glucose, the appropriate temperature range, adaptation to different media, and pH. One of the differences between LAB subgroups is the products formed during fermentation. Among them, there are two groups known as homofermentative (HoLAB) and heterofermentative (HeLAB). The homofermentative group exclusively produces lactic acid, where the enzyme adolase directly ferments glucose into lactic acid. This includes the *Streptococcus* genus (L(+) lactate) and *Pediococcus* (D-L L lactate). On the other hand, the heterofermentative group converts hexoses into pentoses through the enzyme phosphoketolase, resulting in the production of lactic, formic, butyric acids, ethanol, and CO_2. This process also involves the production of diacetyl and aldehydes, which are aroma precursor substances, many of which are important in chocolate manufacturing (Quinatoa, 2017).

2.2.3 Acetic Acid Bacteria

Acetic acid bacteria (AAB) play an important role in the fermentation of cocoa beans. Their multiplication and production of acetic acid are essential for the hydrolysis of proteins in the cotyledon. Additionally, the acid helps in the permeabilization of enzymes secreted by yeasts to the core of the cocoa bean. Together with lactic acid, it reduces the pH to allow enzymatic reactions in the chemo-fermentation phase. It is during this phase that the enzymes inside the seed are activated due to the pH change and the spreading of cellular contents, resulting in the death of the seed, which is now called a cocoa bean (Ardhana & Fleet, 2003; Schwan & Wheals, 2004; Nielsen et al., 2013).

They are Gram-negative microorganisms, aerobic, catalase-positive, and belong to the *Acetobacteriaceae* family. They have a cocci or rod-shaped morphology, can be motile, do not form spores, and oxidize sugars from secondary metabolites. The optimal pH range for growth is 5 to 6.5, although they can grow at pH 3 to 4. The optimal multiplication temperature is 30 °C.

AAB can develop in alcoholic, sugary, and slightly acidic environments. They oxidize sugars and alcohols in these media, resulting in the accumulation of organic acids and the production of acetic acid from ethanol. The most recurring genera in cocoa fermentation are *Acetobacter* and *Gluconobacter*, which encompass more than 40 species. *Acetobacter* preferably metabolizes ethanol, while the others ferment sugars such as glucose and fructose.

Among the two genera, *Acetobacter* was the most common genera found during fermentation in cocoa samples from Indonesia and the Dominican Republic, with *A. lovaniensis* the most common species (Ardhana & Fleet, 2003). In Ghana, the most common species were *A. syzygii, A. tropicalis,* and *A. pasteurianus* (Nielsen et al., 2007). The most common species in Brazil were *A. aceti, A. tropicalis* and *Gluconobacter oxydans* (Schwan et al., 2014).

2.2.4 Association between yeasts and bacteria during fermentation

The pulp that covers cocoa seeds contains an average of 84% water, 13% sugars, 2% citric acid, and 1% pectin. The limited air penetration into the mass due to the compression of the pulp results in a low level of available oxygen, along with a low pH (3.6) due to citric acid. This creates an environment with ideal conditions for the proliferation of yeasts, responsible for alcoholic fermentation (the first stage of cocoa fermentation lasting 24 to 28 hours). Research conducted in Brazil has identified the following yeasts associated with Brazilian cocoa fermentation: *Candida bombi, C. pelliculosa, C. rugopelliculosa, C. rugosa, C. humilis, Yarrowia lipolytica, Kluveromyces marxianus, Kloechera apiculata, K. thermotolerans, Lodderomyces elongisporus, Pichia fermentans, Saccharomyces cerevisiae*, and *Torulaspora pretoriensi* (Schwan & Wheals, 2004).

Some yeasts produce the enzyme pectinase, which reduces the viscosity of the pulp, resulting in a liquid exudate popularly called cocoa honey, consequently contributing to the aeration of the mass. They also produce organic acids such as succinic and acetic acids, which initiate embryo death and contribute to the formation of flavor precursors through the production of volatile compounds such as aldehydes, ketones, terpenes, and esters (Almeida, 2018).

Around 36 hours of fermentation, lactic acid bacteria (LAB) start to multiply due to the microaerobic conditions of the previous phase and the aeration of the mass through stirring. Along with the formation of alcohol, the temperature of the mass increases, and the pH rises to 4. The most recurring bacteria mentioned in the literature are *Lactobacillus plantarum, L. fermentum, Leuconostoc pseudomesenteroides,* and *Enterococcus casseliflavus*. They are responsible for metabolizing the remaining sugars, resulting in the degradation of the pulp and production of lactic acid, reducing sugars to 2%. They also contribute to the diffusion of polyphenols with cellular fluids, undergoing oxidation and complexation of high molecular weight, especially insoluble tannins.

Sugars are hydrolyzed along with anthocyanins, which are hydrolyzed into anthocyanidins, resulting in the lightening of the violet color of the cotyledon. Polyphenol oxidases (mainly anthocyanidins and epicatechins) convert polyphenols into quinones, which form complexes with peptides and proteins, reducing astringency and resulting in the formation of brown color in the seed (Soares, 2001).

The alcohol formed in the first 48 hours of fermentation, along with the increase in pH and aeration of the mass, creates a favorable environment for the development of acetic acid bacteria (AAB), initiating aerobic fermentation. These bacteria oxidize alcohol into acetic acid, causing the cocoa mass to acquire a vinegar-like odor. They follow the metabolic pathway through the periplasm and cytoplasm, facilitated by the action of two enzymes: alcohol dehydrogenase (ADH) and acetaldehyde dehydrogenase (ALDH). This oxidation reaction is highly exothermic, raising the temperature of the cocoa mass to around 50 °C (Bastos, 2016). From the third day of fermentation, there is a decrease in spore-forming bacteria, leading to the appearance of metabolites that, if diffused into the cotyledon nucleus, influence the flavor and aroma of the almonds.

2.2.5 Flavor and aroma precursors

The flavor and aroma precursors of cocoa are directly involved in the primary processing of the beans (fermentation and drying). These precursors are closely related to the microbiota present during fermentation, as well as the action of enzymes on proteins, carbohydrates, and polyphenols in the cocoa bean.

Thus, there is no development of flavor in the beans without fermentation. The role of these microorganisms is to exude the pulp of the bean and produce metabolites (Bastos, 2016).

Undoubtedly, the key structure for obtaining flavor and aroma is the cotyledon of the bean. It is through chemical reactions occurring in this structure that favor development, embryo death, and breakdown of extracellular barriers. The cotyledon is primarily composed of 53% fat (cocoa butter) and consists of two types of cells: pigment cells that store polyphenols and methylxanthines, and parenchyma cells responsible for storing proteins, starch, and lipids. Theobromine and caffeine together account for 1.5% of the dry weight of the bean and contribute to bitterness. Polyphenols account for 11 to 20% of the dry weight of the almond and are responsible for the astringency of the bean. They are divided into three types: procyanidins, catechins, and anthocyanins. However, there is a significant reduction in bitterness and astringency as a result of the diffusion of alkaloids (30% decrease) and polyphenols (20% decrease) during fermentation (Camu et al., 2008).

Polyphenols are stored in pigment cells within cotyledons, which can vary in color from white to purple, depending on the amount of anthocyanins transformed into anthocyanidins. The more anthocyanins, the more intense the purple color. They degrade and condense into flavonoid tannins. The catechins condense into catechic tannins, which have a brown color. When combined with amino acids and proteins, they form important flavor precursors, which will be further developed during the roasting stage through the Maillard reaction. Anaerobic conditions are necessary for the transformation of anthocyanins, which can only develop correctly during the initial phase of fermentation. On the other hand, catechins are favored under aerobic conditions, which is why the mass stirring step is essential (Bastos, 2016).

Anthocyanidins are crucial for the quality of cocoa. The evaluation of anthocyanins through coloration, commonly known as the cut test, is an important parameter used to assess flavor development and the degree of fermentation of cocoa beans. It is based on the color change of the cotyledon from white or purple to a brownish hue.

2.3 Cut test during fermentation

The cut test is the primary methodology for evaluating the fermentation of cocoa beans, both in the fermentation box and in the dried beans. This test not only assesses the quality but also serves as an indicator of the degree of fermentation. Typically, each producer establishes an indicator based on the coloration of the center of the bean. To evaluate the beans during the fermentation process, a small sample of beans is taken from different locations in the fermentation box, and a longitudinal cut is made using a knife (Figure 1f). Over the course of several days, the seed begins to ferment and loses its violet color. Once the embryo dies, it is called a bean, and its color gradually turns brown, indicating that the fermentation process is taking place. Since this analysis is visual, it requires an expert or the implementation of a standardized color scale. The analysis is carried out daily throughout the fermentation process to measure the degree of fermentation. Fermentation should be stopped when the furrows of the bean are well opened and visible, and the bean has a uniform brown color (Copetti et al., 2014).

2.4 Drying

In general, there are two types of drying: artificial drying and natural sun drying, with the latter being more commonly used due to its lower cost, reliance on sunlight, simplicity, and effectiveness. In natural sun drying, the beans are transferred to cement or wooden platforms with a movable roof called "barcaça" and arranged in layers of up to 5 cm. The beans are exposed to the sun for 7 to 14 days, depending on the intensity of sunlight (Figure 2a). Constant stirring with a wooden paddle is necessary to evenly distribute the heat and prevent clumping (Nielsen et al., 2013).

Natural drying can also be carried out in drying rooms (Figure 2b). Both methods, in addition to being more versatile, contribute to the quality of the beans in terms of flavor and acidity, as the slow process enhances the volatilization of acetic acid and contributes to oxidation reactions and the darkening of the cotyledons, resulting in their characteristic color and odor. However, the main goal of the drying process is to reduce moisture content and water activity, as low levels of both factors ensure better preservation of the beans and inhibit fungal growth. The slower the drying process, the lower the water activity, as rapid drying only dries the outer layer of the bean, leading to reduced permeability of the inner part of the bean and hindering the drying process. The final moisture content should be reduced from 40% to 60% to around 6% to 7%, ensuring greater safety and quality of the cocoa. If the beans are dried excessively, they become brittle (Nielsen et al., 2013). Artificial drying is performed using hot air blowers, which contribute to faster drying. However, this method does not produce as many flavor precursors and makes it more difficult to remove moisture and reduce water activity, as its rapid drying speed reduces the beans' permeability.

Figure 2. (a) Drying platform (Barcaça); (b) Drying rooms (source: own author).

2.4.1 Cut test after drying

The second cut test is performed to verify the quality of the beans after primary processing. In this analysis, a sample of 100 beans is placed on a cutting board and cut longitudinally with a knife. Each bean is visually inspected one by one, using a predefined color standard. In addition to quality, the test now aims to measure the number of beans that deviate from the standard, whether they are infected with fungi, germinated, flattened, or contaminated by pests. The official classification standard to determine the quality and soundness of the beans is established in Normative Instruction No. 38, dated June 23, 2008 (Brasil, 2008). This classification takes into account tolerance percentages for defects such as mold, slate beans, flattened beans, smoky odor, and infestation by pests. For bean-to-bar processing, the evaluation becomes even more rigorous. All defects should not exceed 10%. In the next step, a manual selection will be carried out to remove all flattened or germinated beans, further reducing the defect percentage of the batch. These parameters are defined by the producers themselves, with 10% being a joint decision agreed upon.

3 Terroir

The word "terroir" is of French origin and carries multiple meanings, representing the interaction of the environment in which a product is produced. It is associated with the set of characteristics of the environment, such as soil, climate, vegetation, and terrain of the location. It also includes the tradition of producing a specific product, using specific skills and empirical knowledge employed in its creation and production (Cros & Jeanjean, 1989).

These factors define a specific product that acquires a Geographical Indication (GI), which denotes the place of origin of the product. It attributes its own identity and, in turn, the "terroir," distinguishing it from products in the market. Examples of GIs with local identity are Roquefort cheese, Champagne, Vinho Verde, among others. These products can only be produced in a specific region because the climatic factors and production methods must be preserved as they directly impact the flavor and sensory characteristics of the food (Locatelli, 2007).

In the case of fine cocoa, each producer in a specific region employs a specific harvesting, fermentation, and drying process. The microorganisms present in each environment vary between locations, directly affecting the fermentation process. There are also physical factors such as the type of genetic variety, species, exposure to sunlight, local vegetation, or whether the origin is a plantation or if the cocoa is collected in an extractive manner from the forest. This combination of factors results in unique characteristics in cocoa beans, which will be distinct among each producing region (Afoakwa et al., 2008).

4 Bean-to-bar

The bean-to-bar movement emerged in the 2000s in San Francisco, USA, a city known for its new trends, zero waste practices, organic consumption, and artisanal food. The chocolatiers in the city decided to develop artisanal production processes due to a lack of knowledge about the origins of the beans or the chocolates they used, as well as the process and ingredients involved. In the bean-to-bar process, it is important to know the quality of the raw materials, use clean labels, work with small batches, high cocoa content, and have direct contact with the producer to understand the primary processing (Associação Bean to Bar Brasil, 2023).

These companies are concerned with the entire chocolate supply chain, from the farms to the final packaging. They follow the principles of the "slow food" movement, which advocates for good, clean, and fair food and, above all, values small producers. They typically work with equipment suitable for small-scale production, to produce fine chocolates of high quality and value-added.

The cocoa beans of controlled origin and with superior quality are destined for the production of the chocolate bean-to-bar that are fermented to develop characteristic flavor and aroma compounds. In cocoa, pyrazines are a significant group of volatiles known for their distinctive earthy, nutty, roasted, and chocolate flavor notes. The Strecker degradation in the Maillard reaction generates the majority of pyrazines that used precursors (free amino acids and reducing sugars) for their formation (Santander Muñoz et al., 2020). The characteristic aromas at each stage of chocolate processing change in quantity and quality depending on the cocoa variety, the chemical composition of the beans, the specific protein storage content, and the polysaccharides and polyphenols determining the type and quantity of the precursors formed during the fermentation and drying process, leading to the formation of specific chocolate aromas in the subsequent roasting and conching processes. The abundance of peptides present in cocoa beans is important for the formation of aroma and taste and is dependent on geographical origin (Quelal et al., 2023). Caligiani et al (2016) reported that high levels of oligopeptides and amino acids are found in cocoa bean samples from different regions. The difference in peptide concentration in relation to the origin of the cocoa is due to the nitrogen fertilization of the plants and mainly the pH found in the soil.

4.1 Characteristics

To achieve unique characteristics in their products, these companies seek out small-scale producers with more sustainable production systems. In Brazil, one such system is Cabruca, an agroforestry cultivation method for cocoa production, mainly concentrated in Bahia. It involves planting cocoa trees amidst the vegetation of the Atlantic Rainforest, preserving the local ecosystem, and avoiding the use of pesticides. The terroir of each origin ensures unique characteristics in the cultivated cocoa. This is why Brazilian fine cocoa is highly valued worldwide, as its cultural legitimacy carries significant added value. In addition, Brazil has numerous native cocoa varieties, providing unique sensory notes to the product.

Bean-to-bar production, being carried out by small-scale and low-production companies, lacks industrial equipment. As a result, an alternative method of chocolate production is employed, different from the processes used in industrial chocolates. The breaking of the beans is done in grain mills, followed by the use of a blower to remove the husks. From there, all the processing steps to obtain the chocolate mass are carried out in a piece of equipment called a melanger.

4.2 Equipment

The melanger is the equipment used as an alternative source for chocolate production (Figure 3a). It is a tabletop stone mill with rotation and heating (60 °C). The refining is carried out by two natural gray granite stones that rotate in opposite directions, grinding against the bottom of the equipment (made of the same material). This ensures that the particles are reduced to less than 20 micrometers, resulting in a creamy product (Hinneh et al, 2019a).

The equipment is responsible for three stages of the chocolate manufacturing process, which would be performed in separate machines in an industrial scale setup. The first application is the refining of the nibs (peeled and crushed beans), which are directly added to the equipment without any other ingredients until a paste is formed. The second application is the blending of dry ingredients, such as sugar and milk, which are added directly to the cocoa paste, continuing the refining process. Lastly, it is used in the conching stage, which involves stirring the chocolate under heating (60 °C) to impart shine, creaminess, and eliminate undesirable acids originating from fermentation (Hinneh et al, 2019b).

Figure 3. (a) Melanger; (b) Manual selection; (c) Melanger performing refining and conching. Source: own author.

5 Secondary processing - chocolate

The maker carries out the secondary processing of cocoa beans into fine chocolate, and it involves the following steps:

5.1 Manual selection of cocoa beans

The entire batch of cocoa is visually inspected, and defective beans such as flat, slatey, broken, or germinated beans are manually selected and removed from the batch (Figure 3b). This stage also involves removing any foreign bodies or impurities, increasing the selectivity and quality of the final product, while reducing contamination.

5.2 Roasting of cocoa beans

The roasting stage is extremely important as it is where the flavors develop. Cocoa beans contain volatile aromatic compounds, and the time and temperature of roasting influence the flavor and notes of the chocolate.

Therefore, roasting parameters should be applied based on the desired end product. Typically, the beans are roasted at 150 °C for 45 minutes. However, when aiming to obtain fine chocolate and preserve its aromas, temperatures around 120 °C are used. Roasting can be done using ovens or roasters. In addition to being a crucial step for flavor development and characteristic coloration, it is also essential for product safety, as it reduces deteriorating microorganisms due to the high temperatures involved (Silva, 2013).

Compounds that exude chocolate aromas are scarce in dry fermented beans as they arise from Maillard reactions during roasting. The roasting parameters, like temperature, roasting time, and the roasting method, influence the appearance of new compounds and the preservation of those already found in the dry beans. Temperatures above 160 °C for 35 min favor the appearance of pyrazines but reduce the compound concentration responsible for fruity and floral aromas, such as esters and ketones (Quelal et al., 2023).

Roasting can be carried out in three forms: directly on whole beans, on nibs, or on cocoa liquor. For bean-to-bar chocolate production, roasting is performed on whole beans to preserve the aromas and fruity and floral notes, as well as to facilitate the removal of the kernel in the next step. Roasting also aims to reduce undesirable volatile acids, such as acetic, valeric, butyric, and propionic acids. This stage deactivates lipolytic enzymes that can degrade cocoa butter, causing rancidity. Another important factor is the reduction of bean moisture from 8% to 2%, which prevents the proliferation of microorganisms, especially fungi (Hinneh et al., 2019b).

The development of flavor is not solely dependent on this stage; without proper fermentation, the flavor precursors that will manifest during roasting cannot be developed. Roasting reduces the acidity of the chocolate through the partial volatilization of acetic acid. The high temperature triggers the Maillard reaction in the beans, resulting in more pronounced flavor notes. Additionally, it leads to the caramelization of sugars, degradation of proteins, and synthesis of sulfur compounds. Thus, roasting can enhance flavors, create new compounds, and minimize undesirable ones (Copetti et al., 2014).

The main parameters of the cocoa roasting process are time and temperature. These parameters are established according to the desired flavor characteristics, origin and type of almonds, moisture, equipment used, and treatments before roasting (moisture, pH, size of almonds or nibs, and concentration of precursors of the desired aromatic compounds) (Efraim, 2009).

After roasting, the cocoa beans are crushed in roller or cereal mills to break the kernels and release the cotyledon. After the cracking process, the husks (kernel) and germ (embryo) are separated using blowers or specific machines. The kernel is discarded, and the result is the so-called nibs (crushed, husk-free cocoa beans) used for chocolate production (Associação Bean to Bar Brasil, 2018).

5.3 Grinding, mixing, refining and conching

Once the cocoa beans are cracked, the nibs need to be ground to obtain chocolate. The grinding process aims to reduce the particle size to 25 micrometers or less, to achieve creaminess and eliminate the grainy texture of the mass. It also helps to extract as much fat as possible, improving the viscosity of the chocolate. In industrial settings, this step is carried out using roller mills or ball mills, but in bean-to-bar chocolate production, a stone mill called a Melanger is used (Associação Bean to Bar Brasil, 2018).

The abundance of compounds in chocolate is directly related to the conching process, whereby the remaining fraction of moisture and undesirable aroma is eliminated, and the desirable aromas are concentrated (Quelal et al., 2023). The formulation ingredients, such as sugar and milk powder, are added to the cocoa mass for homogenization and to achieve a suitable plastic consistency for refining. This step is performed in the same grinding equipment, the Melanger (Figure 3c), as well as the refining process for approximately 24 hours or until the particles are smaller than 25 micrometers (Clark et al., 2020).

The conching stage aims to round off the flavor of the chocolate, eliminate undesirable acids (acetic acid), induce Maillard reactions (color and flavor), and reduce moisture content. In this stage, the chocolate is agitated for a long time at temperatures ranging from 50 °C to 100 °C. The longer the conching time, the creamier the chocolate becomes, due to the dispersion of solids in the fluid fat (cocoa butter). In industrial chocolate production, conching is divided into three phases: dry phase, plastic phase, and liquid phase. However, in bean-to-bar chocolate, there is only one conching process since there is no addition of excess cocoa butter or additives like lecithin and PGPR. This stage is still carried out in the Melanger, but the stone mill is loosened to reduce stone friction since the particles have already been fully refined (Silva, 2013).

5.4 Aging

There is still limited research on chocolate aging. Typically, aging occurs on the farms, where the beans are stored in sacks to enhance their flavors. However, bean-to-bar chocolate makers have noticed a significant improvement by aging the chocolate before tempering. Floral and fruity notes become more pronounced, and the aroma becomes more intense. It is known that intermolecular interaction in the mixture continues to occur, but there is limited research on the subject. Therefore, aging is a technique practiced only by artisanal producers who store their chocolates for a month or even a year (Burgon et al., 2023).

5.5 Tempering, molding, and cooling

Due to the polymorphic nature of cocoa butter, it is necessary to perform the tempering or pre-crystallization process of chocolate. The first step of tempering is to raise the temperature of the chocolate to 45 °C for complete melting, followed by cooling at a rate of 2 °C per minute, which can be done using a tempering machine or on a marble table with manual stirring. The chocolate must be cooled to the appropriate temperature for the growth of beta crystals, which varies from chocolate to chocolate, but generally falls within the range of 26 °C to 30 °C. During cooling, unstable crystals also form, so it is necessary to reheat the chocolate to 32 °C to achieve the proper rheological properties. Approximately 2% to 4% of the present fat is crystallized during tempering, and the crystallization process continues during the cooling and storage stages (Luccas, 2001).

The characterization of products corresponds to Resolution - RDC No. 723, of ANVISA from July 1st, 2022 (Brasil, 2022) which provides the necessary requirements for chocolate production. The products are defined as follows:

- Chocolate: a product obtained from the mixture of cocoa derivatives (*T. cacao*), cocoa mass, cocoa liquor, cocoa powder, or cocoa butter with other ingredients, and may present various fillings, coatings, shapes, and consistencies;
- White Chocolate: a product obtained from the mixture of cocoa butter with other ingredients such as milk, and sugar, present various fillings, coatings, shapes, and consistencies;
- The basic composition requirements for the products are: chocolate must consist of at least 25% (twenty-five percent) total cocoa solids, while white chocolate must consist of at least 20% (twenty percent) total cocoa butter solids.

Chocolate can also be subdivided according to its composition, based on the Codex Alimentarius (2016). Its composition may also vary according to consumers' dietary preferences. Therefore, the percentage of each ingredient may vary. Among the most consumed chocolates in Brazil are milk chocolate, dark chocolate, and white chocolate, defined by the Codex Alimentarius (2016) as follows:

- Semi-bitter chocolate shall contain, based on a dry matter basis, no less than 30% total cocoa solids (including a minimum of 15% cocoa butter and a minimum of 14% fat-free cocoa solids);
- Bitter chocolate shall contain, based on a dry matter basis, no less than 40% total cocoa solid (including a minimum of 22% cocoa butter and a minimum of 18% fat-free cocoa solids);

- Milk Chocolate: shall contain, based on the dry matter, no less than 25% cocoa solids (including a minimum of 2.5% fat-free cocoa solids) and a minimum content of milk solids between 12% and 14% (including a minimum milk fat content between 2.5% and 3.5%);
- White Chocolate: shall contain, based on the dry matter, no less than 20% cocoa butter and no less than 14% milk-derived solids (including a minimum milk fat content between 2.5% and 3.5%).

6 Conclusions

Bean-to-bar processing involves meticulous control from raw material selection, fermentation, and processing, ensuring higher sensory quality in chocolate. The terroir of each chocolate origin imparts unique characteristics to the cultivated cocoa, as each region encompasses a distinct range of microorganisms and genetic varieties

The microbiology of cacao significantly influences the bean-to-bar chocolate production process. Understanding the dynamics of microbial communities, their interactions, and potential safety concerns is crucial for ensuring high-quality and safe chocolate. Further research and advancements in microbial control strategies will continue to improve the overall quality and safety of bean-to-bar chocolate.

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