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FUNDAMENTALS AND FRONTIERS

2nd Edition

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Food Microbiology: Fundamentals and Frontiers, 2nd Ed.
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35 Cocoa and Coffee

Cocoa and coffee are two of the many foods that rely on a microbial curing process or fermentation for flavor development. The popularity and worldwide appeal of these products are due primarily to their unique flavor and aroma. Although a primary curing process is conducted in the preparation of each product before marketing, fermentation of cocoa is absolutely essential for flavor development, whereas with coffee, the curing process is less crucial to flavor and more important for the removal of pulp. Consequently, this chapter will focus mainly on the more comprehensive role of fermentation in cocoa curing and to a lesser extent in the production of coffee.

COCOA PROCESSING

Commercial cocoa is derived from the seeds (beans) of the ripe fruit (pods) of the plant *Theobroma cacao*, which is native to the Amazon region of South America. It has been used by the Amerindians to produce a beverage since time immemorial and was introduced to Europe in the 15th century by Cortés during the period of discovery and colonization of the Americas. Its popularity and demand led to the establishment and spread of rootstock to virtually all of the European colonies located between 15 degrees north and south of the equator with climates that could support cocoa production.

Of the *Theobroma* species, only *T. cacao* produces beans suitable for chocolate manufacturing. Immediately following the harvest of ripe fruit, or following a brief storage period, the seeds are removed and subjected sequentially to a fermentation and drying process often referred to as "curing," which is carried out on farms, estates, or cooperatives in the producing countries. The origin of this process has been lost in antiquity, but it was believed at one time that fermentation was conducted simply to aid in removing the mucilaginous pulp surrounding the seed so as to facilitate drying and storage, as in the case of coffee. This in fact is true, but the main reason for fermentation of cocoa is to induce biochemical transformations within the beans that lead to formation of the color, aroma, and flavor precursors of chocolate. Without this treatment, cocoa beans are excessively bitter and astringent and, when processed, do not develop the flavor that is characteristic of chocolate. The character and strength of chocolate flavor are

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governed primarily by the genetic constitution of the cocoa variety, while the fermentation process releases and develops this flavor potential (30). The inherited characteristics of the bean therefore set a limit to what can be achieved by fermentation. It is impossible to improve genetically inferior material by superior processing techniques, yet, on the other hand, it is quite easy to ruin good-quality cocoa by careless or inadequate curing.

The cocoa fruit varies among varieties in size, shape, external color, and appearance. These characters have often been used in classifying cocoa, but as far as the flavor quality is concerned, the only really important morphological differences are those that distinguish between the white-seeded Criollo variety of South and Central America and the purple-seeded Forastero variety of the Amazon. The former type is the source of the original "fine" cocoa which has almost disappeared from the market because of its susceptibility to disease, its lower productivity, and its replacement by the hardier, more prolific Forastero varieties and their varietal crosses, which now account for over 95% of the world production. Hence, the following discussion refers primarily to the processing of Forastero cocoa.

Flowers are produced seasonally from cushions that emerge on the bark of the trunk and stems. Fertilized flowers bear fruit 170 days from pollination, a period during which the fruit grows to maturity and changes color from green or dark red-purple to yellow, orange, or red, depending on the variety. The mature fruits are thick walled and contain 30 to 40 beans, each enveloped in a sweet, white, mucilaginous pulp and loosely attached to an axial placenta. Only the beans are used in chocolate manufacturing. For the purpose of describing the curing process, the bean may be envisaged as comprising two main parts, namely, the testa (seed coat) together with the attached sugary, mucilaginous pulp that surrounds it, and the embryo or the cotyledons contained within. The mucilage, containing sugars and citric acid (58), serves as a substrate for microorganisms that are involved in the natural fermentation process; the cotyledons, referred to as the "nib" in the cured bean, are used in chocolate manufacturing.

Processing begins with the harvesting of healthy ripe fruits, an operation carried out over a period of 3 to 4 days at a frequency which varies according to the size of the farm and yield. Fermentation is a batch-type process, and harvesting is conducted to allow for the accumulation of sufficient material for each batch while taking precautions that, in the process, pods do not overripen and the seeds within do not germinate. The pods are usually collected in piles in the field and broken open on site or at the processing plant (fermentary) at the end of the harvesting operation. The beans are removed manually or mechanically on some large estates in West African countries, Mexico, and Brazil, where pod breaking and bean extraction are mechanized (35). Once removed from the pods, the seeds are aggregated in heaps or in receptacles of various types and left to ferment for a period of 2 to 8 days. During this interval, microorganisms which are transferred to the seeds from laborers' hands, fruit surfaces, and containers used in transport and fermentation degrade the cells of the mucilage that surround the bean (39). The collective microbial activity resulting from the accidental inoculation by a multitude of microorganisms is referred to as "fermentation" or "sweating." This process results in the liberation of pulp juices from which alcohols and acids are produced with the evolution of heat. During fermentation, concentrations of ethanol and lactic and acetic acids sequentially increase and decrease. This can result in an excess of acetic acid that remains at the end of fermentation (59). Together, these factors bring about changes and affect the curing of the bean. The two principal objectives of fermentation are to remove mucilage, thus allowing aeration during fermentation of the beans and facilitating drying later on, and to provide the heat and acetic acid necessary to inhibit germination, which assures proper curing of the beans (31).

Methods of Fermentation

The manner of fermenting cocoa varies considerably from country to country, and in many instances, even adjacent farms may adopt different curing methods. Much effort has been made to standardize fermentation practices. Today, many of the primitive methods such as fermentation in banana leaf-lined holes in the ground, in derelict canoes, and in makeshift banana and bamboo frames are the exception rather than the rule. In general, large farms with adequate cocoa fruit production will have permanent facilities specifically constructed for this purpose. In such instances, fermentation is carried out in batteries of wooden or fiberglass boxes. However, most of the world's cocoa is produced on small holdings under very rural conditions; here, relatively small volumes are produced and do not always merit permanent processing facilities. In this case, cocoa is fermented in any convenient receptacle such as fruit boxes, baskets, plastic buckets, or fertilizer bags, or when these are not readily available, the beans are simply piled on a sheet and covered with any handy material. On the whole, however, the majority of the world's cocoa is fermented on drying platforms, in heaps covered with banana leaves, in baskets, or in an assortment of wooden boxes (13, 26, 50).

Approximately one-half of the world's crop is fermented in some type of box, and the remaining half is fermented in heaps or by using other primitive methods.

Fermentation on Drying Platforms

Fermentation on drying platforms is practiced in parts of Central America where Criollo cocoa was once grown. Wet cocoa beans are spread directly on drying platforms where they ferment and dry during the day and are heaped into piles each night to conserve heat and retard the growth of surface molds. Criollo cocoa requires only a short period of fermentation (about 2 to 3 days) for flavor development. Forastero varieties require a fermentation time of 5 to 8 days for the development of flavor (14). Although Criollo cocoa has been largely replaced by Forastero hybrids in Central American countries, in many instances, the old method of fermentation still persists (46). This practice preserves the fine flavor characteristics of Criollo beans; however, it is inappropriate for Forastero varieties, which require longer fermentation times for optimal flavor development. Fermenting cocoa on the drying floor is convenient, but unless properly managed, the process tends to produce underfermented cocoa with the added danger of undesirable mold growth and its consequences of off flavor development.

Fermentation in Heaps

Fermentation in heaps is a popular method among smallholding farmers in Ghana and many other African cocoa-producing countries. It also has been observed sporadically in the Amazon region of Brazil. This method does not require a permanent structure and is well suited to family holdings with a small production. Judging from Ghanaian cocoa, fermentation in heaps can produce good-quality products. Varying quantities of cocoa beans from 25 to 1,000 kg are heaped in the field on plantain leaves and covered with the same material. The beans are mixed (turned) periodically to ensure even fermentation and to decrease the potential for mold growth. This is often done daily or every other day by forming another heap. Mixing is laborious and small heaps may not be turned at all. The duration of fermentation is from 4 to 7 days.

Fermentation in Baskets

Fermentation in baskets is practiced principally by small-scale producers in Nigeria, the Amazon region, the Philippines, and some parts of Ghana. Small lots of cocoa are placed in woven baskets lined with plantain leaves. The surface is covered with plantain leaves and weighted down. The turning procedure and fermentation process are similar to that used for small heaps. Basket fermentation is often used when cocoa fermented in heaps is vulnerable to predial larceny.

Fermentation in Boxes

Fermentation in boxes is considered to be an improvement over other methods. This batch process requires a fixed volume of cocoa and is the method of choice on large estates. The size of the containers varies from region to region, but the design and function are standard. The container or "sweat box" may be a single unit or one of a number of compartments within a large box created by subdividing the space into units measuring approximately 1 by 1 by 1 m, with either fixed or movable internal partitions. These boxes hold between 600 and 700 kg of freshly harvested (wet) cocoa beans. The box is always raised above ground level, over a drain which carries away the pulp juices (sweatings) liberated by the degradation of the mucilage during fermentation. The wooden floor of the box generally has holes or spaces between the boards or slats to facilitate drainage and aeration. Sweat boxes vary considerably in size from that of a small fruit box (0.4 by 0.4 by 0.5 m) to some measuring 7 by 5 by 1 m, used on some Malaysian estates (24). Large estates and cooperatives often have batteries of 20 to 30 sweat boxes arranged in tiers in three to seven rows, one below the other to facilitate mixing or turning. Mixing is achieved by simply removing a dividing wall and shoveling the beans into the next box or, in the case of the tier design, into the box below. On some Malaysian estates, boxes are built on pallets, and a forklift is used to transfer the contents into an empty box. Variations occur not only in the size of sweat boxes but also in the type of wood used in their construction and in drainage and aeration methods and the duration of fermentation. The recommendation is to ferment a 1-m^3 volume for 6 to 7 days with two to three mixings during this period. In the majority of cases, boxes are filled to within 10 cm of the top, and the surface is covered with a padding of banana leaves or jute sacking to help retain the heat and prevent the surface beans from drying.

In some countries, fermentation norms have been modified in an attempt to overcome problems such as acidity by varying the prefermentation treatment and the depth of beans in the sweat boxes (30). In Malaysia, for instance, harvested cocoa pods are stored up to 15 days before breaking to remove the beans, or the beans may be pressed or predried to reduce the pulp volume before fermentation (4, 10).

Fermentation progess is assessed by the odor and the external and internal color changes in the beans. When the process is judged complete, the beans are dried in the sun or in mechanical dryers.

Microbiology of Cocoa Fermentation

Fermentation begins immediately after the beans are removed from the pods, as they become inoculated with a variety of microorganisms from the pod surface, knives, laborers' hands, containers used to transport the beans to the fermentary, dried mucilage on surfaces of the fermentation box (tray, platform, or basket) from the previous fermentation, insects, and banana or plantain leaves (18, 19, 26, 36, 39, 48, 55). It is the pulp surrounding the beans, not the cocoa bean, that undergoes microbial fermentation. Chemical changes take place within the bean as a result of the fermentation of the pulp. The testa of the bean acts as a natural barrier between microbial fermentation activities outside the bean and chemical reactions within the bean. However, there is a migration of ethanol, acetic acid, and water of microbial origin from the outside to the inside of the bean. After the bean dies, soluble bean components are leached through the skin and lost in the drainings. The pulp consists of about 85% water, 2.7% pentosans, 0.7% sucrose, 10% glucose and fructose, 0.6% protein, 0.7% acids, and 0.8% inorganic salts (21), making it a rich substrate for microbial growth. The concentration of sucrose, glucose, and fructose is influenced by the age of the pod (56).

The initial microbial population is variable in number and type; however, the key groups active during fermentation of beans are yeasts, lactic acid bacteria, and acetic acid bacteria (28, 39, 48, 61). Climatic conditions may influence the sequence of microorganisms involved in the fermentation (61). It is theorized that *Bacillus* species play an important role during the latter stages of the fermentation and become the dominant group during drying (61). More research is needed to confirm this theory. Over 100 aerobic spore-forming bacteria were isolated from cocoa bean fermentations in Bahia. Bacteria were identified as *Bacillus subtilis*, *B. licheniformis*, *B. firmus*, *B. coagulans*, *B. pumilus*, *B. macerans*, *B. polymyxa*, *B. laterosporus*, *B. stearothermophilus*, *B. circulans*, *B. pasteurii*, *B. megaterium*, *B. brevis*, and *B. cereus*. *B. subtilis*, *B. circulans*, and *B. licheniformis* were encountered more frequently than the other *Bacillus* species during fermentation (61).

During fermentation, yeasts, lactic acid bacteria, and acetic acid bacteria develop in succession. Species of microorganisms that have been detected in cocoa during fermentation in Ghana, Malaysia, and Belize are listed in Table 35.1. At the onset of fermentation, a pH of 3.4 to 4.0, a sugar content of 10 to 12%, and a low oxygen tension favor the growth of yeasts (56, 60). Yeasts utilize the carbohydrates in the pulp under aerobic and anaerobic conditions and may form 40 to 65% of the microflora when the fermentation begins (9, 40). The yeast phase lasts 24 to 48 h, during which populations may increase to 90% of the total microflora. Yeast populations have been determined in several investigations (28).

Some yeasts produce various pectinolytic enzymes that degrade the cocoa pulp, thereby aiding in the drainage of juices (16, 48). In addition to metabolizing sugar to produce ethanol, yeasts utilize citric acid, causing the pH to increase (48). All yeast species that contribute to fermentation are not present simultaneously, but follow a succession which is influenced by the turning step (aeration) and the fact that fermenting bean masses are not homogeneous (28). Several genera of yeasts are involved in fermentation (Table 35.1). In one study (9), of the 142 yeast genera detected in fermenting bean masses, 105 were asporogenous and 37 were ascosporogenous. Between 48 and 72 h of fermentation, the yeast population begins to decrease so that by day 3, it is reduced to 10% of the total microbial population (4). Three factors are responsible for the rapid decline in the dominance of yeasts. First, yeasts rapidly metabolize sucrose, glucose, and fructose in the pulp to form carbon dioxide and ethanol, causing a reduction in energy source. Second, the production of ethanol produces a toxic environment that suppresses yeast growth. For example, Schwan et al. (61) reported that a decline in the opulation of *Kloeckera apiculata* was associated with an ethanol concentration greater than 4%. A small amount of heat is developed simultaneously with ethanol production (2). Third, acetic acid, which is produced from ethanol by the acetic acid bacteria, is also toxic to yeasts. The acetic acid concentration may reach 1 to 2% (6).

The anaerobic conditions created by yeasts make the environment suitable for lactic acid bacteria (40). Lactic acid bacteria prefer a low oxygen concentration or, if oxygen is present, a high concentration of carbon dioxide (28). Such an environment develops as the pulp collapses and the yeast population decreases. The population of lactic acid bacteria increases rapidly, but large numbers may be present for only a brief period (30, 48, 50). The lactic acid bacteria population has been observed to reach 10^6 to 10^7 CFU/g in a typical fermentation in Belize and a high of 20% of the total microflora after 1.5 days in fermentations in Trinidad (55). In Brazil, the lactic acid bacteria population is about 65% of the total microflora after 14 h of fermentation. The lactic acid bacteria population remained high up to 3 days, at which time it decreased to less than 10% of the total microflora (40).

Table 35.1 Microorganisms isolated from fermenting cocoa beans

Microorganism	Country		
	Ghana[a]	Malaysia[a]	Belize[b]
Lactic acid bacteria	*Lactobacillus plantarum* *Lactobacillus mali* *Lactobacillus collinoides* *Lactobacillus fermentum*	*Lactobacillus plantarum* *Lactobacillus collinoides*	*Lactobacillus plantarum* *Lactobacillus fermentum* *Lactobacillus brevis* *Lactobacillus buchneri* *Lactobacillus cellobiosus* *Lactobacillus casei* subsp. *pseudoplantarum* *Lactobacillus delbrueckii* *Lactobacillus fructivorans* *Lactobacillus kandleri* *Lactobacillus gasseri* *Leuconostoc mesenteroides* *Leuconostoc paramesenteroides* *Leuconostoc oenos*
Acetic acid bacteria	*Acetobacter rancens* *Acetobacter ascendens* *Acetobacter xylinum* *Gluconobacter oxydans*	*Acetobacter rancens* *Acetobacter lovaniensis* *Acetobacter xylinum* *Gluconobacter oxydans*	*Acetobacter* spp. *Gluconobacter oxydans*
Yeasts	*Candida* spp. *Hansenula* spp. *Kloeckera* spp. *Pichia* spp. *Saccharomyces* spp. *Saccharomycopsis* spp. *Schizosaccharomyces* spp. *Torulopsis* spp.	*Candida* spp. *Debaryomyces* spp. *Hanseniaspora* spp. *Hansenula* spp. *Kloeckera* spp. *Rhodotorula* spp. *Saccharomyces* spp. *Torulopsis* spp.	*Brettanomyces claussenii* *Candida* spp. *Candida boidinii* *Candida cocoai* *Candida intermedia* *Candida guilliermondii* *Candida krusei* *Candida reukaufii* *Kloeckera apis* *Kloeckera javanica* *Pichia membranaefaciens* *Saccharomyces cerevisiae* *Saccharomyces chevalieri* *Schizosaccharomyces* spp. *Schizosaccharomyces malidevorans*

[a] From Carr et al. (7).
[b] S. S. Thompson and J. Pfeifer, unpublished data.

Both homofermentative and heterofermentative lactic acid bacteria occur in cocoa fermentations; however, the majority are homofermentative (61). Lactic acid bacteria detected in traditional box fermentation of cocoa beans in Brazil were isolated and characterized as being homofermentative and heterofermentative. The homofermentative species included *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus delbrueckii*, *Lactobacillus acidophilus*, *Pediococcus cerevisiae*, *Pediococcus acidilactici*, and *Streptococcus* (*Lactococcus*) *lactis*. The heterofermentative species included *Leuconostoc mesenteroides* and *Lactobacillus brevis* (41). Citric acid is metabolized either to acetic acid, carbon dioxide, and lactic acid by heterofermentative species or to acetylmethylcarbinol and carbon dioxide by homofermentative species. Lactic acid bacteria may be more important in cocoa fermentation in Brazil where, during the first 48 h of fermentation, their population is consistently larger than the yeast population (40). This differs from most other fermentations where yeasts are the dominant microorganism during the first 48 h. If lactic acid bacteria remain as a high percentage of the total microbial population during the fermentation, high concentrations of lactic acid will be produced. Since lactic acid is not volatile, it will remain in the chocolate after manufacturing, producing an undesirable chocolate (52, 64, 72).

As the beans are turned to aerate the mass, more of the pulp is metabolized, and conditions become aerobic. The population of lactic acid bacteria decreases and the population of acetic acid bacteria increases with increased aeration. The population of acetic acid bacteria generally reached 10^5 to 10^6 CFU/g in a typical fermentation in Belize. Two genera of acetic acid bacteria, *Acetobacter* and *Gluconobacter*, have been isolated from fermenting cocoa beans. *Acetobacter* species occur more frequently than *Gluconobacter* species (6, 7, 39). The acetic acid bacteria population has been observed to make up 80 to 90% of the total microflora after 2 days in fermentations in Trinidad (55). Acetic acid bacteria oxidize ethanol to acetic acid exothermally (2), causing the temperature of the bean mass to rise to 45 to 50°C. Turning the beans periodically facilitates oxidation of the ethanol to acetic acid and conserves the high temperature of the bean mass. When all of the ethanol is oxidized to acetic acid and then carbon dioxide and water, fermentation subsides and the temperature of the bean mass decreases quickly.

During the later stages of fermentation and while drying, aerobic spore-forming *Bacillus* species develop and may become dominant (6, 39). *Bacillus* species are present during the first 72 h of the fermentation, but during this early stage, their population remains constant. They become dominant later in the fermentation, making up over 80% of the microbial population (60, 62). Development of *Bacillus* species in the bean mass is favored by increased aeration, an increased pH (3.5 to 5.0) of the pulp, and an increase in temperature to 45 to 50°C (6, 39, 62).

Bacillus species can produce several compounds that may contribute to the acidity and off flavors of fermented cocoa. The C_3, C_4, and C_5 free fatty acids that are present in the bean mass during the aerobic phase of fermentation may contribute to the development of some of the off flavors of chocolate (33, 44). The importance of *Bacillus* species in cocoa bean fermentation is not well established, but they are reputed to produce acetic and lactic acids, 2,3-butanediol, and tetramethylpyrazine, which can affect the flavor of chocolate (33, 62, 73).

A key factor that must be considered when deciding on a fermentation scheme is when to remove the beans from their fermentation environment and begin drying. Extending the fermentation can result in undesirable microbial activity, leading to putrefaction and the production of compounds such as butyric and valeric acids that contribute to off flavors (34). Forsyth and Quesnel (13) suggested that the following factors may collectively indicate when fermentation is optimum: (i) external color of the beans; (ii) time schedule; (iii) decrease in temperature; (iv) bean cut test and the internal color used as a criterion; (v) aroma of the fermenting mass; (vi) plumping or swelling of the beans.

A more desirable measurement of optimum fermentation would be a chemical method that is relatively rapid, inexpensive, and easy to perform and interpret. We have observed that the end point of fermentation can be determined by the pH of the beans, provided a normal temperature curve is established. The minimum pH that gives acceptable cocoa liquor is 5.2; however, the actual fermentation pH may be slightly lower. Other methods to measure the qualitative and quantitative changes that occur during cocoa fermentation have been reviewed by Shamsuddin and Dimick (63).

Fermentation Using Pure Culture Seeding

Fermentation of cocoa beans continues to be a "natural process"; however, studies have been conducted to determine the potential application of a defined microbial inoculum to improve the natural fermentation technique (11, 57, 59). A seeding fermentation study was conducted using *Saccharomyces chevalieri*, *Candida zeylanoides*, and *Kluyveromyces fragilis*, isolates obtained from previous fermentations. Of the three yeasts used, the fermentation using *S. chevalieri* produced the better quality chocolate (57). *Torulopsis candida* (ATCC 20031), *Candida norvegensis* (ATCC 22971), *K. fragilis* (ATCC 8601), and *S. chevalieri* were evaluated for their ability to increase the yield of the cocoa sweatings for use in the development of products such as soft drinks, jams, and marmalades. These controlled fermentations did not have a negative effect on the physicochemical properties of the cocoa sweatings or the quality of the chocolate (11). An inoculum cocktail consisting of *Sacchoromyces cerevisiae* var. *chevalieri*, *Lactobacillus lactis*, *Lactobacillus plantarum*, *Acetobacter aceti*, and *Gluconobacter oxydans* subsp. *suboxydans* was successfully used to produce an acceptable quality chocolate (59). We conducted bench-top seeding studies using an inoculum mixture isolated from natural fermentation studies in Belize. The mixture contained 11 microorganisms consisting of yeasts and lactic and acetic acid bacteria. The final chocolate was judged to be acceptable by a trained taste panel (S. S Johnson and J. Pfeifer, unpublished data). Several issues and questions must be resolved to determine whether a starter culture approach can supplement or replace the traditional fermentation.

Biochemistry of Cocoa Fermentation

The actual production of chocolate flavor precursors occurs within the cocoa bean and is primarily the result of biochemical changes that take place during fermentation and drying. The mode of fermentation and the microbial environment during these stages of cocoa production provide the necessary conditions for complex biochemical reactions to occur. Although flavor compounds such as lactic and acetic acids are produced external to the bean by microbial activity, chocolate flavor development is largely dependent on the enzymatic formation of flavor precursors within the cotyledon that are unique to cocoa. Such classes of compounds include free amino acids, peptides, reducing sugars, and polyphenols. When fermented dried cocoa beans containing these flavor precursors are subjected to roasting during chocolate manufacture, a necessary step in flavor development, a series of complex nonenzymatic browning reactions occurs to produce flavor and colored compounds characteristic of chocolate (23, 29, 51, 53, 54). However, if unfermented cocoa beans lacking these precursor compounds are roasted, very little chocolate flavor is produced. It is, therefore, important that these flavor precursor compounds be formed inside the cocoa bean during fermentation.

The initiation of fermentation also corresponds to an incipient germination phase which is necessary for mobilization of the enzymes and hydration of bean components in preparation for growth. However, the germination phase is undesirable in cocoa beans used to make chocolate.

Bean Death

Bean death is a critical event during cocoa fermentation which allows the biochemical reactions responsible for flavor development to occur within the cocoa bean. Although rising temperatures and increasing acetic acid concentrations during fermentation have been implicated in causing seed death (42), more recent data (27) indicate that the production of ethanol during the anaerobic yeast growth phase correlates very closely with death of the seed. Total inability of the seed to germinate occurs about 24 h after maximum concentrations of ethanol are attained within the cotyledon (Fig. 35.1). As a result, events associated with germination and certain quality defects, e.g., the utilization of valuable seed components such as cocoa butter and the opening of the testa by hypocotyl extension, will not occur. This produces a more stable, desirable end product.

From a flavor perspective, events associated with the death of the seed also cause cellular membranes to leak and permit enzymes and substrates to react to form flavor precursor compounds important to chocolate flavor development. Activity of these enzymes in the cotyledon results in significant increases in free amino acid and reducing sugar contents (glucose and fructose) (Fig. 35.1). While the temperature of the beans increases, the concentration of organic acids increases, causing a decrease in pH. All of these factors influence the biochemistry within the bean and have an impact on cocoa flavor and quality.

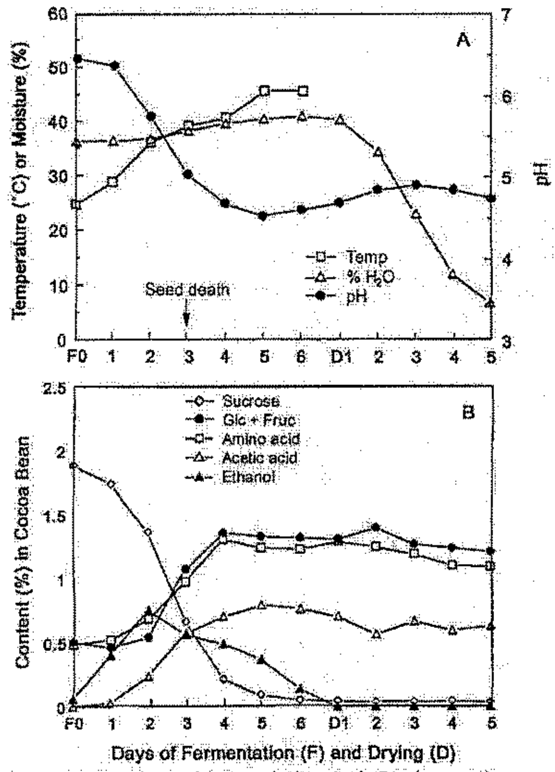

Figure 35.1 Physical (A) and chemical (B) changes in cocoa beans during fermentation and drying in Belize. Fermentation was conducted with 2,000 lb of wet cocoa beans from ripe pods in wooden boxes that were turned daily. Drying was conducted in flat-bed dryers indirectly heated with hot air. Data represent an average of 11 fermentation trials using composite samples collected daily. (A) Temperature was measured in the whole bean mass. Moisture (%) and pH analyses are based on shell-free cotyledons. (B) Sucrose, glucose (Glc), fructose (Fruc), total amino acid, acetic acid, and ethanol contents (%) were determined by analysis of water extracts of shell-free cotyledon samples. Data from Lehrian (27).

Environmental Factors

There are several environmental factors, viz., pH, temperature, and moisture, in the fermenting mass that influence cocoa bean enzyme reactions. Each enzyme has an optimum pH at which it is most active, and within a defined range, an enzyme reaction accelerates as temperature increases. In addition, a certain amount of moisture is necessary to allow enzymes and their substrates to react to form products. Significant changes in pH, temperature, and moisture occur during cocoa fermentation and drying processes (Fig. 35.1) that influence the type and quantity of flavor precursor compounds produced by enzymatic action.

Moisture content within the cotyledon during fermentation is usually more than 35% and will permit adequate migration of enzymes and substrates for enzymatic activity. However, once the drying process begins, moisture content gradually decreases, making it increasingly difficult for enzymes and substrates to react. When a moisture content of 6 to 8% is achieved, virtually all enzyme activity ceases.

The pH of the unfermented cotyledon is about 6.5 and may decrease to as low as 4.5 by the end of the fermentation. This lowering of pH occurs after seed death and is primarily due to the diffusion into the bean of organic acids produced by lactic and acetic acid bacteria. It is the growth of these and other microorganisms that also contributes to the increasing temperature of the mass of fermenting beans. Typically, bean mass temperature will rise from 25°C to about 50°C, followed by a slight decrease as bacterial growth subsides. An increase in temperature of more than 20°C during fermentation can have a profound impact on enzyme activity. If very little change in temperature occurs, enzyme activity is reduced, resulting in fewer flavor precursors and poor chocolate flavor. Likewise, if appropriate amounts of organic acids are not produced during fermentation, the pH of the cotyledon will not be suitable for optimal enzyme activity, and the flavor profile of the resulting cocoa will be affected. However, too much acid will produce excessive sourness that can mask the chocolate flavor.

Consequently, there is a delicate balance among the length of fermentation, environmental factors, and microbial activity that influences enzyme activity within the cotyledon. Hydrolytic and oxidative enzymes play a major role in reactions that produce flavor precursors. A summary of cocoa bean enzymes, their substrates, and pH optima is given in Table 35.2 (30). More recently, Hansen et al. have studied cocoa bean enzymes and their activities during the fermentation and drying process in an effort to understand their impact on cocoa flavor and quality (20).

Hydrolytic Enzyme Reactions

Hydrolytic enzymes such as invertase, glycosidases, and proteases have highest activity during the anaerobic phase of cocoa fermentation. The products of these enzyme activities during cocoa fermentation fall into three basic categories: sugars, amino acids/peptides, and cyanidins. Sugars and amino acids/peptides participate in nonenzymatic browning reactions during roasting to form important chocolate flavor precursors, whereas the cyanidins have more of an impact on color development and some minor flavor components.

Sucrose is the major sugar in unfermented cocoa beans. It is not a reducing sugar and therefore does not participate in nonenzymatic browning reactions that occur during roasting to contribute to chocolate flavor. However, sucrose is converted to glucose and fructose by invertase during the fermentation process. These reducing sugars represent more than 95% of the total reducing monosaccharides in cocoa beans, and their concentrations increase almost three fold during fermentation, while sucrose is depleted (Fig. 35.1).

Another class of hydrolytic enzymes within the cocoa bean that contributes to both flavor and color during fermentation is the glycosidases. The substrates for these enzymes are the purple-colored anthocyanins located in specialized vacuoles within the cotyledon and are responsible for the characteristic deep purple color of the unfermented bean. The actions of specific glycosidase enzymes begin at seed death and are responsible for cleaving the sugar moieties, galactose, and arabinose attached to the anthocyanins. This results in a bleaching of the purple color of the beans as well as the release of reducing sugars that can participate in flavor precursor reactions during roasting (12). Pigments themselves do not carry any flavor potential (12, 33, 49). Cocoa beans that still contain significant purple color are considered to have been poorly fermented and are less desirable.

Although there are small amounts of free amino acids present in unfermented cocoa beans, the total free amino

Table 35.2 Characteristics of the principal enzymes active during the curing of the cocoa bean[a]

Enzyme	Location	Substrate	Product	pH	Temp (°C)	Reference(s)
Invertase	Testa	Sucrose	Glucose and fructose	4.0 5.25	52 37	32
Glycosidases (β-galactosidase)	Bean	Glycosides (3-β-D-galactosidyl cyanidin and 3-α-L-arabinosidyl cyanidin)	Cyanidin and sugars	3.8–4.5	45	12
Proteases	Bean	Proteins	Peptides and amino acids	4.7	55	5, 43
Polyphenol oxidases	Bean	Polyphenols (epicatechin)	o-Quinones and o-diquinones	6.0	31.5, 34.5	45

[a] Information taken from Lopez (30).

acid pool increases significantly during fermentation due to the action of both endo- and exoproteases on cocoa bean proteins. After seed death occurs, these proteolytic enzymes are free to act on protein substrates within the bean, and their activity becomes dependeant on pH and temperature. A vicilin-like globular storage protein within the cotyledon is the primary target of these proteolytic enzymes, and ratios of free amino acids and peptides that are unique to cocoa are produced (70). These flavor precursor compounds contribute to the development of cocoa flavor when roasted in the presence of reducing sugars.

Oxidative Enzyme Reactions

Significant oxidative enzyme activity also occurs, being most prevalent late in the aerobic phase of fermentation but continuing well into the drying of cocoa. Polyphenol oxidase is the major oxidase in cocoa and is responsible for much of the brown color that occurs during fermentation as well as some flavor modifications. This enzyme becomes active during the aerobic phase of the fermentation as a result of oxygen permeating the cotyledon. Events that contribute to activity include seed death, subsequent breakdown of cellular membranes, reduction in the amount of seed pulp, and aeration of the bean mass by agitation. Oxygen continues to penetrate the beans during the drying process, enabling polyphenol oxidase activity to continue until rising temperatures and insufficient moisture become inhibiting factors.

Catechins and leucocyanidins are the major classes of polyphenols that are subject to oxidation in cocoa beans. Epicatechin makes up more than 90% of the total catechin fraction and is the major substrate of polyphenol oxidase (17). Oxidation of epicatechin during the aerobic phase of fermentation and drying is largely responsible for the characteristic brown color of fermented cocoa beans. Polyphenols in the dihydroxy configuration are oxidized to form quinones which in turn can polymerize with other polyphenols or complex with amino acids and proteins to yield characteristic colored compounds and high-molecular-weight insoluble material. This complexation also has an impact on flavor. The formation of these less soluble polyphenolic complexes reduces astringency and bitterness associated with native polyphenols present in unfermented cocoa (14, 47). In addition, the ability of polyphenols to complex with proteins results in the reduction of off flavors associated with the roasting of peptide and protein material (22, 71).

Flavor and Quality Implications

The ultimate goal of biochemical changes during fermentation is to produce cocoa beans with desirable flavor and color characteristics. Good chocolate flavor potential is achieved by the production of specific amino acids, peptides, and sugars through the action of proteases and invertases on cocoa bean substrates. Proper control of fermentation conditions and microflora assures that concentrations of organic acids are maintained at reasonable levels to minimize sour and putrid off flavors while still developing the pH and temperature environment for enzyme-substrate reactions that produce chocolate flavor precursors. Enzyme-mediated conversion of polyphenol materials during fermentation and drying processes reduces astringency and bitterness and produces the desirable brown color typical of properly fermented cocoa beans. The drying process will then preserve the flavor and color characteristics of the beans until they are made into chocolate.

Although cocoa has been successfully produced for centuries, flavor characteristics of specific varieties of cocoa are becoming diluted due to the prevalence of genetic hybrids. While these hybrids are being selected for high crop yield and disease resistance, certain flavor attributes are being lost. The actual identity of the flavor precursors responsible for chocolate flavor has not yet been confirmed. However, recent work has focused on characterizing cocoa bean enzymes and proteins that yield breakdown products unique to cocoa during the fermentation process (69). This work needs to continue in order to understand flavor development and maintain the high quality of chocolate flavor the consumer expects.

Drying

After the beans are fermented, they have a moisture content of about 40 to 50%, which must be reduced to 6 to 8% for safe storage. A higher final moisture content will result in mold growth during storage. The drying process relies on air movement to remove water. This environment favors aerobic microorganisms which proliferate at rates that decrease with moisture loss. Sun drying is the preferred method, but in regions where harvesting coincides with frequent rainfall, some form of artificial drying is necessary and desirable. In general, sun drying is employed on small farms, whereas large estates may resort to both natural and artificial drying. Sun drying allows a slow migration of moisture throughout the bean, which transports flavor precursors that had been formed during fermentation.

During sun drying, beans are placed on wooden platforms, mats, polypropylene sheets, or concrete floors in layers ranging from 5 to 7 cm thick. The beans are constantly mixed to promote uniform drying, to break agglomerates that may form, and to discourage mold growth. Under sunny conditions, the beans dry in about a week, but under cloudy or rainy conditions, drying times may be prolonged to 3 or 4 weeks, increasing the risk of mold development and spoilage.

Various types of artificial dryers employed to overcome the dependence on weather conditions have been described by McDonald et al. (37). Hot air dryers of one form or another, fueled by wood or oil as a source of cheap, readily available energy, are generally employed. The beans may be heated by direct contact with the flue gases; however, the preferred method relies on indirect heating via heat exchangers. Improperly used or poorly maintained heating systems present the danger of contamination with smoke which results in smoky or hammy off-flavors characteristic of beans from some countries. Platforms, trays, and rotary dryers of various designs, coupled to furnaces, are used, but in every instance, the initial drying must be slow and with frequent mixing to obtain uniform removal of water. This results in volatilization of acids and sufficient time for oxidative, biochemical reactions to occur. For this reason, temperatures should not exceed 60°C and drying times should take at least 48 h. Elevated temperatures also tend to produce cocoa with brittle shells and cotyledons which crumble during handling. In short, the drying rate should be controlled so as to remove moisture at a rate that will avoid case hardening (rapid drying on the bean surface with moisture retention inside the bean) or excessive mold growth, while still allowing sufficient time for biochemical oxidative reactions and loss of acid to occur.

Storage

Due to marketing practices and manufacturing procedures, fermented, cured, dried beans are stored for periods of 3 to 12 months in warehouses on farms, at wharfs in exporting and receiving countries, and at factories before being processed into chocolate. The efficiency of the drying process will determine the shelf life of the product. Uniformly dried beans with a moisture content of 7 to 8%, when stored at a relative humidity of 65 to 70%, will generally maintain that moisture, resist mold growth and insect infestation, and not require repeated fumigation. The cocoa quality can change during storage depending on temperature, relative humidity, and ventilation conditions. Slow oxidation and acid loss continue to enhance product quality somewhat, but prolonged storage results in a noticeable staling (31).

COFFEE PROCESSING

Coffee beans may also undergo a fermentation step to prepare the fruit for commercial use (3, 8, 66). Like cocoa, the fermentation of coffee has the important goal of breaking down the pulp layer surrounding the beans in order to aid in the processing of the fruit into a desirable finished product. Unlike cocoa, however, the role of fermentation in coffee is less critical in the development of coffee flavor, although improperly fermented fruit can result in undesirable off flavors. Coffee beans are produced by the genus *Coffea*. Over 40 species are known, but only a few are used to produce coffee. *Coffea arabica*, *C. canephora*, *C. robusta*, *C. liberica*, and *C. excelsa* are the most important species. The coffee fruit is a fleshy berry approximately the size of a small cherry (8).

It takes approximately 1 year from the flowering stage for a fruit, or coffee cherry as it is commonly called, to reach maturity. As the fruit ripens, it changes color from green to cherry red. The ripe, red coffee cherry consists of two green beans surrounded by a pulp layer and enclosed in an outer skin. It is necessary to first remove the outer skin and pulp layer to obtain the green coffee beans, which are then dried, roasted, milled, and used for making coffee beverages. The removal of the pulp layer can be accomplished by either a natural drying step yielding "natural coffee" or a wet fermentation step yielding "washed coffee." The percent yield of dried coffee from ripe cherries varies among species: Arabica produces 12 to 18%, Robusta produces 17 to 22%, and Liberica produces about 10%.

Natural Coffee

The "natural" drying process relies on partial dehydration of the pulp layer while the coffee fruit is ripening on the tree. The ripe fruit is then harvested and subjected to additional drying, and the dried pulp layer and outer skin are removed by hulling. Some mucilage will penetrate the coffee bean during drying, and for this reason, the natural process produces a light-brown-colored bean instead of the blue-green color of wet-processed coffee. Drying must be carefully controlled to avoid excessive fermentation during the initial stages which can result in reduced product quality. Molds that may develop on slowly drying coffee can produce off flavors typical of a butyric fermentation. Coffee is dried to a moisture content below 13%.

Washed Coffee

The wet process is accomplished by harvesting ripe coffee fruit and immediately removing the majority of pulp by mechanical squeezing or depulping. This is followed by a fermentation step which is used to convert the remaining mucilage to water-soluble products that can be removed by washing. In general, wooden or concrete bins are used for this fermentation step. The white, sticky, partially depulped beans are held under water for 12 to

60 h, depending on environmental conditions, ripeness of the cherries, and the variety being processed. Microbial by-products are periodically washed away during the fermentation step to avoid the development of excessive off flavors. Once the sticky pulp layer is converted to water-soluble products, the beans are washed with water and dried to a moisture content of about 12%.

Microbiology and Biochemistry of Coffee Fermentation

As stated above, the purpose of fermentation is removal of the mucilage from around the seed. A natural fermentation, which involves several different microorganisms, is the primary method used to remove the mucilage. Three nonmicrobial methods, namely, the addition of enzymes, chemical treatment (sodium hydroxide), and mechanical force, may also be used to remove mucilage (25). However, only the natural fermentation used in the wet process to produce washed coffee will be discussed.

Coffee is generally fermented in wooden or concrete tanks. Mucilage is easily metabolized by most of the microorganisms that have been identified in coffee fermentation studies. Mucilage is composed of pectin, pectic acids, reducing sugars, sucrose, caffeine, chlorogenic acid, amino acids, and hydrolytic and oxidative enzymes (25).

Many coffee fermentation studies have been conducted over the years, but researchers do not agree on which microorganism(s) is responsible for digestion of the mucilage. However, there is agreement that several microorganisms are present during the fermentation. Molds, yeasts, several species of lactic acid bacteria, coliforms, and other gram-negative bacteria have been isolated. These microorganisms originate from the surface of the fruit and the soil (1, 15, 67).

Since the mucilage composition consists largely of pectic substances (25), the microorganisms responsible for colonization and utilization of this material must be capable of producing pectinases. Coffee fermentation studies have demonstrated that the highest microbial activity occurs during the first 12 to 24 h after the beans are harvested (68). Most of the microorganisms detected during this early stage belong to the genera *Aerobacter* (*Enterobacter*) and *Escherichia*. Populations of these bacteria increase from 10^2 to 10^9 CFU/g during that first 24-h period. Pectinolytic species of *Bacillus*, *Fusarium*, *Penicillium*, and *Aspergillus* have also been detected. Only a few yeast species have been identified. Fermentation studies conducted on Kona coffee beans demonstrated that *Erwinia dissolvens* was the main cause of mucilage decomposition (15).

The pH of unfermented coffee beans can range from 5.4 to 6.4. During fermentation, the pH may decrease to 3.7 (1, 38). Low levels of ethanol have been detected under both aerobic and anaerobic conditions (38). However, ethanol may reach slightly higher levels under anaerobic fermentation conditions (25).

Duration of a natural fermentation will depend on climate conditions, regional factors, coffee variety, degree of anaerobiosis of the ferment, and the microorganisms present (25). An optimal fermentation time is considered to be 16 to 24 h (66). Extending the fermentation will result in development of flavor defects and bean discoloration (38, 46). Once the fermentation is complete, any remaining mucilage is removed by washing. Washing the beans is required to avoid excessive fermentation and enhance the drying process.

CONCLUSION

Although serving somewhat different purposes, microbial fermentation plays a critical role in the production of both cocoa and coffee. A common goal of fermentation of both of these products is the breakdown and removal of fruit pulp. This process aids in the proper drying of coffee and, in the case of cocoa, provides a suitable environment for flavor and color development. As in most natural curing processes, there is a delicate balance between environmental factors and conditions enabling microbial activities, all of which influence the biochemical changes that take place in processed foods. Coffee and cocoa are no exceptions, and it is the proper control of the fermentation process that largely determines the color and flavor quality of final products. Consequently, understanding the microbiology and biochemistry of cocoa and coffee production, as well as the factors that influence them, is critical to quality control.

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