

# How Can We Get The Healthy Chocolate Products?

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Fermentation starter using combination of yeast, acetic acid bacteria, and mangosteen rind extract, and combination of yeast, lactic acid bacteria, and acetic acid bacteria can increase physical quality and inhibit the mycotoxigenic fungi and their mycotoxins in cocoa beans as basic material for chocolate products.

### Chocolate Products And Cocoa Beans

Chocolate is a food product made from roasted and ground cocoa beans. There is three types of chocolate, i.e. dark chocolate, white chocolate, and milk chocolate. Dark chocolate contains cocoa liquor, cocoa butter, lecithin, sugar and vanilla, meanwhile white chocolate contains everything dark chocolate does (except chocolate liquor), milk fats and solids. Milk chocolate has all of the dark and white chocolates content [1]. Cocoa liquor is the paste of cocoa beans that contains non-fat cocoa solids and cocoa butter, meanwhile cocoa powder is made by removing some of the cocoa butter from liquor [2]. Lecithin is a group of fatty substances that can be found in animal and plant tissue that has a range of functions within the human body.

Chocolate products can not be already processed, if we have not many good cocoa beans. Cocoa beans are obtained from cocoa trees (*Theobroma cacao*). Cocoa trees are native to the Amazon and Orinoco river basins in South America [1]. Now, Indonesia is the third cocoa beans exporter countries after Ivory Coast and Ghana [3]. Cocoa beans production in exporter countries were Ivory Coast (> 2 000 000 tonnes), Ghana (883 652 tonnes), Indonesia (659 776 tonnes), Nigeria (328 263 tonnes), and Cameroon (295 028 tonnes) [4]. The highest value of Indonesian cocoa beans production in 2021 originated from Central Sulawesi (130 650 tonnes), Southeast Sulawesi (114 828 tonnes), and South Sulawesi (107 075 tonnes) [5].

There are three types of cocoa beans can be found in Indonesia, such as *criollo*, *forastero* and *trinitario*, but the most widely grown cocoa in Indonesia is *forastero*. Characteristic of *criollo* or fine cocoa are 1) oval and red cocoa pods, 2) big and round kernels, 3) weight of dried kernel > 1.2 g, and 4) white cotyledon, 5) fat content is lower that 56%, 6) size and weight of kernels are similar relatively, and 7) aroma and taste are better than *forastero* and *trinitario*. The morphology of *forastero* or *lindak* are 1) round until oval and green cocoa pods, 2) flat and small kernels, 3) the average of weight of dried kernels is 1 g, 4) purple cotyledon, 5) fat content is more than 56%, 6) size and weight of kernels are heterogeny, and 7) aroma and taste are not better than *criollo*. *Trinitario* is hybrid cocoa from *criollo* and *forastero* [6]. Hybrid is the offspring of two plants or animals of different varieties or species.

### Nutritional Facts And Benefits Of Cocoa Beans And Chocolate Products

Cocoa beans contains more phenolic antioxidants are called flavonoids, including catechin, epicatechin, and procyanidins [2]. Phenolic antioxidants or flavonoids are a wide group of metabolites that originate from the

secondary metabolism of plants as providing antioxidants roles. Nutritional compositions of cocoa powder are 81.6 % non-fat cocoa solids (NFCS), 15.0 % fat, 52.4 % total polyphenols, 1.9 % mg/g epicatechin, and 0.6 % mg/g catechin. Dark chocolate contains 23.4 % NFCS, 34.7 % fat, 13.0 % total polyphenols, 0.3 % epicatechin, and 0.2 % catechin; meanwhile milk chocolate contains 6.2 % NFCS, 32.6 % fat, 4.4 % total polyphenols, 0.1 % epicatechin, and 0.04 % catechin [7]. White chocolate has 12.6 % total polyphenols, while the epicatechin and catechin were below than 0.03 and 0.01 mg/ml [8]. Common nutritional composition in white chocolate are 472 cal, 2 g protein, 29.8 g fat, 62.7 g carbohydrate, 63 mg calcium, 287 mg phosphor, 2.8 g iron, 30 mg vitamin A, 0.03 vitamin B1, and 1.4 g water; meanwhile dark chocolate contains 504 cal, 5.5 g protein, 52.9 g fat, 29.2 g carbohydrate, 98 mg calcium, 446 mg phosphor, 4.4 mg iron, 60 mg vitamin A, 0.05 mg vitamin B1, 2.30 g water. Milk chocolate has 381 cal, 9 g protein, 35 g fat, 53.6 g carbohydrate, 200 mg calcium, 2 mg iron, 30 mg vitamin A, 0.08 mg vitamin B1, and 1 g water [6].

Nutritional composition in cocoa beans has many benefits for human health. Chocolate consumption can reduce risk of diabetes, because the antioxidant effect of cocoa beans may directly influence insulin resistance. Insulin is a hormone produced by pancreas that controls the amount of glucose in the bloodstream. Not only that, chocolate also may stimulate changes in redox sensitive signaling pathway involved in gene expression and the immune response, can protect nerves from injury and inflammation, protect the skin from UV radiation, cognitive function and mood [7].

### Problems In Cocoa Beans And Chocolate Products

Chocolate products come from cocoa beans are processed through several stages of processing, i.e. preharvesting, harvesting, postharvesting, manufacturing, and distribution. In fact, many farmers has been guided by instructors from Ministry of Agriculture and some private companies to conduct good agricultural practices, but some farmers and collectors are still ignoring good handling practices after the cocoa beans are harvested.

This condition may cause decreasing of cocoa beans postharvest quality, especially during harvesting, fermentation, drying, storing, and packaging process that facilitates fungal contamination.

The international standard for cocoa beans based on the Food and Drug Administration (FDA) requires the cocoa beans such as: 1) should be fermented and had moisture content of 7%, 2) have no insects,

and 3) had uniform quality without foreign materials such as pods or pulp [4]. Physical quality of cocoa beans can be detected from grade of cocoa beans based on total kernels, slaty kernels, and moldy kernels. Grade of cocoa beans based on total kernels in 100 g of cocoa beans are : 1) AA (maximum 85 kernels), 2) A (maximum 86 – 100 kernels), 3) B (maximum 101 – 110 kernels), 4) C (maximum 111 – 120 kernels), and 5) S (maximum > 120 kernels) [5]. Grade of *lindak* cocoa beans based on slaty kernels are : 1) I – B (maximum 3%), 2) II – B (maximum 8%), and 3) III – B (maximum 20%) [9]. Grade of *lindak* cocoa beans based on moldy kernels are : 1) I – B (maximum 2%), 2) II – B (maximum 4%), and 3) III – B (maximum 4%), while the moisture content of cocoa beans should be lower than 7.5% [9]. Dried cocoa beans during storage can be infested by insect and contaminated by fungi. Some postharvest fungi can produce mycotoxins, such as *Aspergillus flavus*, *A. niger*, *A. ochraceus*, *A. parasiticus* and *Penicillium citrinum*. Fungal infection may cause decreasing of germinability, discoloration, decreasing of physical quality and nutritional content, heating of cocoa beans, and mycotoxin production.

Some fungi were isolated from unfermented cocoa beans in Central Sulawesi, i.e. *A. flavus*, *A. niger*, *A. fumigatus*, *Fusarium* sp., *Geotrichum* sp., *Mucor* sp., *Penicillium* sp., *Rhizopus* sp., *Trichoderma* sp., *T. viride*, dan *Verticillium* sp.. *Aspergillus flavus* population was isolated in cocoa beans at farmer, collector, and exporter levels were 7.2 x 10<sup>8</sup>, 4.5 x 10<sup>5</sup>, and 4.1 x 10<sup>3</sup> cfu/ml. Aflatoxin B1 (AFB1) content in cocoa beans were collected from farmers (104.80 ppb), collectors (61.31 ppb) and exporters (47.74 ppb) [10]. Aflatoxigenic fungi also were found in 226 cocoa beans were collected from Brazillian farms, i.e. *A. flavus*, *A. parasiticus*, and *A. nomius* [11]. Mycotoxigenic fungi were also found in cocoa beans were collected from Flores, such as *A. flavus*, *A. fumigatus*, and *A. niger*. The range of AFB1 content in fermented cocoa beans were collected from Flores was 2.21 – 3.65 ppb, while the range of ochratoxin A (OTA) was not detected – 0.38 ppb [12].

There are three kinds of mycotoxins are usually found in foodstuff, i.e.



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### Relationship Between Fungal Infection, Mycotoxins Contamination, and Human Health



Figure 1. Mechanisms of mycotoxins contamination in foodstuff influence to human and animal health  
(Source : Nijma Nurfadila)

aflatoxins, fumonisin, and ochratoxin A [13], but aflatoxins and ochratoxin A are commonly found in cocoa beans. Mycotoxins are toxins produced by some fungal species or fungal strains, meanwhile the mycotoxigenic fungi is a kind of fungal species or fungal strains that can produce mycotoxins. Aflatoxin is a kind of mycotoxins produced by *A. flavus* and *A. parasiticus* that causes liver cancer in human and animal. There are four types of aflatoxins, i.e. B1, B2, G1, and G2, but the most dangerous for human health is aflatoxin B1 (AFB1) [13]. The maximum tolerable limit (MTL) for aflatoxin in cocoa beans, cocoa butter, and cocoa powder in Bulgaria are 5 ppb, while the MTL in Uruguay and Malaysia are 10 ppb [14]. Ochratoxin A (OTA) is a potent nephrotoxic mycotoxin that has been linked to kidney problems in both livestock and human [13]. The MTL of ochratoxin A in cocoa beans in Brazil is 10 ppb [15]. Mechanisms of mycotoxins contamination in cocoa beans influence to human health is initiated by fungal infection in foodstuff, for example cocoa beans. Some mycotoxigenic fungi contaminated in foodstuff (example : cocoa beans), then the fungi produce the mycotoxins in available condition. Then, the contaminated foodstuff (example : cocoa beans or chocolate products) are consumed by human or animal that will give negative effect to human or animal health, such as cancer, indigestion, and etc (Fig. 1).

Fungi and mycotoxins contamination in cocoa beans can be prevented using conventional biotechnology is called fermentation. Fermentation is also useful for omitting the pulp and forming of the delicious taste as well as reducing bitter taste. The physicochemical properties of dried cocoa beans also can be improved using fermentation.

### Appropriate Fermentation Starters and Their Advantages for Cocoa Beans

There are two processing of cocoa beans in Indonesia, i.e cocoa beans within fermentation and without fermentation. Fermentation is divided into three stages, i.e.: 1) anaerobic stage, 2) lactic acid bacteria stage, and 3) acetic acid bacteria stage. In aerobic stage, yeast will convert glucose into alcohol in low oxygen condition and pH value <4 at 24 – 36 first hours. In lactic acid bacteria (LAB) stage, the LAB will convert glucose and organic acid into lactic acid at 48 and 96 hours. In acetic acid bacteria (AAB) stage, AAB will convert alcohol into acetic acid as the effect of wide exothermic during temperature increase.

As much as 90% unfermented cocoa beans were exported with lower price compared to the fermented cocoa beans. The quality of unfermented dried cocoa beans can be improved by artificial fermentation. Artificial fermentation is carried out using useful microorganisms to increase the quality of cocoa beans. It means that fermentation is one of important processes for cocoa beans. Cocoa beans fermentation process involved some microorganisms, i.e. yeast, AAB, LAB, Bacillus and several other bacteria, as well as filamentous fungi [16]. Combination of microorganisms are used for fermentation process is called fermentation starter.

The addition of *Saccharomyces cerevisiae*, *Acetobacter aceti* and *Lactobacillus lactis* [17] and the combination of *S. cerevisiae*, *Lactobacillus plantarum*, *A. aceti* [18] had been processed as fermentation starter, but the quality of cocoa beans were still not increased. *Saccharomyces cerevisiae* is a kind of yeasts, meanwhile *A. aceti* is a



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kind of acetic acid bacteria (AAB) and *L. lactis* is a kind of lactic acid bacteria (LAB). Therefore, formulating and concocting combination treatment to increase quality of cocoa beans, specially physical quality and to inhibit the mycotoxigenic fungi in cocoa beans is necessary.

As much as 75% samples were included into grade B based on total kernels, except controls and *Issatchenkia orientalis* BIO 211291 + *A. aceti* (grade B into grade C). Mean of total kernels in 1st and 3rd day after fermentation was 107 kernels, while the mean of those in 6th and 11th day after fermentation was 110 kernels per 100 g. Based on the percentage of slaty kernels, as many as 58% samples were included into grade II-B. Based on the percentage of moldy kernels in Indonesian National Standard 2008, all treatments were included into grade I-B (total maximum of moldy kernels is 2%). The differences of slaty, fermented and moldy kernels were shown in Fig. 2 [19]. Slaty kernel is a kind of cocoa kernels that has not been fermented.

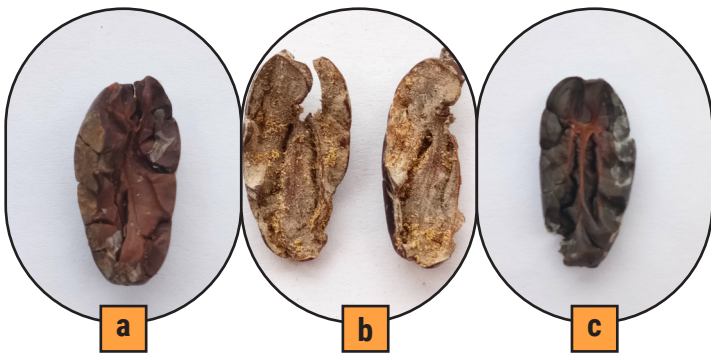


Figure 2. (a) Slaty kernel, (b) fermented kernel, and (c) moldy kernels of cocoa beans (Source : Nijma Nurfadila)

The lowest population of aflatoxigenic *A. flavus* at the beginning of inoculation until 11 days after inoculation was found in treatment with *Issatchenkia orientalis* BIO 211288 + *Acetobacter aceti* + mangosteen rind extract (MRE) (0.48 log cfu/g into not detected) and *I. orientalis* BIO 211291 + *I. orientalis* BIO 211288 + *A. aceti* + MRE (3.75 log cfu/g into not detected), while the aflatoxins could not be detected in all treatments, because their aflatoxin were still lower than limit of detection [20]. It means that the appropriate fermentation starter to increase physical quality and inhibit aflatoxigenic *A. flavus* and aflatoxins are *I. orientalis* BIO 211291 + *I. orientalis* BIO 211288 + *A. aceti* + MRE and *I. orientalis* BIO 211288 + *A. aceti* + MRE. Mangosteen rind extract (MRE) as addition of fermentation starter is used for antioxidant and anticancer, thus two appropriate fermentation starters are very important for human health.

In other research, there is microorganisms combination can be used as fermentation starter to inhibit ochratoxigenic *A. niger* YAC-9 and ochratoxin A (OTA) synthesis, i.e. indigenous *Lactobacillus plantarum* HL-15 either

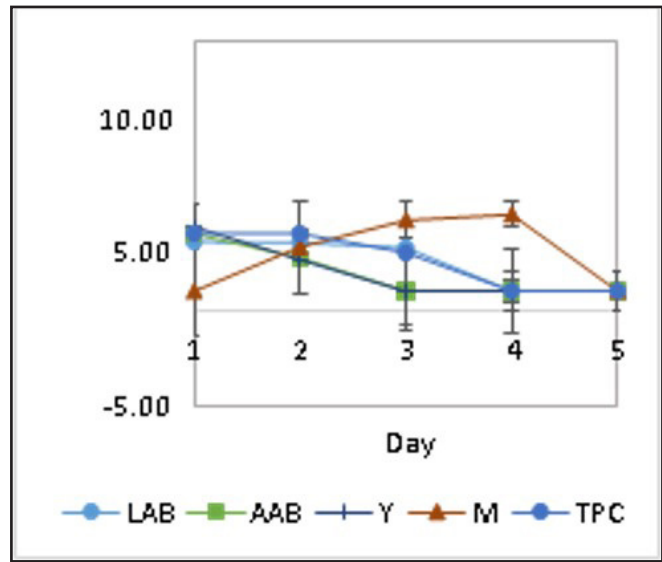
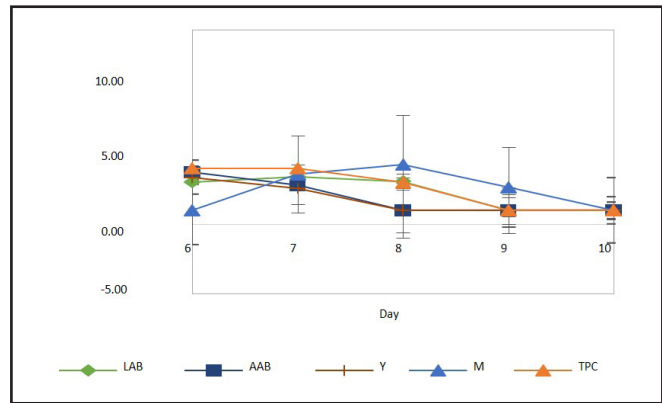


Figure 3. Growth of yeast, LAB, AAB, and mold during the course of drying in : (a) cocoa beans were inoculated by *L. plantarum* HL-15 and *A. niger* YAC-9; and (b) cocoa beans were inoculated by *L. plantarum* HL-15, *C. famata* HY-37, *Acetobacter* spp. HA-37, and *A. niger* YAC-9 (Source : Rahayu et al. 2021).

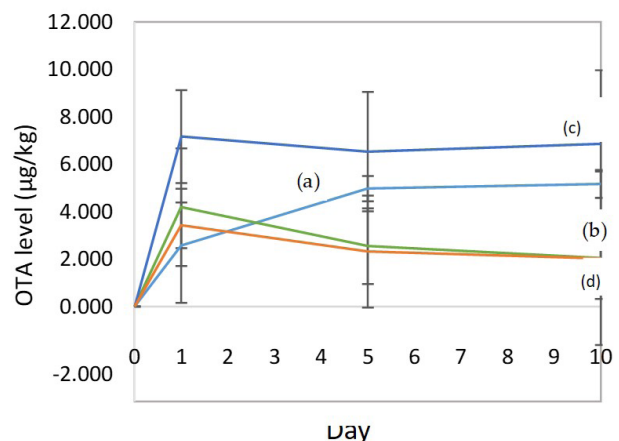


Figure 4. The OTA concentration during fermentation (0, 1st and 5th day) and drying (10th day) in cocoa beans with: (a) natural fermentation, (b) fermentation starter (*L. plantarum* HL-15) + *A. niger* YAC-9, (c) natural fermentation + *A. niger* YAC-9, (d) fermentation starter (*L. plantarum* HL-15 + *C. famata* HY-37 + *Acetobacter* spp. HA-37) + *A. niger* YAC-9 (Rahayu et al. 2021)

individually or in combination with *Candida famata* HY-37 and *Acetobacter* spp. HA-37. Population of ochratoxigenic *A.niger* YAC-9 decreased in cocoa beans were inoculated by *Lactobacillus plantarum* HL-15 as starter culture during fermentation and drying (Fig.3a). *Aspergillus niger* population also decreased during fermentation and drying in cocoa beans were inoculated by *L. plantarum* HL-15 + *C. famata* HY-37 + *Acetobacter* spp. HA-37 (Fig. 3b) [21]. Not only ochratoxigenic *A. niger*, but also ochratoxin was inhibited by those (Fig.4).

### Way Forward Strategies

Fermentation starter using combination of *I. orientalis* BIO 211288 + *I. orientalis* BIO 211290 + acetic acid bacteria (*Acetobacter aceti*) + mangosteen rind extract (MRE); and *I. orientalis* BIO 211288 + *A. aceti* + MRE can be used as appropriate postharvest handling of dried cocoa beans in exporter countries to increase the physical quality and inhibit aflatoxigenic *A. flavus* and aflatoxins, especially in Indonesia. Other microorganisms combination of *L. plantarum* HL-15 + *C. famata* HY-37 + *Acetobacter* spp. HA-37 also can be used as fermentation starter to inhibit ochratoxigenic *A. niger* and OTA. These appropriate fermentation starters should be introduced to farmer, collector and exporter levels of cocoa beans through the campaign, thus they will be applied in the fermentation process of cocoa beans. The collaboration of all stakeholders, such as scientists, government, lecturers, economic agents (farmers, collectors, traders, exporters, and customers) should be built to solve many problems in cocoa beans. Campaign of fermented cocoa beans is one of methods to keep our quality of cocoa beans and cocoa products from upstream (farmers) to downstreams (customers), thus the customers health will also be maintained.

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