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Prevalence of Toxygenic Micro-Organisms in Cocoa Peppers after the Fermentative and Drying Processes in the Agro-Forest System

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Abstract: ***Notably during the cultivation of cocoa seeds, fungal contamination can occur due to favorable environmental conditions and this contamination remains until cultivation. Otherwise, the bacterial presence can arise during fermentation and remain until post-cultivation. Drying methods are used during the processing of cocoa beans and contribute to the reduction of microbial contamination. The main objective of the work was to evaluate the presence of toxigenic fungi and bacteria in cocoa almonds after fermentation and drying. The methodology artificial drying of heate dair flow, was the methodology demanded less fungal growth. However, bacteria of the genus bacillus were those that appeared in all drying methods surviving after. Therefore, there are few studies that report the presence of microorganisms after the fermentation and drying process of the cocoa seed contamination of cocoa beans in the Amazon region.***

Keywords: cocoa beans; fungi, bacteria, toxigenics, Amazon region

1. Introduction

The Agroforestry System (SAFs) is currently being widespread in the Amazon due to its characteristics of providing economic development in environmental conservation. Its implementation in the Amazon region is aimed at recovering degraded areas with a view to local development with an emphasis on integrated management of natural resources. By presenting these characteristics, the SAFs increasingly draws the attention of public policy programs and national and international researchers (PORRO, 2009).

Seeds or grains recently removed from the field have a high water content, making it impossible to store them in these conditions. For this reason, it is necessary to subject them to drying processes in order to maintain the physical and chemical properties of the grains or seeds and prolong their quality, as well as reducing the activity of insects and proliferation of fungi (CARVALHO & NAKAGAWA, 2012).

According to research carried out, it points to the significant appearance of microorganisms in the cocoa bean, such as bacteria and fungi, and its growth that causes damage to it and consequently harms human productivity and health, as the presence of toxicity has been verified. population (COPETTI, 2009).

The drying process is extremely important for obtaining quality cocoa beans. For this reason, fermentation must be carried out immediately to prevent the proliferation of fungi, as the unwanted presence of fungi causes an unpleasant taste in the almond and is impossible to be used in subsequent processing steps (EFRAIM, 2004).

Cocoa fermentation presents lactic bacteria of *Lactobacillus sp.,* including *L. fermentum and L. plantarum,* are isolated together with several species of *Leuconostoc spp., Lactococcus spp. and Pediococcus spp.* and yeasts as the main acting agents.

Several species of Bacillus, other bacteria and filamentous fungi can also grow during fermentation (GARCIA-ARMISEN et al., 2010; LIMA et al., 2011), especially when this process is performed in boxes (PASSOS et al., 1984; THOMPSON et al., 2013; SCHWAN & WHEALS, 2004). BALs in fermented foods not only contribute to the taste, aroma and texture, but they also promote their quality and safety (KOPERMSUB & YUNCHALARD, 2010).

Previous research has shown the different types of fungi that have been isolated cocoa beans like *Mucor, Penicillium, Rhyzopus, Fuzarium and especially Aspergillus*. (COPETTI et al, 2011; MOUNJOUENPOU et al. 2008; SANCHES – HERVAS et al. 2008).

The objective of this work is identified and to evaluate strategies to reduce the presence of fungi and bacteria resulting from the pulping process (fermentation) and three type soft drying ("Terreiro", "Estufa" and "Hot Air") during the processing of cocoa beans.

2. Materials and Methods

Sampling and drying method analysis

The almonds samples were donated already fermented by the owner Michinori Konagano, in a total of 6 kilos. They were divided into three parts of 2 kilos and a sample of 20 almonds was separated for each one, where before beginning the drying process the samples were weighed. Then

submitted to three types of drying: Greenhouse, Terreiro and mixed drying of greenhouse plus prototype artificial dryer with finned type resistance, as shown in figure 01.

Drying Method

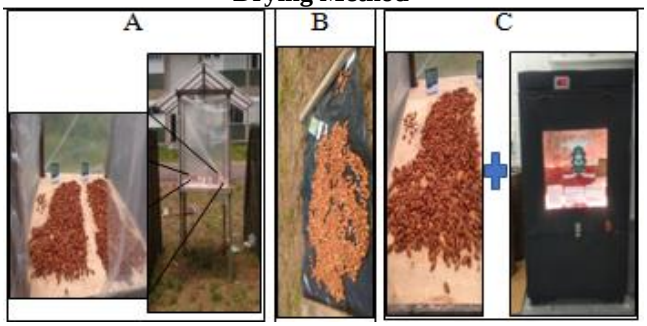

Figure 1: A: Greenhouse drying; B: drying in terrace; C: Greenhouse drying + Prototype

The green houses were built to dry cocoa beans with a size of 1 m² each, so the following materials were used: 8 X 2m stilts, 12 X 1m ripples, 6 X 1.5m ripples, 12 meters of canvas for greenhouses 150 microns, 1 sheet of 40 mm plywood and caught (BRASIL, 2008). To build the dryer on the terrace, only 2.25 m² of black canvas was used. Finally, 3 thermo hygrometers were purchased to measure the temperature and humidity of the air, in order to monitor the drying process of the cocoa bean. The Greenhouse drying plus prototype artificial dryer with U-type finned resistance "artificial drying of heate dair flow", consists of drying the cocoa almond from 06:00 to 18:00 in the green house, using solar energy and from 6:01 pm to 5:59 am in the artificial drying prototype artificial drying of heate dair flow to keep the drying process constant.

Isolation of fermenting bacteria and toxigenic fungi

Subsequently, 10 samples of each treatment were taken and sent to the Microscopy laboratory for identification of the fungus and bacteria and their incidence for each drying method used in the study (LEVY, 2004).

Nevertheless, the isolation of the bacteria was carried out from the collected samples, one of the procedures applied was the crushing of the dried cocoa bean. These scraping samples were inserted in the culture medium Agar Mac Conkey and Blood Agar, grown for 48 hours in the microbiological oven, (figure 02). Cultures were analyzed by counting the colony-forming units (CFU / mL) (LEVY, 2004).

Bacteria Culture Medium

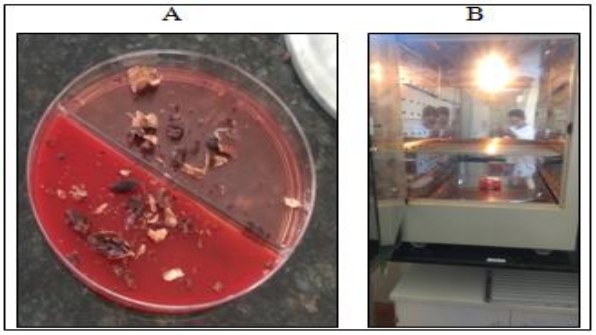

Figure 2: A: MacConkey Agar and Blood Agar; B: Greenhouse Microbiological.

Consequently, to evaluate the ochratoxigenic fungi contaminating the cocoa samples, the almonds were moistenedhen scraped and placed in the culture medium Sabouraud agar with Chlorphenicol and Sabouraud agar with Chlorphenicol and Cyclohexemide, incubated at 25 ° C, figure 03, (PITT et al. 2009), for later identification.

Fungal Culture Media

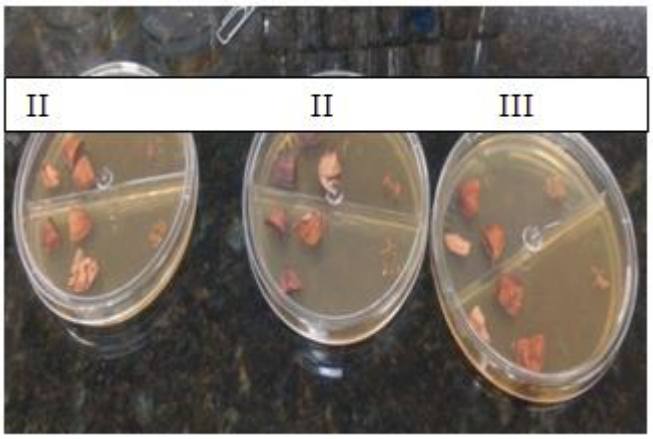

Figure 3: I-Sabouraud agar with Chlorphenicol and II and III: Sabouraud agar with Chlorphenicol + Cyclohexemide.

3. Results and Discussion

Several studies seek to establish which factors lead to the development of mycotoxins in food, but so far there is no accuracy of all contaminating factors, only that they are correlated to humidity, temperature, time, physical condition of the grain, levels of O_2 and CO_2, product water activity and microbiological interaction (HOELTZ, 2005).

For this reason, Enfraim et al. (2010) apud Crespo (1985), says that the drying process of cocoa beans should be done as soon as possible after the cocoa fermentation stage is finished and should be well conducted, in order to avoid unpleasant taste and possible proliferation. fungal.

After 14 days from the start of the fungi incubation process in cocoa beans, growth was observed in the three drying methods, figure 04.

Fungal growth Drying Method

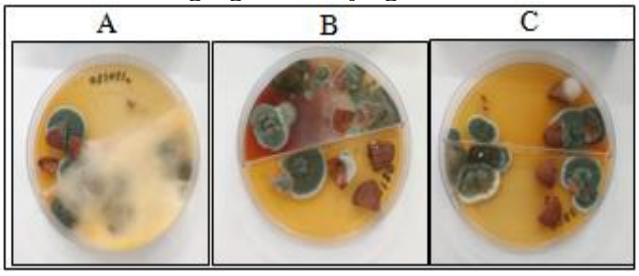

Figura 4: A: Terrace; B: Greenhouse; C: Greenhouse + Prototype.

Figure 04 shows the fungal growth, and it was observed that the cleanest culture medium was the artificial drying of heate dair flow, as the drying methodology demanded less time and presented less exposure to weather conditions. The fungal growth was to the group *Penicillium spp and Aspergillus* spp.

During the drying process of the cocoa beans, chemical-physical reactions occur, causing the final product to taste like chocolate, the drying temperature is in the ideal temperature range between 35°-50°C, avoiding organoleptic changes and product quality (SALES and CÂNDIDA, 2016). According to Schabo (2014), due to the fact that cocoa is dried at relatively low temperatures, it does not show efficiency in the process of elimination of fungi of the genus Aspergillus spp, Penicilliumspp. and Fusarium spp that develop when growing under excellent conditions at these temperatures.

COPETTI ET AL. (2011) AND SANCHES - HERVAS ET AL. (2008), reported the presence of Aspergillus species in all different processing phases primary effect of cocoa beans, with emphasis on the species producing ochratoxin A in the section Nigri, which increased after fermentation.

The presence of aflatoxins in cocoa beans is already known to contaminate agricultural areas grown with cocoa, coffee, peanuts and corn (COPETTI et al., 2011). A high percentage of *A. flavus* (almost 60%) was able to produce aflatoxins in cocoa beans (COPETTI et al., 2011; SANCHES - HERVAS et al., 2008).

Currently, Gram-positive and gram-negative bacteria were found in cocoa beans <100 CFU / ml, figure 05. The bacteria identified were gram-positive bacteria of the genus Bacillus spp.

Bacteria Colony

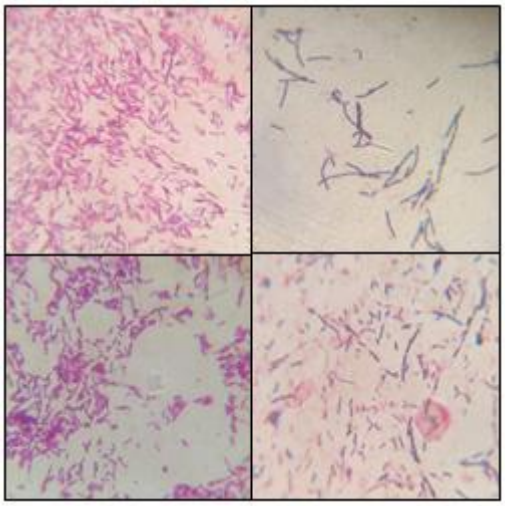

Figura 5: Bacillus gram positive

According to Granados 2016, the bacteria found in the cocoa beans were Bacillus, *B. brevis and B. megaterium*

The verification of *Bacillus spp.* in the cocoa almond maybe associated with soil contamination during the drying step, as Ferrari Filho (2011) indicates that the practice of drying agricultural products is considered one of the most important steps, since it is a determining factor in the control of microorganisms. And it is subject to the action of microorganisms from soils, air, handling by producers (RIBEIRO et al., 1986; LOPEZ & DIMICK, 1991; SCHWAN, 1996; OETTERER, 2004).

This diversity of microorganisms presents in almonds cocoa beans come from the air, from their direct contact with the hands and tools of workers, contact with other cocoa beans, the trough itself fermentation, among other sources (Ferrari Filho, 2011).

4. Conclusion

It is possible to conclude that in the Amazon region there are few studies regarding the contamination of cocoa beans resulting from drying processes, and through there search it was verified the presence of this microorganism in the almonds, no matter how much the drying methodology variation has been done. Was able to prevent the proliferation of undesirable microorganisms in cocoa beans.

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