

## Physical and Chemical Degradation of B-aflatoxins during the Roasting and Dutching of Cocoa Liquor

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### ABSTRACT

To evaluate the effect of roasting and Dutching processes on the stability of B-aflatoxins (AFB1+AFB2), experimental units of cocoa beans contaminated with aflatoxin at a concentration of 220.7 ng g<sup>-1</sup> were roasted at 250°C for 15 minutes. Roasting conditions caused a notable reduction in the aflatoxin content (up to 71%). The resulting cocoa liquors contaminated with 63.9 ng g<sup>-1</sup> were thermal-alkaline treated with sodium, potassium, and calcium hydroxide at three different concentrations (10, 20, and 30 g kg<sup>-1</sup>). The effects of the two variables (alkali type and concentration) were analyzed as a completely randomized factorial 3x3 design. At a concentration of 10 g kg<sup>-1</sup>, the aflatoxin reduction was more effective when using NaOH and Ca(OH)<sub>2</sub> (up to 94%) than when using KOH (up to 88%); however, at concentrations of 20 and 30 g kg<sup>-1</sup>, all of the three chemicals were almost equally effective for aflatoxin degradation (up to 98%). According to these results, higher reductions in aflatoxin levels were achieved during the roasting and an effective extra-reduction occurred during the Dutching process. Treatment of cocoa liquors with these alkalinizing agents not only improved their physicochemical properties, but also enhanced their sanitary quality through the reduction in the aflatoxin content.

**Keywords:** Alkalinization, *Aspergillus flavus*, Cocoa beans, Mycotoxins, Sanitary quality.

### INTRODUCTION

Mesoamerica native populations have made significant contributions to the world food supply. Mexico has been the source of many food crops that are now enjoyed by humans all over the world, such as many varieties of maize, bean, green tomato, avocado, pineapple, guava, cinnamon, chili, and cocoa, among others (Tannahill, 1995). *Theobroma cocoa* is a tree native to humid-tropical regions of the northern part of South America and, according to some reports, of Central America (Miranda, 1962). Indeed, there is still some controversy about the origin and domestication of cocoa; however, Cuatrecasas (1964)

hypothesized that wild plants from the “Selva Lacandona” from Chiapas-Mexico were possible ancestors of domesticated cocoa.

Despite its international reputation, the process of cocoa transformation in Mexico - from harvesting until drying- is quite ancient. The cocoa pod is harvested by hand and cocoa seeds are removed manually, then fermented in wooden boxes, sun-dried, and transported to processing facilities, where they are roasted and shelled. The roasted cocoa beans are then usually ground into a suspension (called cocoa liquor), which contains butter and non-fat fine brown particles. In Mexico, the best beans come from the fertile forest of the southeast states such as Tabasco, Chiapas, Guerrero, and Oaxaca, where the climate conditions are

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favorable for their development. National cocoa production is estimated at 25,000 tons per year (SAGARPA, 2010).

Now, with the worldwide proliferation of free trade agreements, the key word in the new realm of competition has become “quality”; any quality alteration (nutritional, industrial, or sanitary) automatically diminishes the potential commercial value and reflects substandard postharvest systems. Thus, the quality of Mexican’s cocoa can best be appreciated when additional technology is applied to the processing in order to improve its functional quality. Alkalization, a 183-year old process also known as “Dutching”, first used by Conrad Van Houten (Minifie, 1989), is one of these technologies. This process darkens the cocoa, changes the taste by reducing bitterness, and increases the dispersibility of cocoa powder for various applications, and it can be performed using alkali solutions (e.g., sodium or potassium carbonate, sodium bicarbonate, sodium hydroxide, among others). However, the use of this technology for the production of cocoa liquor, elaborated with aflatoxin-contaminated beans has not been evaluated.

Unfortunately, neither storage nor processing conditions of cocoa are strictly controlled, thus, fungi contamination is inevitable. The beans are susceptible to fungal spoilage both during and after fermentation. Fungal species belonging to the genera *Aspergillus*, *Penicillium*, *Fusarium*, *Mucor*, *Geotrichum*, *Trichoderma*, and *Rhizopus* have been observed on dried fermented cocoa beans (Mounjouenpou *et al.*, 2008; Sánchez-Herváz *et al.*, 2008). Many of these fungi, principally species from the genera *Aspergillus* and *Penicillium*, produce mycotoxins that can cause acute or chronic intoxication and damage to humans and animals (Manafi, 2012). Among the mycotoxins, aflatoxins are of special interest, given their high occurrence as well as their toxicological and carcinogenic potential (Mohammadi, 2009). Mexican regulations for aflatoxin levels in cocoa are imposed by a set of guidelines published by the Ministry of Health. Regulations have established the maximum level of aflatoxins

allowed in cocoa, as well as the sanitary requirements for their transport and storage. Action levels are set to 20 ng g<sup>-1</sup> total aflatoxins (Norma Oficial Mexicana NOM-186-SSA1/SCF1-2002).

Evidence from the literature has shown that the roasting process reduces the aflatoxin content in some commodities up to 75% (Méndez-Albores *et al.*, 2004a; Yazdanpanah, 2005; Bokhari and Aly, 2009). However, no information is available about the fate of aflatoxins during the roasting and Dutching processes of aflatoxin-contaminated cocoa beans. Therefore, the present research was conducted to determine the efficacy of the roasting conditions as well as the effect of alkalization on aflatoxin degradation and on certain physicochemical properties of cocoa liquors.

## MATERIALS AND METHODS

### Safety Precautions

Procedures used for handling aflatoxin contaminated materials were adopted from recommendations published by the International Agency for Research on Cancer (Castegnaro *et al.*, 1981).

### Chemicals

AFB1 and AFB2 were obtained from Sigma Chemical Co. Ltd (St Louis MO, USA). Other chemicals such as sodium, potassium, and calcium hydroxide were obtained from JT Baker (J. T. Baker, Mallinckrodt Baker, Mexico).

### Cocoa Beans

Cocoa beans from the Soconusco region (Chiapas-Mexico), with 3.3% moisture content (MC) were used. The beans were aflatoxin-free, as tested with the

immunoaffinity column (IAC) method described below.

### Fungus Isolate

The fungus *A. flavus* Link (UNIGRAS-1231) was obtained from the culture collection of the Grain and Seed Research Unit of the National Autonomous University of Mexico. The fungus was plated in Petri dishes containing MSA medium (%: malt extract, 2; sodium chloride, 6; and agar, 2) for 7 days at 25°C. This strain only produces AFB1 and AFB2 (Pérez-Flores *et al.*, 2011).

### Inoculation Technique

To inoculate the beans, fungal spores were removed from the Petri dishes with a spatula; a sterile-water spore suspension (1.793 L) was prepared with approximately 100,000 conidia mL<sup>-1</sup>, and this suspension was used to raise the MC of the bean. This amount of inoculum (approximately 18,000 spores g<sup>-1</sup> of cocoa bean) was determined to eliminate competition with other storage fungi that can potentially grow under the same incubation conditions. The total amount of cocoa beans was 20 kg. The MC of the beans was adjusted to 18% (adding the inoculum for the fungus-inoculated experimental unit or the same amount of sterile-water for the fungus-free control), and stored in plastic bottles (10 kg of beans per treatment). Bottles were covered with thin polyethylene film to minimize the loss of humidity from the grain; however, 10 perforations with a pin were made to each film to avoid the accumulation of carbon dioxide generated by the respiration of grains and fungi. Bottles were incubated at 28°C under 70-75% relative humidity, mixed every 12 hours at low speed (60 rev min<sup>-1</sup>) for 15 minutes in a mixer (model C-100, Hobart Corp. Troy, Ohio, U.S.A.) for the fungus inoculated experimental unit; as well as for the fungus-free control. After the incubation period (21 days), the bean was put under a 1,000 mg L<sup>-1</sup> ethylene oxide gas atmosphere

for 5 hours, to stop further development of the toxigenic fungus and to avoid the dispersal of viable spores (Pérez-Flores *et al.*, 2011). Finally, the bean was oven-dried at 60°C for 48 hours until reaching ~3.5% MC, then transferred to plastic bags, and stored at 4°C for further analysis.

### Aflatoxin Quantification

Aflatoxin content was determined according to the 991.31 AOAC method (AOAC, 2000) using monoclonal antibody columns for aflatoxins B1 and B2 (VICAM, Milford, Mass., USA). Samples (25 g) were extracted by blending with 100 mL methanol and 5 g NaCl, using a laboratory blender (Mod. 51BL30; Waring, New Hartford, Conn., USA). The mixture was filtered through a Whatman No. 1 filter paper and a 10-mL portion was diluted with 40 mL of Zn(O<sub>2</sub>CCH<sub>3</sub>)<sub>2</sub>/AlCl<sub>3</sub> solution. The diluted preparation was filtered through a micro-fiber filter, and 10 mL were applied to an IAC (Aflatest-P; VICAM Science Technology, Watertown, Mass., USA). Subsequently, the column was washed twice with 10 mL of methanol-water (20:80 v/v) and dried in a sterile air flow chamber. The toxins were then eluted with 1 mL of high-performance liquid chromatography (HPLC)-methanol and quantified in a fluorometer VICAM Series-4 (VICAM Source Scientific, Irvine, Calif., USA) after reaction with 1 mL of 0.002% bromine solution. Aflatoxin detection limit with the IAC via fluorescence measurement is approximately 0.5 ng g<sup>-1</sup>. When total aflatoxin concentration was greater than 25 ng g<sup>-1</sup>, dilutions from the extracts (1 mL of the methanolic extract was diluted with 49 mL of zinc acetate/aluminum chloride solution) were made before being passed through the IAC.

### Recovery Checks

The performance of the 991.31 AOAC method was tested by measuring the percentage of aflatoxin recovery using the



HPLC method on spiked cocoa liquor, injecting 4 replicates of 6 different aflatoxin concentrations (from 0.78, 1.56, 3.13, 6.25, 12.50 to 25 ng g<sup>-1</sup>). A Waters HPLC equipment with two pumps (Model 510, Waters Associates, Milford, MA), and a Waters nova-pak C18 reverse phase column (5 µm, 3.9 mm, 150 mm) was used. Samples collected from the IAC (20 µL) were injected into a HPLC and eluted isocratically with a mobile phase of 12.5 mM acetic acid:acetonitrile (1:1, v/v) at a flow rate of 1 mL min<sup>-1</sup>. Aflatoxins were fluorometrically detected using a fluorescence detector (Waters model 470), the excitation and emission wavelengths were 338 and 425 nm, respectively. Aflatoxins were identified by their retention time (Rt), compared with those for a pure aflatoxin standard solution under identical conditions. The aflatoxin recovery for the IAC method was 92%, with a standard error of 1.2, and a coefficient of variation value of 4.4%. These results indicated that the method used was reliable.

### Roasting Procedure

To produce the cocoa liquor, four batches (2.5 kg each) of whole contaminated bean samples, as well as the control (aflatoxin-free beans), were roasted in an oven at 250±5°C for 15 min, and steeped 1 hour at room temperature (22±1°C). The roasted beans were manually decorticated, milled in an electric plate-style grain mill type C-11-1 (Glen Mills, Inc. Clifton NJ, USA) and sieved to provide ground material with a particle size of < 500 µm (40 mesh).

### Alkalization (Dutching)

To perform the alkalization, samples of cocoa liquor (250 g) were dispersed in the corresponding solution (500 mL of solution containing 10, 20 or 30 g kg<sup>-1</sup> of each alkali) in a 1 L round-bottom flask and heated in an agitated water bath (Bellco Glass Inc. NJ, USA) at 85°C during 1 hour. The neck of the

flasks was connected to a reflux condenser in order to maintain constant volume and minimal evaporation loss. Once the alkalization was attained, the alkalized liquor was oven-dried at 60°C for 48 hours, until reaching a constant MC (~3%). Finally, the dried alkalized liquor was milled (Glen Mills, Inc. Clifton NJ, USA) and sieved to provide ground material (40 mesh).

### Composition Analysis

The following analyses were performed in triplicate according to AOAC official methods (AOAC, 2000): MC, pH, crude protein (N×6.25), and crude fat (methods 931.04, 970.21, 970.22, and 963.15, respectively).

### Color Analysis

Cocoa liquors were subjected to surface-color analysis with a MiniScan XE model 45/0-L colorimeter (Hunter Associates Laboratory, Reston, VA, USA). The colorimeter was calibrated with a white porcelain plaque (L= 97.02; a= 0.13, b= 1.77). Samples were placed in a glass cup and readings were made in triplicate. Three derived functions (ΔE, chroma, and hue value) were computed from the L\*, a\*, and b\* readings, as follows:

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \quad (1)$$

In which, (symbol) ΔE = total difference of color between reference and sample. (symbol) ΔL, (symbol) Δa, and (symbol) Δb, = absolute differences between values L, a, and b of the reference and sample.

$$\text{Chroma} = (a^{*2} + b^{*2})^{1/2} \quad (2)$$

$$\text{Hue angle} = \arctan (b^*/a^*) \quad (3)$$

### Experimental Design and Statistical Analysis

The experiment was conducted as a completely randomized 3×3 factorial design; the first factor corresponded to the alkali

type (sodium, potassium and calcium hydroxide), and the second to the alkali concentration (10, 20 and 30 g kg<sup>-1</sup>). The experimental conditions were carried out with three replicates. Data were assessed by analysis of variance (ANOVA) and means comparisons were performed according to the Tukey test using the Statistical Analysis System (SAS, 1998). A significance value of  $\alpha=0.05$  was used to distinguish significant differences between treatments.

## RESULTS AND DISCUSSION

### Aflatoxin Degradation

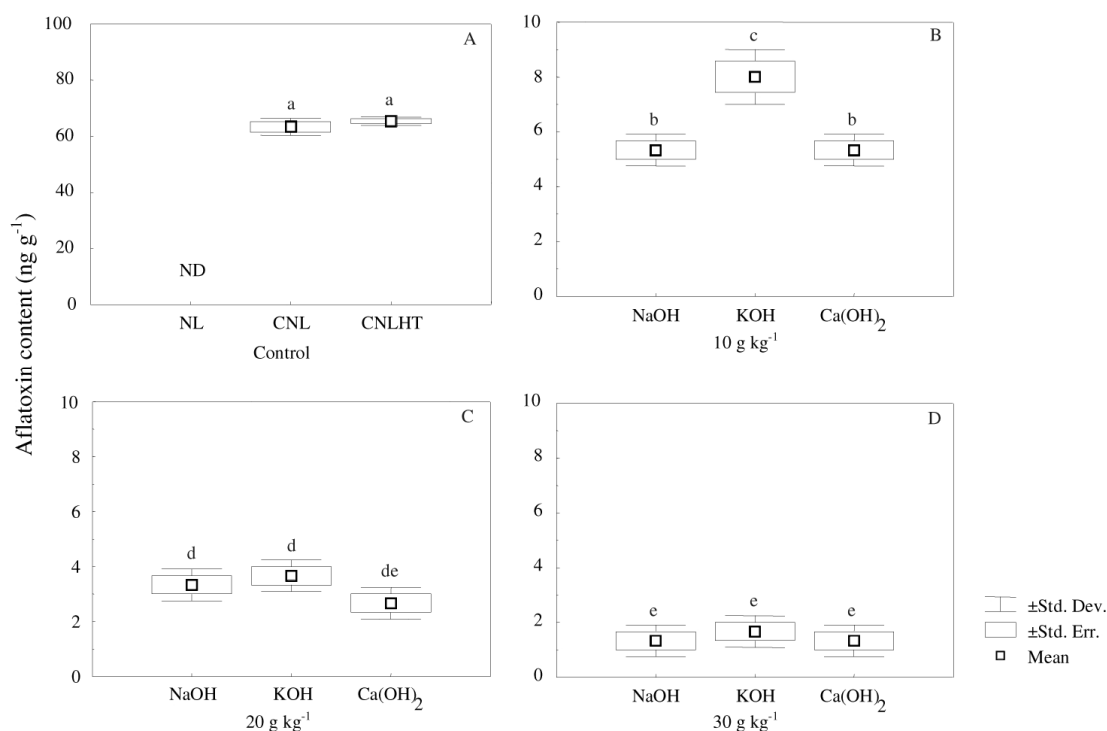
Regarding the total aflatoxin concentration, no aflatoxins were detected in the control bean at any time, the experimental bean received the same treatment during incubation (27°C, 18% MC, 21 days) in the absence of spores of the aflatoxin-producing fungus. However, for the inoculated bean, the aflatoxin concentration was 220.7±12.2 ng g<sup>-1</sup>. On the other hand, the percentage of aflatoxin reduction in the cocoa beans after roasting was 71%; thus, cocoa liquor presented a final aflatoxin concentration of 63.9±2.6 ng g<sup>-1</sup>. This result was in close agreement with that reported previously by Méndez-Albores *et al.* (2004a) who roasted aflatoxin-contaminated maize at 285±2°C for 7 minutes, reducing the aflatoxin concentration by 70-81%.

Aflatoxins have been found to be unstable up to their melting point of around 250-270°C (Pohland *et al.*, 1982). Consequently, MC in combination with heat might well induce chemical reactions in some mycotoxins, thus reducing their concentration. It is well known that heat treatments degrade mycotoxins to a certain extent during the processing of foodstuffs; however, the prediction of the extent of mycotoxin reduction in relation to heat treatment depends on several of many possible factors, including mycotoxin concentration, the extent of "binding"

between mycotoxin and food constituents, heat penetration, and MC, among others. In this research, roasting conditions (250±5°C, 15 minutes) were not sufficient for total aflatoxin degradation, so that a residual content of aflatoxins always remained in the cocoa liquor (63.9 ng g<sup>-1</sup>). It is quite probable that the toxins deposited inside the kernels by the penetrating mycelia were protected to a greater extent than when present only on the shell of the cocoa beans.

Figure 1 shows the effect of the type of alkali and its concentration on the aflatoxin degradation on cocoa liquors. As observed, contaminated natural liquor heat-treated (CNLHT) at 85°C during 1 hour (without alkali), did not have effect on the aflatoxin content (profile A). However, alkalization had a significant effect on aflatoxin degradation (profiles B, C and D). At a concentration of 10 g kg<sup>-1</sup>, sodium and calcium hydroxide presented no statistical difference; samples had an aflatoxin concentration of 5.3 ng g<sup>-1</sup>. Nevertheless, in the case of potassium hydroxide, statistical differences were observed: those samples presented an aflatoxin concentration of about 8 ng g<sup>-1</sup> (profile B). Samples treated with alkali at 20 g kg<sup>-1</sup> did not show differences among them in the aflatoxin content; all samples presented an average aflatoxin value of 3.2 ng g<sup>-1</sup>. The same trend was observed in samples treated with the three different alkalis at a concentration of 30 g kg<sup>-1</sup>; however, the aflatoxin content was lower (1.4 ng g<sup>-1</sup>) than those observed at 20 g kg<sup>-1</sup> (profiles C and D, respectively). Results indicated a significant reduction in the aflatoxin content in cocoa liquors, due to the thermal-alkaline treatment (up to 98%). In general, as the alkali concentration increased, lower aflatoxin values were registered (Figure 1).

The first step in the degradation during alkalization is likely to be the base-induced lactone ring-opening of aflatoxin, yielding a water soluble salt including decarboxilation. Consequently, aflatoxin fluorescence, attributable to the coumarin moiety,



**Figure 1.** Effect of the thermal-alkaline process (Dutching) on aflatoxin degradation in cocoa liquors. Box and whiskers with same letter are not significantly different (Tukey > 0.05). ND: Not detectable (below immunoaffinity column detection limit, 0.5 ng g<sup>-1</sup>) NL: Natural liquor; CNL: Contaminated natural liquor, CNLHT: Contaminated natural liquor with heat treatment.

diminishes or even disappears in alkaline treatments (Méndez-Albores *et al.*, 2004b).

### Moisture Content

Heat treatment (80°C, 1 hour) and alkalization with NaOH did not have effect on the MC of the cocoa liquor; the average MC for those samples was 3.36 and 3.46%, respectively (Table 1). However, samples alkalized with KOH and Ca(OH)<sub>2</sub> showed a significant effect on MC; those samples presented an average MC of 2.64%, similar to those reported for Venezuelan cocoa liquor alkalized with NaHCO<sub>3</sub>, Na<sub>2</sub>CO<sub>3</sub> and NaOH, respectively (Rodríguez *et al.*, 2009).

### Proximate Composition (Protein and Fat)

Natural liquor (NL) presented an average crude protein value of 13.09%, and this

parameter was not affected by heat treatment: samples presented an average value of 13.03%. However, the type of alkali and its concentration had a significant effect on crude protein. In general, as the alkali concentration increased, lower protein values were registered (Table 1). Odunsi and Longe (1999) reported similar results and the authors argued that protein diminution was due to the oxidative destruction of proteins by deamination. The same phenomenon was observed in the case of crude fat, i.e., samples treated with NaOH at 30 g kg<sup>-1</sup> presented the lowest average crude fat value (37.64%) as compared to the natural liquor (53.32%). Previous reports have also shown that the reduction in fat content by alkalization is due to hydrolysis and saponification of triglycerides with the consequent formation of salts (Odunsi and Longe, 1998; 1999). This fact should be considered, because excessive alkali addition could lead to the generation of

**Table 1.** Some physicochemical properties of the non-treated and alkalized cocoa liquors.<sup>a</sup>

Sample	Moisture content (%)	Crude protein (%)	Crude fat (%)	pH
NL	3.27±0.05 a	13.09±0.02 a	53.32±0.29 a	5.52±0.006 a
CNL	3.29±0.11 a	13.10±0.03 a	53.36±0.15 a	5.52±0.005 a
CNLHT	3.36±0.06 a	13.03±0.02 a	53.75±0.19 a	5.54±0.004 a
Na-1	3.48±0.10 a	12.16±0.06 b	50.61±1.20 b	8.71±0.005 b
Na-2	3.42±0.18 a	11.33±0.03 c	44.27±0.14 c	9.82±0.001 c
Na-3	3.49±0.16 a	9.56±0.05 d	37.64±0.32 d	10.64±0.010 d
K-1	2.68±0.07 b	12.14±0.03 b	52.47±0.26 e	7.92±0.005 e
K-2	2.61±0.20 b	11.29±0.08 c	50.63±0.30 b	8.82±0.004 f
K-3	2.91±0.19 b	9.52±0.08 d	48.62±0.27 f	9.77±0.049 g
Ca-1	2.47±0.15 b	12.14±0.04 b	52.37±0.29 e	8.33±0.050 h
Ca-2	2.65±0.13 b	10.39±0.09 e	45.48±0.24 c	9.18±0.051 i
Ca-3	2.51±0.19 b	9.56±0.05 d	40.49±0.19 g	10.09±0.006 j

<sup>a</sup> Mean of three replicates ±SE, Mean with the same letter in the same column are not significantly different (Tukey > 0.05). NL: Natural liquor; CNL: Contaminated natural liquor; CNLHT: Contaminated natural liquor with heat treatment; Na-1: NaOH at 10 g kg<sup>-1</sup>; Na-2: NaOH at 20 g kg<sup>-1</sup>; Na-3: NaOH at 30 g kg<sup>-1</sup>; K-1: KOH at 10 g kg<sup>-1</sup>; K-2: KOH at 20 g kg<sup>-1</sup>; K-3: KOH at 30 g kg<sup>-1</sup>; Ca-1: Ca(OH)<sub>2</sub> at 10 g kg<sup>-1</sup>; Ca-2: Ca(OH)<sub>2</sub> at 20 g kg<sup>-1</sup>; Ca-3: Ca(OH)<sub>2</sub> at 30 g kg<sup>-1</sup>.

soapy flavor. In this research, due to the nature of the cocoa liquor, a sensorial analysis was not performed; however, Rodríguez *et al.* (2009) reported no soapy flavor on alkali-treated cocoa liquor with sodium bicarbonate, sodium carbonate, and sodium hydroxide at concentrations up to 30 g kg<sup>-1</sup>.

### pH

Table 1 also summarizes the effect of alkali type and its concentration on the pH of the non-treated and alkali-treated cocoa liquors. As expected, with increase in alkali concentration, higher pH values were observed. Contaminated natural liquor (CNL) presented an average pH value of 5.52, similar to the natural liquor (NL). This value was consistent with those reported in cocoa liquor from Ecuador (Luna *et al.*, 2002). However, other authors have reported pH values in the range of 5-5.4 for Venezuelan cocoa liquor (Rodríguez *et al.*,

2009). Those differences could be related to the origin of cocoa, whether post-harvest system or even processing conditions of the cocoa beans. In this research, the pH of the liquor treated with NaOH at the rate of 30 g kg<sup>-1</sup> was higher than in those samples alkalized with Ca(OH)<sub>2</sub> and KOH at the same concentration. These pH increments were principally due to neutralization of free acids, which favor the formation of browning compounds, due to partial deamination of proteins (Serra and Ventura, 2002).

### Color

In this research, heat and alkalization significantly affected the *L\** value of cocoa liquors (Table 2). Natural liquor had an average *L\** value of 46.83, similar to that recorded for contaminated natural liquor (46.90); however, this parameter was reduced (41.92) by heat treatment.



In general, the alkali treatment, which produced liquor with a low  $L^*$  value, was done with a concentration of  $30 \text{ g kg}^{-1}$ ; consequently, that sample appeared darker than the others, including the control (non-alkalized liquor). It is well known that an inverse relationship exists between alkali concentration and luminosity of samples. Terink and Brandon (1984) alkalized cocoa using a 1:1.3 ratio of cocoa:water with KOH at a concentration of  $34 \text{ g kg}^{-1}$  in open containers. They reported a value of  $L^*=22.1$  for cocoa powder without alkalization, and  $L^*=12$  for alkalized cocoa powder. In the present research, drastic reductions in the  $L^*$  values were observed; however,  $L^*$  values were considerably higher and lighter than those reported by the aforementioned authors. These differences were probably due to the differences in cocoa origin, as well as on alkalization conditions, since this investigation was performed in closed containers and losses by evaporation were thus minimized.

On the other hand, there was no statistical difference in the total color change ( $\Delta E^*$ ) among natural liquor (NL) and contaminated natural liquor (CNL); these samples

presented an average value of 50.75 (Table 2). However, heat treatment significantly affected the total color difference such that the samples had a  $\Delta E^*$  value of 55.41. The maximum  $\Delta E^*$  value was observed in samples treated with  $30 \text{ g kg}^{-1}$  alkali. Table 2 also shows the chroma and Hue angle values for the non-alkalized and alkali-treated cocoa liquors. Natural liquor (NL) and contaminated natural liquor (CNL) presented an average chroma value of 8.36, which indicated that color was more pure in these samples, since chroma represents color "purity". In this research, heat treatment as well as alkalization significantly affected the chroma value in cocoa liquors. As the alkali concentration increased, lower chroma values were observed. In the case of Hue, heat treatment had no effect on this parameter; i.e., liquor samples presented an average value of 30.49. However, alkalization significantly affected the Hue value in cocoa liquors, which means that the thermal-alkaline treatment improves the red-brown color. Most of the cocoa liquors are located in the red zone, making them darker as a function of the increment in alkali concentration. Silva *et al.* (2005) treated

**Table 2.** Surface-color analysis of the non-treated and alkalized cocoa liquors. <sup>a</sup>

Sample	Color			
	$L$	$\Delta E$	Chroma	Hue
NL	46.83±0.35 a	50.73±0.30 a	8.33±0.32 a	30.86±1.65 a
CNL	46.90±0.22 a	50.77±0.26 a	8.39±0.17 a	30.39±1.11 a
CNLHT	41.92±0.08 b	55.41±0.09 b	6.73±0.25 b	30.22±1.00 a
Na-1	36.82±0.41 c	60.34±0.39 c	4.02±0.33 c	5.91±0.90 b
Na-2	36.18±0.23 cd	60.89±0.23 cd	2.02±0.01 d	0.75±0.81 c
Na-3	35.81±0.36 d	61.25±0.36 d	1.80±0.04 d	3.19±1.22 b
K-1	41.36±0.31 b	56.13±0.28 b	8.33±0.31 a	34.77±0.39 d
K-2	36.89±0.03 c	60.26±0.02 cf	4.53±0.09 e	10.43±0.40 e
K-3	36.82±0.09 c	60.29±0.09 c	2.93±0.12 f	15.16±1.03 f
Ca-1	37.35±0.32 e	59.05±0.31 ef	5.23±0.19 g	24.59±0.64 g
Ca-2	36.06±0.15 d	61.02±0.15 d	2.99±0.05 f	4.51±2.82 b
Ca-3	35.99±0.23 cd	61.60±0.23 cdf	2.65±0.06 f	10.63±1.14 e

<sup>a</sup> Mean of three replicates ±SE, Mean with the same letter in the same column are not significantly different (Tukey > 0.05). NL: Natural liquor; CNL: Contaminated natural liquor; CNLHT: Contaminated natural liquor with heat treatment; Na-1: NaOH at  $10 \text{ g kg}^{-1}$ ; Na-2: NaOH at  $20 \text{ g kg}^{-1}$ ; Na-3: NaOH at  $30 \text{ g kg}^{-1}$ ; K-1: KOH at  $10 \text{ g kg}^{-1}$ ; K-2: KOH at  $20 \text{ g kg}^{-1}$ ; K-3: KOH at  $30 \text{ g kg}^{-1}$ ; Ca-1:  $\text{Ca(OH)}_2$  at  $10 \text{ g kg}^{-1}$ ; Ca-2:  $\text{Ca(OH)}_2$  at  $20 \text{ g kg}^{-1}$ ; Ca-3:  $\text{Ca(OH)}_2$  at  $30 \text{ g kg}^{-1}$ .



cocoa nibs with a solution of 47.8 g kg<sup>-1</sup> (w/w) of K<sub>2</sub>CO<sub>3</sub> at 108°C over a 54 minutes period, obtaining a darker brown color than those alkalized with solutions of 12.2 or 30 g kg<sup>-1</sup> (w/w) K<sub>2</sub>CO<sub>3</sub>. However, some researches have pointed out that the reaction would not develop below a concentration of 10 g kg<sup>-1</sup> (w/w), due to the low alkalinity. The characteristic color of cocoa liquor is due to the polyphenoloxidase enzyme action, which has optimal activity at pH 8 (Razzaque *et al.*, 2000). This enzyme acts by oxidizing polyphenolic compounds and producing melanoidines (pigments of brown color), thus, degrading and reducing the polyphenolic substances. As the pH increases, the phenolic compounds develop a reddish-brown to black color. The higher the pH, the darker should be the cocoa; thus, cocoa that has undergone alkalization has reduced natural bitterness and enhanced color, making it darker.

### CONCLUSIONS

The roasting and Dutching processes with relatively high levels of aflatoxin contamination may have been effective in reducing the aflatoxin content in cocoa liquors. Moreover, other experimental conditions, such as roasting time/temperature, and alkali type and concentration, could be proven to determine effects on aflatoxin levels without compromising cocoa quality. More research, however, pertaining to the possible effect of this thermal-alkaline process on the toxicity and mutagenic/carcinogenic activity of alkali-treated aflatoxin sub-products needs to be conducted.

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## تجزیه فیزیکی و شیمیایی افلاتوکسین-ب در طی برشته کردن دانه کاکائو و داچینگ (قلیایی کردن حرارتی) کاکائوی مایع

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### چکیده

برای ارزیابی اثر برشته کردن و فرایند داچینگ در پایداری افلاتوکسین-ب (AFB1+AFB2)، تعدادی دانه های کاکائو آلوده به افلاتوکسین با غلظت  $220.7 \text{ ng/g}$  در حرارت  $250^\circ\text{C}$  درجه سانتی گراد به مدت ۱۵ دقیقه برشته شدند. شرایط برشته شدن منجر به کاهش چشمگیر (تا ۷۱٪) محتوی افلاتوکسین شد. مایع کاکائوی به دست آمده که غلظت آلودگی افلاتوکسین آن  $63.9 \text{ ng/g}$  بود تحت فرایند قلیایی-حرارتی قرار داده شد. در این فرایند از هیدروکسید سدیم، پتاسیم، و کلسیم با سه غلظت (۱۰، ۲۰، و  $30 \text{ g/kg}$ ) استفاده شد. اثر این دو متغیر (نوع عامل قلیا و غلظت آن) به صورت فاکتوریل  $3 \times 3$  در طرح تصادفی کامل تجزیه آماری شد. در غلظت  $10 \text{ g/kg}$ ، کاهش افلاتوکسین در تیمارهایی که NaOH و  $\text{Ca(OH)}_2$  به کار رفته بود (۹۴٪ کاهش) در مقایسه با KOH (۸۸٪) موثرتر بود. اما، در غلظتهای بالاتر هر سه ماده شیمیایی اثر تجزیه کنندگی و کاهش دهندگی مشابهی روی افلاتوکسین داشتند (تا ۹۸٪). بر پایه این نتایج، بیشتر کاهش افلاتوکسین در طی برشته کردن به دست آمد و در طی فرایند قلیایی کردن کاهشی موثر و افزون بر آن رخ داد. افزودن این مواد قلیایی کننده به کاکائوی مایع نه تنها خواص فیزیکی-شیمیایی آن را بهبود بخشید بلکه با کاهش مقدار افلاتوکسین کیفیت بهداشتی آن را نیز بالا برد.