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MICROORGANISMS IN COCOA BEANS DURING POST-HARVEST PROCESS AND DAMAGES CAUSED BY MICROORGANISMS: A REVIEW

***Elazmanawati Lembong, Edy Subroto, Mohamad Djali, Gemilang Lara Utama, Alda Marsha Maheswari**

Department of Food Industrial Technology, Faculty of Agro-Industrial Technology, Universitas Padjadjaran, Jl. Raya Bandung-Sumedang Km. 21, Jatinangor, Sumedang 40600, Indonesia.

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***Corresponding Author**
Elazmanawati Lembong
Department of Food Industrial Technology, Faculty of Agro-Industrial Technology, Universitas Padjadjaran, Jl. Raya Bandung-Sumedang Km. 21, Jatinangor, Sumedang 40600, Indonesia.

ABSTRACT

Cocoa (*Theobroma cacao*, L.) is a plantation crop that is the basis for making chocolate. To produce good quality cocoa beans, microorganisms must play the role of creating flavor precursors to improve the post-harvest cocoa process. In practice, some cocoa producers still do not correctly apply the pre-harvest and post-harvest processing stages, so the cocoa beans produced are dominated by unfermented cocoa beans, with high levels of bacterial, fungal, and mycotoxin contamination. This review aimed to know what microorganisms play a role in the post-harvest production stage of cocoa and the damage that can occur due to microorganisms at the pre-harvest and post-harvest processing stages. Microorganisms, whether bacteria, fungi, or yeast, are always involved in the processing of cocoa beans. Its role can help improve the quality of cocoa beans so that the final cocoa product has a distinctive taste such as yeast, lactic acid bacteria, and acetic acid bacteria. However, microorganisms also have a detrimental role at several processing stages, such as those caused by mycotoxins and bacterial pathogens.

KEYWORDS: Microbiology, Post-harvest, Cocoa.

INTRODUCTION

Cocoa (Theobroma cacao, L) is a plantation crop from Central America and South America (Afoakwa, 2014). The cacao plant is 4 to 8 meters high, the stem is straight, and the wood is light and has a thin bark. Cocoa pods have a size of 30 x 10 cm with a weight of 0.2-1 kg, fruit that is yellow, red, orange, green, or brown, and when ripe, there are seeds covered in lender pulp that reach 40 seeds (de Jesús Umbarila et al., 2020). Cocoa beans are essential for making chocolate products (Lima et al., 2011).

Indonesia is the sixth-largest cocoa-producing country after Ivory Coast and Ghana, with total cocoa production reaching 767.28 thousand tons in 2018. Central Sulawesi province contributed the highest cocoa production figure of 125.47 thousand tons, or 17.19 percent of the total cocoa production in Indonesia in 2018 (BPS, 2019). Although it has a high annual cocoa production, in fact, it is not in line with the quality of cocoa beans.

Good quality cocoa beans must follow SNI standards in which cocoa beans must be fermented and dried, as well as physical, microbiological, chemical, and organoleptic characteristics (Basri, 2010). One of the foundations for forming good quality cocoa beans is the role of microorganisms in creating flavor precursors so that there is an increase in the post-harvest cocoa process.

In Indonesia, the cocoa produced comes from smallholder plantations, large private plantations, and large state plantations. In the eyes of the international market, the quality of Indonesian cocoa beans has a negative image, especially cocoa produced from smallholder plantations. This is because the cocoa beans produced are dominated by unfermented cocoa beans, with high levels of contamination by insects, fungi, and mycotoxins.

Microorganisms in Cocoa Post-Harvest Processing

Cocoa Fruit Cutting

Before fermentation, the cocoa pods must be split first to remove the seeds. This cleavage can be done either manually or mechanically (Arya Bima Senna, 2020). Before splitting, healthy, undamaged, and intact cocoa beans can be categorized as sterile fruit or have only a small population of microorganisms. When fruit splitting is carried out, the seeds covered by the pulp can be contaminated by various types of microorganisms because cocoa beans have a high sugar content and high humidity. This can affect the subsequent processing. This microorganism originates from the skin of the cocoa pods, workers' hands, knives, tubs used to transport cocoa beans, residual mucus in the fermentation tank, and various types of plants that come into contact with cocoa pods (Nielsen et al., 2005). Research conducted by Salazar et al. (2020), stated that freshly split cocoa beans and exposed to air contained 5.61 ± 2.04 log CFU/g aerobic microorganisms and 4.20 ± 0.23 log CFU/g yeast, and 2.83 ± 2.59 log CFU/g fungi were also found.

Fermentation

One of the most crucial processes in processing cocoa beans is fermentation because fermentation can add quality value and provide a distinctive aroma and taste (Arya Bima Senna, 2020). Fermentation is also intended to prevent physical and chemical changes caused by the growth of cotyledons (Hartuti et al., 2020). Raw cocoa beans have an astringent taste that is not favored, so fermentation is carried out with the help of microorganisms to modify the components in cocoa. In addition, cocoa beans that have gone through the fermentation process will be easier to dry because the pulp layer attached to the cocoa beans has been eroded. Various microorganisms involved in the spontaneous fermentation of cocoa beans come from workers, fruit skins, equipment used during harvesting, insects, and fermentation boxes (Ozturk & Young, 2017). However, not all these microorganisms will assist in fermentation (Camu et al., 2007).

Various factors affect the type of microbes that work in the fermentation process, such as cultivar, location, type of fermentation, and length of fermentation time (Ozturk & Young, 2017). Microbial activity during cocoa fermentation involves complex biochemical reactions in the cotyledons. This includes the growth of several species of yeast, lactic acid bacteria (LAB), acetic acid bacteria (BAA), Bacillus species, and filamentous fungi to some extent. The bacteria that work in the early stages of fermentation are yeast.

Yeast is a eukaryotic microorganism slightly more significant than bacteria, which is 5×10µm (Montes de Oca et al., 2016). In fermentation, yeast has a vital role in degrading the pulp. Strains identified in the cocoa fermentation process include *Saccharomyces spp.*, *Candida spp.*, and *Pichia spp*. strains, but the most common strain found in all cocoa species is *Saccharomyces cerevisiae* (Koné et al., 2016; Magalhães da Veiga Moreira et al., 2017). Yeast can live for approximately 48 hours, and the peak activity of yeast is at 12 hours. At that time, the yeast activity had changed the pulp condition, so other microorganisms began to grow and carry out their activities (Ordoñez-Araque et al., 2020).

The next stage is followed by the emergence of lactic acid bacteria, which play an essential role in degrading pulp, accumulating taste, and inhibiting the growth of spoilage microorganisms (Sarbu & Csutak, 2019). Lactic acid bacteria appear in the early stages of fermentation, and as long as sugars and pulp begin to hydrolyze and leave the fermentation system, their numbers will continue to increase and be active. Species of lactic acid bacteria have been found in several cocoa fermentation processes, such as *Lactobacillus plantarum, Lactobacillus fermentum, Lactobacillus cellobiosus, Leuconostoc spp*, and several types of *Bacillus* species.

Acetic acid bacteria are gram-negative bacteria and belong to the *Acetobacteraceae* family. The Acetobacter genus can grow in an environment containing ethanol and oxidize some types of carbohydrates, which release various metabolites such as aldehydes, ketones, and organic acids into the medium through an oxidative fermentation process (Mamlouk & Gullo, 2013). Acetic acid produced by acetic acid bacteria comes from ethanol released by yeast which is then converted into acetaldehyde using alcohol dehydrogenase, which is then reduced by aldehyde dehydrogenase (Illeghems et al., 2013). The most common acetic acid bacteria encountered were the genera *Acetobacter* and *Acetobacter pasteurianus* (Ordoñez-Araque et al., 2020).

During fermentation, the proliferation of microorganisms varies greatly, and each group of microorganisms has a role in the development of specific characteristics in cocoa beans. These microbes are sources or producers of ethanol, lactic acid, and acetic acid, which are essential precursors to converting raw cocoa beans to the required conditions before proceeding to the following process.

Fermentation begins when the oxygen in the medium is reduced due to the large amount of pulp surrounding the cocoa beans, creating anaerobic conditions. Cocoa beans are wrapped in a layer of slimy pulp containing 82-87% water, 10-15% sugars (glucose, fructose, and sucrose), 2-3% pentose, 1-3% citric acid, 1-1.5% pectin, and about 1-2% hemicellulose as well as protein, amino acids vitamins (especially vitamin C), and other minerals in small amounts (Pettipher, 1986; Rahmadani et al., 2020). Because it is rich in nutrients, the pulp is a suitable place for microorganisms to grow.

At this stage, the yeast will metabolize simple sugars through glycolysis to produce pyruvate and glycerol. This will increase the pH of the pulp, which previously had a pH of 3.0-4.0, and limit it to further proliferation and further processing to produce ethanol and CO_2 (Luc De Vuyst & Leroy, 2020). Glycerol produced by yeast during glycolysis will give a sweet taste and increase mouthfeel (Dzialo et al., 2017). The ethanol produced will then be absorbed into the cotyledons and oxidized to acetic acid by lactic acid and acetic acid bacteria. This ethanol formation process is exothermic in which the temperature during fermentation increases to 35-40^oC from 25-30^oC for up to two days (L. De Vuyst & Weckx, 2016).

Pectin is also degraded due to the hydrolysis of pectin assisted by several enzymes such as polygalacturonase, pectin methylesterase, and pectin lyase, causing changes in seed viscosity and helping to penetrate oxygen and reduce citric acid (Afoakwa, 2014; L. De Vuyst & Weckx, 2016; Ordoñez-Araque et al., 2020; Schwan & Fleet, 2014). The pulp will release a yellowish-white to a reddish cellular fluid which causes its viscosity to decrease and allows air to enter. The citric acid in cocoa pulp causes a pH of 3.0-4.0 in the pulp (Ardhana & Fleet, 2003; Vásquez et al., 2019). Although, generally, lactic acid bacteria metabolize citric acid, there are several types of yeast that are also involved in reducing the amount of citric acid (Sarbu & Csutak, 2019). According to Leal et al. (2008), if the yeast does not contribute to the fermentation process, the cocoa beans will be heavier, more sticky, and moist, indicating that the pulp has not been completely degraded. Yeast also significantly influences the aromatic quality of cocoa compared to bacterial strains (Hamdouche et al., 2019).

Research by Ardhana & Fleet (2003) showed that during the first 24 hours of fermentation, the yeast population shot from 105-107 to 108-109 CFU/g, and the number was constant for the next 12-24 hours. The yeast population will decrease to 10-102 CFU/g at the end of fermentation. This condition will be followed by the growth of lactic acid bacteria, which has increased due to consuming the remaining cocoa pulp sugar and producing lactic acid. Luc De Vuyst & Leroy (2020) stated that the yeast species most often isolated from the beginning to the middle of the fermentation process was *Hanseniaspora opuntiae*, while *Saccharomyces cerevisiae* and *Pichia kudriavzevii* in the middle to specific points of the fermentation process.

As mentioned earlier, the loss of cell fluid during the ethanol formation process will reduce the acid content and open cavities that allow air to enter the pulp. At this stage, there is a transition from anaerobic to aerobic conditions. This condition causes an increase in pH and an increase in the ethanol content produced, and glucose levels in the pulp are also reduced due to the ethanol formation process so that the amount of yeast decreases and triggers lactic acid bacteria to grow because these conditions are ideal conditions for lactic acid bacteria.

Lactic acid bacteria play several roles in fermentation, including fermenting sugar, utilizing citric acid for conversion, and converting fructose into mannitol (L. De Vuyst & Weckx, 2016; Schwan & Fleet, 2014). The homofermentative lactic acid bacteria such as *L. plantarum*, *Pediococcus acidilactici*, and *Lactococcus lactis* ferment the remaining sugars in the pulp such as glucose and fructose by the Embden-Meyerhof (EM) pathway to produce lactic acid, while the heterofermentative groups such as *L. fermentum*, *Leuconostoc spp.*, and *Weissella spp.* will ferment sugar through the hexose monophosphate/pentose phosphate pathway to produce lactic acid, ethanol, acetic acid, and CO_2 (Schwan & Fleet, 2014). Fructose that is still available will also be converted into mannitol by making fructose as an alternative external electron acceptor by heterofermentative lactic acid bacteria (L. De Vuyst & Weckx, 2016; Luc De Vuyst & Leroy, 2020).

Lactic acid bacteria will convert the available citric acid into acetic acid and oxaloacetic acid. Oxaloacetic acid is converted to pyruvate and produces lactic acid, acetic acid, 2,3-butanedione (diacetyl), 3-hydroxy-2-butanone (acetoin), and 2,1-butanediol, of which diacetyl and acetoin are part of the taste profile. on cocoa bean products (Lefeber et al., 2011; Vinderola et al., 2019). This process raises the pulp's temperature and pH, creating ideal conditions for acetic acid bacteria.

Aerobic conditions in cocoa beans cause a decrease in the population of lactic acid bacteria and an increase in acetic acid bacteria. In fermentation, acetic acid bacteria, especially *Acetobacter* species, will oxidize the ethanol produced by the yeast to acetic acid. *Acetobacter* uses ethanol as an energy source, and lactic acid is produced by lactic acid as the primary carbon source (Pelicaen et al., 2019). Some of the acetic acid is then reduced through acetyl CoA, which then enters the citric acid cycle, oxidizing it to CO_2 and water (Luc De Vuyst & Leroy, 2020; Sarbu & Csutak, 2019).

This acetic acid fermentation is a highly exothermic process, so the temperature can exceed 50^{o}C (Luc De Vuyst & Leroy, 2020; Peláez et al., 2016). The population of acetic acid bacteria, which previously reached 107 to 108 CFU/g, decreased due to the high temperature caused by acetic acid fermentation (Ordoñez-Araque et al., 2020; Schwan & Fleet, 2014). Based on the data summarized by L. De Vuyst & Weckx (2016), *Acetobacter ghanensis* and

Acetobacter senegalensis were replaced at the beginning of the cocoa by *Acetobacter pasteurianus* in the final fermentation process.

After increasing the temperature and decreasing the pH value of cocoa beans from 6.5 to 4.8 due to the acetic acid fermentation process, the resulting acetic acid is absorbed into the beans causing the cotyledons to die. The death of the cotyledons will activate endogenous hydrolytic enzymes, which this enzyme will form a chocolate flavor precursor (Figueroa-Hernández et al., 2019; Sarbu & Csutak, 2019).

These conditions also trigger the growth of some spore-forming bacteria, such as the genus *Bacillus*. Most of *Bacillus spp*. such as *B. stearothemophillus, B. coagulans*, and *B. circulans* are resistant to hot conditions and can even be identified during the drying, roasting, and final cocoa products (Figueroa-Hernández et al., 2019; Ordoñez-Araque et al., 2020; Schwan & Fleet, 2014; Thanh Binh et al., 2017). *Bacillus* can produce several chemical components, both proteolytic and lipolytic enzymes that can catalyze reactions, tetramethylpyrazine, and other pyrazines, which are essential components in the cocoa aroma. However, some bacteria such as *B. subtilis, B. cereus*, and *B. megaterium* produce C3-C5 free fatty acids, which can cause off-flavor in chocolate (Afoakwa, 2014; Moreira et al., 2018).

Drying

The previously fermented cocoa beans are then dried. Drying is a process to reduce the water content from 60% to 7% or until the time when the water contained does not reduce the quality and grow microorganisms (Hartuti et al., 2020). Cocoa beans that are not completely dry will damage the quality, so the selling price will decrease. This loss can be caused by the activity of aerobic microorganisms, especially fungi (Afoakwa, 2014).

When drying with the help of sunlight, cocoa beans undergo several changes such as temperature and relative humidity that trigger changes in the microbial ecology in cocoa beans slowly. This change will lead to lower volatile acidity. This stage is very susceptible to the development of xerophilic fungi that, like substrates with low water activity, can produce mycotoxins. Because sun-drying occurs outdoors, cocoa can be contaminated by various factors such as insects, birds, and rodents which carry fungi that were not previously present in cocoa beans during the fermentation stage. Thus, fungal contaminants in dried cocoa beans are often found. Therefore, prolonged drying increases the likelihood of fungal growth and spoilage (Delgado-Ospina et al., 2021).

Abs. corymbifera, P. paneum, Aspergillus sp. nov., A. flavus, A. parasiticus, A. candidus, A. niger, and *Eurotium chevalieri*. is the most frequently isolated fungus during the drying process using the sun drying method for 7-14 days. The presence of *A. flavus* and *A. niger* must be considered because they are potential fungi producing aflatoxins and ochratoxins. Fungi belonging to the toxigenic fungal species most commonly found in the cocoa bean drying process are *A. niger, A. flavus, A. parasiticus*, and *A. carbonarius* (Copetti et al., 2011; Delgado-Ospina et al., 2021). The presence of mycotoxins can increase during the drying process due to improper procedures.

Hamdouche et al. (2015) stated that there are several yeast species that are still found in the drying process of fermented cocoa beans, including *Hanseniaspora opuntiae, Candida insectorum, Pichia kudriavzevii, Pichia sporocuriosa,* and *Issatchenkiahanoiensis*. The most common and dominant yeast species during cocoa beans' fermentation and drying processes are *Pichia kudriavzevii* and *Saccharomyces cerevisiae.* This is because some yeasts are protected by polyphenol compounds contained in the seeds so that the yeast can withstand high-temperature processes such as drying.

Packaging and Storage

The dried cocoa beans will be packaged and stored. Cocoa beans with good quality can last up to several years. Microorganisms such as fungi can grow during storage if the moisture content of cocoa beans exceeds 8% (Fowler & Coutel, 2017). High moisture will increase humidity and provide suitable environmental conditions for mold to grow. This high humidity is caused by the incomplete drying process, moisture absorption in the humid atmosphere, and leakage of the cocoa beans. These things can trigger the metabolism of some fungi to produce mycotoxins in cocoa beans. Airborne contamination is the primary source by which fungal spores can be transmitted from different media to the final product during storage. The fungus that was isolated in the research of Akinfala et al. (2020) are the genera *Aspergillus, Paecilomyces, Talaromyces, Penicillium, Pseudopithomyces,* and *Simplicillium*. There are also several xerophilic species, such as the *Eurotium* (Copetti et al., 2011).

Damage by Microorganisms During Pre-Harvest Cocoa

In general, as much as 30% of cocoa plants experience crop failure due to damage caused by pests and diseases. Several diseases that often occur in cocoa plants include black pod rot (BP), witches' broom disease (WB), and frosty pod rot (FPR) (Afoakwa, 2014; Fowler & Coutel, 2017).

Black Pod Disease

Black pod disease (BP) is a disease that causes a large number of harvest failures because it can reduce the quality of cocoa to be harvested. The characteristics of this disease are the appearance of brown spots or parts on certain parts of the plant, dryness, and spots on the bark to produce a reddish fluid. According to Purwantara et al. (2015), BP is very common in Indonesia, up to 70-80%. The cause of this disease is the contamination of the microorganism *Phytophthora palmivora*, an oomycete that can attack various parts of the cocoa plant (Komalasari et al., 2018; Perrine-Walker, 2020). Masanto et al. (2019) found that there were 52 isolates of *Phytophthora palmivora* from 55 regions in Indonesia. During 2013-2017, most of the cocoa seeds/seedlings originating from Java and Sulawesi were transported to other cocoa-producing areas. This allows for the spread of *Phytophthora palmivora* in several areas in Indonesia.

Figure 1: ***Black pod disease on unharvested cocoa pods*** **(Afoakwa, 2014).**

Figure 2: ***Black pod disease on harvested cocoa pods*** **(Afoakwa, 2014).**

Witches' Broom Disease

Cocoa infected with Witches' Broom Disease (WB) will show green spots that give the impression of uneven maturity of the cocoa pods and the growth of the "extra" part of the leaf bud (Fowler & Coutel, 2017). If the infected cacao pods have not yet reached the stage of complete maturity, the pods will fail to be harvested because the infection will destroy the pods. WB disease is caused by the Basidiomycota fungi *Monilophthora perniciosa* and *Marasmus perniciosus,* which are indigenous microorganisms in South America (Afoakwa, 2014; Barsottini et al., 2020).

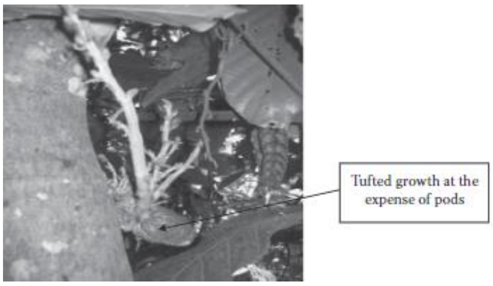

Figure 3: ***Witches' broom disease on harvested cocoa pods*** **(Afoakwa, 2014).**

Frosty Pod Rod

Frosty Pod Rod (FPR) is a disease of cocoa plants caused by the fungus *Moniliophthora roreri*. The fungus *Moniliophthora roreri* can only attack fruit species of the genera *Theobroma* and *Herreania* and cause damage both internally and externally so that it can result in total damage to cocoa pods. FPR is twice as lethal as BP and more challenging to control than WB (Jaimes et al., 2019). This fungal contamination often occurs in intertropical areas of America, such as Colombia, and Central America, such as Mexico (Fowler & Coutel, 2017).

Damage by Microorganisms in Post-Harvest Cocoa

One of the parameters determining the final quality of cocoa beans is their handling during post-harvest processing. The quality of cocoa beans can decrease due to several factors, including the lack of a post-harvest system and contamination of microorganisms in cocoa beans. Fermentation is one of the crucial post-harvest processing stages, but its application is rarely carried out in Indonesia. According to Syahri & Hastuti (2017), almost 90% of the cocoa beans produced by Indonesian farmers do not go through the fermentation process but directly dry the beans after splitting. Cocoa beans that do not undergo the fermentation process will have low acidity, germinated beans, less dominant cocoa flavor due to the absence of flavor precursors, and microorganism contamination (Atmaja et al., 2016). Some organic acids such as acetic acid, lactic acid, and citric acid produced from the fermentation process can act as antimicrobials, especially fungi, in the drying and storage process of cocoa beans (Sarbu & Csutak, 2019; Schwan & Fleet, 2014; Setyabudi et al., 2017).

Ochratoxin A (OTA) is a secondary metabolite toxin produced by several species of *Aspergillus* fungi such as *Aspergillus carbonarius, A. niger*, and *A. ochraceus* and *Penicillium* species such as *Penicillium verrucosum and P. nordicum* (Afoakwa, 2014). OTA is nephrotoxic, immunotoxic, teratogenic, and carcinogenic, so it is dangerous when consumed (Setyabudi et al., 2017). OTA production depends on several factors such as temperature, water activity, and medium composition. During the post-harvest processing phase of cocoa beans, the amount of OTA will increase if storage conditions and other processes are not adequately controlled.

Based on research on OTA isolation conducted on fermented and unfermented cocoa beans by Setyabudi et al. (2017), unfermented cocoa has a higher OTA concentration than fermented cocoa. This contamination is influenced by environmental conditions, where cocoa bean storage is carried out at a 91% RH environment to stimulate mycoflora growth and mycotoxin production. It is known that the fungi that contaminate unfermented cocoa beans are species *A. carbonarius* and *A. niger*, which are mycotoxin-producing fungi and are thought to grow on unfermented cocoa beans due to the availability of sugars such as glucose and sucrose in the pulp where sugar is the substrate for growth. Fermented cocoa has lower OTA content because acetic acid inhibits OTA growth.

In addition to contamination with mycotoxin-producing fungi, *Salmonella* were also detected in the post-harvest processing phase due to poor hygiene conditions. *Salmonella* is a bacterial pathogen responsible for a foodborne illness that causes many cases of death worldwide. Based on research (Nascimento et al., 2013), the number of *Salmonella* increased between the end of fermentation and the beginning of the drying process. This is influenced by pH during fermentation and aw during drying. To minimize the risk of Salmonella contamination in cocoa beans during the post-harvest phase, is to implement good agricultural practices starting from maintaining contamination such as the cleanliness of tools and workers.

CONCLUSION

Microorganisms, whether bacteria, fungi, or yeast, are always involved in the processing of cocoa beans. Its role can help improve the quality of cocoa beans so that the final cocoa product has a distinctive taste such as yeast, lactic acid bacteria, and acetic acid bacteria.

However, microorganisms also have a detrimental role in several processing stages, causing damage to both the pre-harvest and post-harvest stages. The damage caused can result in crop failure or a decrease in the quality of cocoa beans. The majority of damage caused by microorganisms comes from fungi that produce mycotoxins as well as pathogenic bacteria.

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