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# Effect of cocoa bean drying methods on polycyclic aromatic hydrocarbons contamination in cocoa butter

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## Abstract

Numerous polycyclic aromatic hydrocarbons (PAHs) are carcinogenic, making their presence in foods and the environment a health concern. This study assessed the contamination and or/formation of PAHs in cocoa butter as affected by their cocoa bean drying methods. Three major drying methods were evaluated, including full sundrying, full artificial drying and the drying combination of sundrying followed by artificial drying. Result of the study showed that PAHs contamination in cocoa butter originated mostly from smoke contamination during their bean's drying and small amount intentionally synthesized during fermentation and drying. Longer drying time promoted a higher concentration of PAHs, although still below the maximum limit of 2 ppb. PAHs concentration significantly higher in cocoa butter extracted from whole cocoa bean compared with that extracted from cocoa cotyledon, indicating the contaminant moved from cocoa shell into the bean cotyledon. Uses of artificial dryer with wood as fuel showed a higher risk of contamination compared with the uses of kerosene and industrial diesel oil (IDO). The highest risk of contamination during artificial drying was obtained when there was a leak of gas separator facilitating a contact of firing gas with cocoa beans.

## Introduction

Polycyclic aromatic hydrocarbons (PAHs) are organic compound containing only hydrogen and carbon. Benzo[a]pyrene (BaP) is a PAH that is a pollutant and carcinogen, usually used as marker of the contamination. It can be found in coal tar (after a forest fire), after eruption of volcanoes, in cigarette smoke, and in burnt foods such as coffee. Evidence exists to link benzo[a]pyrane to the formation of lung cancer (IARC, 1987; The European Cocoa Association, 2005). Studies in European countries showed that the main contributors for PAH are smoked fish and meat, cereals and vegetables (DG-SANCO SCOOP, 2006).

A number of PAHs have been shown to be genotoxic carcinogens. In 2002, the Scientific Committee on Food (SCF) reviewed PAH toxicity. For 15 compounds it concluded that there was clear evidence for their toxicity. In view of the non-threshold effects of genotoxic substances the Committee recommended that the levels of PAH in food should be reduced to as low as reasonably achievable (European Union, 2006). Joint FAO/WHO Expert Committee on Food Additives (JECFA) performed a risk assessment on PAHs, basically agreed with the SCF selection, downgraded one substance from the SCF list, and nominated one further compound for observation in food (JECFA, 2006; European Union, 2006). About 660 different compounds belong to the PAH group of which some show carcinogenic properties (Sander and Wise, 1997). The combined list nominated by either SCF or JECFA would thus comprise of 16 substances. Maximum levels of benzo[a]pyrane, used as a marker for PAH contamination, in a range of foodstuffs are now specified in Commission Regulation (EC) 1881/20061. However, there are some uncertainties in relation to the accuracy of benzo[a]pyrane as a general indicator for overall PAH contamination and the selection of food groups specified in the regulation (European Communities, 2005; European Food Safety Authority, 2008).

Scientific Committee on Food, the European Communities (2005) and Standing Committee on Foods (2002) stated that benzo[a]pyrene can be used for marker in the detection of PAH covering benzo[a]anthracene,benzo[b]fluoranthene, benzo[j]fluoranthene,benzo[k]fluoranthene, benzo[g,h,i]-perylene,chrysene,cyclopenta[c,d] pyrene,dibenz[a,h]anthracene,dibenzo[a,e] pyrene,dibenzo[a,h]pyrene,dibenzo[a,i]pyrene, dibenzo[a,l]pyrene,indeno[1,2,3-cd] pyrene and 5-methylchrysene. Benzo[a]pyrene and DMBA[7,12-dimethylbenz[a]anthracene) were also suggested as effective tumor initiator and perfect carcinogen in a serial research on mice skin (DiGiovanni, 1992; Baer-Dubowska, 1997; EFSA/DATEX/002, 2008).

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Cocoa product is one of the foodstuffs having attention to be free or less from maximum limit of PAH contamination. Commission Regulation (EC) No 208/2005 of 4 February 2005 amends Regulation (EC) No 466/2001 as regards to polycyclic aromatic hydrocarbons (PAHs) in food set up oils and fats, intended for direct human consumption or use as an ingredient in foods (excluding cocoa butter until April 1, 2007), have been set up not to exceed 2.0 µg/kg. Within the chocolate manufacturing procedure, there are some critical steps during which cocoa may be contaminated with PAH and as a consequence chocolate may be also contaminated with (Ziegenhals *et al*., 2009).

PAHs are generally a result of the incomplete combustion of organic material and consist of two or more condensed aromatic carbon rings (Smith, 1984). The most critical step is the drying the cocoa beans in their respective country of origin. The contamination of cocoa beans can occur by drying cocoa on asphalt, on bitumen in the sun, or by using direct firing drying model. Furthermore, cocoa beans can be contaminated with PAH during storage and transport in jute or sisal bags that had been treated with batching oil (Grob *et al*., 1993).

However, since 1998, international standards recommend that ingredients of batching oils must be non-toxic and approved for use in packaging materials (Ziegenhals *et al*., 2009). It has also been postulated a possible contamination of cocoa beans by lube andhydraulic oils (DG-SANCO SCOOP, 2006; The European Cocoa Association, 2005; Ziegenhals *et al*., 2009). This research evaluated occurrence of PAH contamination in cocoa bean during their drying which in turn being available to mitigate and prevent the contamination.

## Materials and Methods

### *Cocoa beans preparation*

Cocoa beans were dried as much as possible close to the drying method used by cocoa producers. The experiment was mainly consisted of cocoa bean drying under sun (sundrying), drying in combination of sundrying and artificial drying, and full artificial drying. Cocoa beans used for sundrying experiment were prepared from bulk cocoa type produced from center production areas of West Sulawesi, South Sulawesi and Bali Provinces of Indonesia. Cocoa beans were sundried at different time ranged from 2.5 to 4.5 days, conducted during dry months between May and July. Drying time was around 9 hours per day. The drying was carried out on three types of mate, namely cement, plastic net on cement and plastic net on ground. After sundrying, cocoa samples were then dried in Heraeus Electric Glass Drying Oven (Tokyo, Japan) at temperature of 80$^{o}$C to reach final moisture content of 7.5%.

Other experiments in terms of drying combination method and full artificial drying method were conducted separately in two cocoa estates in East Java, Indonesia. The first experiment was drying of fermented cocoa beans pre-dried with sundrying and then followed with artificial drying. Another experiment was the drying of fermented cocoa beans fully using artificial drying method. These two methods is the common practice in the production of cocoa bean in big plantations, cooperatives or cocoa bean processors.

The drying combination method was performed by spreading cocoa bean on cement, plastic sheet on ground, woven plastic sheet on ground, bamboo mate on ground or woven plastic net on cement with beans layer varied from 2 to 7.5 cm. Drying time varied from 1 to 6 days with 8 – 9 hours drying time per day. Subsequently, the beans resulted (moisture content was 10 – 25%) were dried with artificial dryer to reach moisture content of 7.5%. The types of artificial dryer used were static, vertical, rotary and circular. Four kinds of fuel were evaluated in this research, including firewood, kerosene, IDO (Industrial Diesel Oil) and vegetation waste (branches wood). The dryers used either direct or indirect firing, differentiated by whether their burning gas is come in contact with cocoa beans dried or not. The dryings were run at temperature between 65 to 85$^{o}$C. Experiment on full artificial drying method was conducted in similar way with in the drying combination method, but in the artificial drying was started directly with wet cocoa beans from fermentation. All the drying experiment is described in Table 2.

### *PAHs analytical method*

PAHs concentration were analyzed by using LC-LC coupling on line method according to Stijn (Stijn *et al*., 1996)] in ADM's Laboratory, Amsterdam - Holland. This analytical method performed a trace hold of 0.1 µg/kg and identification of 12 PAH compounds. Cocoa butter was extracted from cocoa bean samples with hydraulic press. The butter was then purified by adding 1 g activated carbon into 20 g cocoa butter, heated for two hours at 60°C whith gentle stirring, then filtered through a pleated filter. Filtrate was passed through a membrane filter of 0.45 µm (PTFE, Millex™-LCR, Millipore) and the resulting purified butter samples were stored at 4°C before analyses. Determination of PAHs was carried out in UltiMate 3000 ×2 Dual system consisting of DPG-3600A pump with SRD-3600 Air Solvent Rack, WPS-3000TSL autosampler, TCC-3200 thermostatic column compartment with two 2p-6p valves, equipped with RF2000 fluorescence detector. Two SUPELCOSIL™ LC-PAH columns, 4.6 × 250 mm (Supelco Cat.# 58229) with On-Line SPE and ChromSpher Pi, 3.0 × 80 mm (Varian P/N: CP28159) column were used for separation. Mobile phases consisted of A. Water, B. Acetonitrile for both loading and analysis pumps and C. Isopropanol for loading pump. Flow rate was adjusted at 1 mL/min, injection volume at 80 µL (100 µL injection loop). Column temperature set at 30°C. Detection was including phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b] fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene, benzo[g,h,i]perylene and indeno[1,2,3-cd]pyrene. Benzo[b]chrysene was used as an internal standard.

## Results and Discussion

### *Absorption of PAH in cocoa beans*

Cocoa butter is the most important and value compound in cocoa beans, constitutes up to 57% in the cocoa bean cotyledon (Lass, 1999). Result of the analysis of Benzo[a]pyrene (BaP) in cocoa butter extracted from whole cocoa beans (Figure 1) showed that all cocoa bean samples dried under the combination of sundrying and artificial drying exceed the maximum limit of 2 ppb required in EU regulation. However, by deshelling these cocoa beans, the concentration of the BaP in their cocoa butter was reduced into maximum of 0.81 ppb. This finding indicated that most PAHs content in cocoa bean originated from outside penetrate into cocoa cotyledon. The PAHs were suggested from smoke produced from wood and or/ fuel burning. Cocoa bean shell effectively absorb the PAHs passing through and contacting with.

These evidences showed a risk for the PAH contamination in cocoa bean cotyledon from the contaminant in their shell. In the practical aspect the shell contamination can give raise the possibility an increase in PAHs concentration in cocoa beans cotyledon through butter (plus PAHs) migration especially during beans roasting and micronizing. The shell debris also may contaminate cocoa cotyledon during deshelling step in the secondary cocoa processing. PAHs are aromatic compounds formed from incomplete burning processes, contained in the crust (tar), asphalt, some pesticides, smoke and exhaust gases. PAHs may be formed in edible oils by pyrolytic processes, such as incomplete combustion of organic substances. PAHs in foods can also result from petrogenic contamination (DIONIX, 1998).

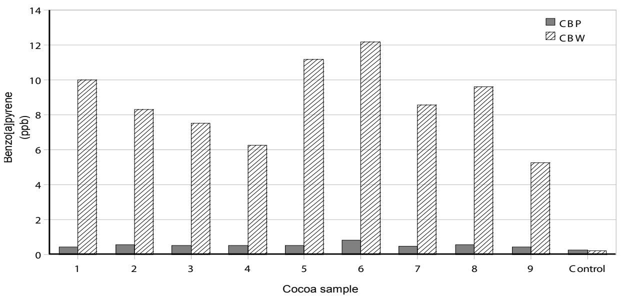

Figure 1. Benzo[a]pyrene concentration in cocoa butter extracted from whole (CBW) and peeled cocoa bean (CBP)

Table 1. Benzo [a] pyrane concentration in cocoa butter extracted from cocoa bean cotyledon prepared with different time and drying mate

| Sundrying time | Drying mate | | | Average |
|---|---|---|---|---|
| | Cement | Plastic net on cement | Plastic net on ground | |
| 2.5 days | 0.33; 0.25<br>χ = 0.29 | No sample | 0.11 | 0.23 |
| 3 days | 0.30; 0.37<br>χ = 0.34 | No sample | 0.20; 0.34; 0.36; 0.39<br>χ = 0.32 | 0.33 |
| 3.5 days | 0.29; 0.15<br>χ = 0.22 | 0.21; 0.22<br>χ = 0.22 | 0.17; 0.38<br>χ = 0.28 | 0.36 |
| 4 days | 0.71 | 0.58 | 0.18; 0.44; 0.55; 0.37<br>χ = 0.38 | 0.47 |
| 4.5 days | No sample | 0.73 | 0.26 | 0.50 |

Note:
Data were collected from West Sulawesi, South Sulawesi and Bali Province smallholders, Indonesia

### *Benzo [a] pyrane in cocoa beans prepared with sundrying*

Result of the PAH contamination analysis represented by benzo [a] pyrene (BaP) in 23 samples of cocoa butter extracted from sundried cocoa beans (Table 1) showed that all contamination less than the maximum limit of 2 ppb. All the cocoa butter contained BaP of less than 1 ppb, 19 of the 23 samples contained BaP <0.5 ppb, although they are still higher than that of Ghanaian cocoa butter with BaP of 0.28 ppb. BaP concentration in cocoa butter samples ranged from 0.11 to 0.71 ppb with average of 0.38 ppb. Roughly, there was no correlation between drying mate and BaP concentration.

All the cocoa beans samples were collected from smallholders who dried their cocoa beans ranged from 2.5 to 4,5 days before sold the bean into cocoa beans village collector. Cocoa collector would follow additional sundrying up to moisture content ca. 11% in which the bean will be then sent to district trader. In this stage, before the bean are deliver to processor (exporter), the beans will be re-dried by using

Table 2. Benzo[a]pyrene concentration in butter of cocoa bean cotyledon dried with artificial drying and its combination with sundrying

| Sundrying | | | | Artificial drying | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Drying base | Duration, day | Bean layer, cm | Moisture content at the end of drying/start of artificial drying, % | Type of dryer | Heating | Fuel | Temperature, °C | Duration, hour | BaP (ppb) |
| **Combination of Sundrying and Artificial Drying** | | | | | | | | | |
| Cement | 1 | 5 | 20.0 | tunnel | indirect | firewood | 70 | 16 | 0.45 |
| Cement | 1 | 5 | 20.0 | tunnel | indirect | firewood | 70 | 16 | 0.55 |
| Cement | 1 | 7.5 | 25.0 | tunnel | indirect | firewood | 85 | 28 | 0.54 |
| Cement | 1 | 7.5 | 25.0 | tunnel | Indirect | firewood | 85 | 24 | 0.51 |
| Cement | 1 | 3 | 20.0 | tunnel | Indirect | firewood | 70 | 11 | 0.54 |
| Cement | 1 | 7.5 | 25.0 | tunnel | Indirect | firewood | 70 | 20 | 0.81 |
| Cement | 1 | 7.5 | 25.0 | tunnel | Indirect | firewood | 70 | 22 | 0.47 |
| Cement | 1 | 2.5 | 20.0 | tunnel | Indirect | firewood | 80 | 20 | 0.59 |
| Plastic sheet | 1 | 2.5 | 20.0 | tunnel | Indirect | firewood | 80 | 20 | 0.45 |
| Woven plastic net | 6 | 2 | 18.0 | static | Static | kerosene | 70 | 5 | 0.35 |
| Bamboo mate | 3 | 2.5 | 16.0 | static | Static | firewood | 70 | 5.5 | 0.36 |
| Woven plastic net | 1 | 3 | 18.0 | static | Static | kerosene | 75 | 1.5 | 0.45 |
| Cement | 1 | 7.5 | 17.0 | static | Static | kerosene | 75 | 1.5 | 0.41 |
| Cement + woven plastic net | 1 | 5 | 15.0 | static | Static | kerosene | 75 | 3 | 0.38 |
| Cement | 1 | 5 | 16.0 | static | Static | kerosene | 75 | 3 | 0.22 |
| Cement | 1.5 | 6.5 | 15.0 | static | Direct | kerosene | 75 | 72 | 0.36 |
| Woven plastic net | 1.5 | 3 | 10.0 | static | Static | kerosene | 75 | 3.5 | 0.35 |
| **Full Artificial Drying** | | | | | | | | | |
| | | | Start from wet bean | static | Indirect | firewood | 65 | 27 | 1.13 |
| | | | Start from wet bean | static | Indirect | firewood | 65 | 27 | 1.18 |
| | | | Start from wet bean | vertical | Direct | IDO | 80 | 8 | 0.51 |
| | | | Start from wet bean | rotary | Direct | IDO | 75 | 22 | 0.25 |
| | | | Start from wet bean | circular | Indirect | vegetation wastes | 65 | 38 | 0.39 |
| | | | Start from wet bean | circular | Indirect | vegetation wastes | 65 | 38 | 0.38 |
| | | | Start from wet bean | circular | Indirect | vegetation wastes | 65 | 38 | 0.35 |
| | | | Start from wet bean | circular + rotary | Indirect | IDO | 65 | 36 | 0.34 |
| | | | Start from wet bean | circular + rotary | Indirect | IDO | 65 | 36 | 0.32 |
| | | | Start from wet bean | circular + rotary | Indirect | IDO | 65 | 36 | 0.36 |
| | | | Start from wet bean | circular + rotary | Indirect | IDO | 65 | 36 | 0.30 |
| Control (fermented cocoa bean dried in electric oven, n = 8) | | | | | | | | Average :<br>SD :<br>Max – Min : | 0.26<br>0.08<br>0.15 – 0.42 |

Note: M.c.: moisture content; IDO: Industrial diesel oil; NA: not analyzed; SD: standard of deviation

sundrying or artificial drying into moisture content of ca. 7.5% and mixed with other cocoa beans origin.

The data obtained also showed a trend of the longer sundrying the higher BaP concentration in the cocoa butter. Despite this trend still needs further deep research, the evidence offers hypothesis that PAHs may formed inside the bean derived from intrinsic compound. This was also confirmed by the presence of BaP in full sundried of Ghanaian cocoa bean used as control. PAH compounds shows similarity structure with phynolic compounds abundance in cocoa bean cotyledon, thus there is a possibility that the PAH compounds may be derived from the phynolic compound, especially during cocoa beans drying and fermentation. There are 35 parent compounds of PAH with ring numbers of 1 to 11 and molecule weight of 78 – 478 (Sander and Wise, 1997). Some of the parent compound showed similar structure with phynolic compound derivates present in cocoa bean, such as phenanthrene, anthracene chrysene, picene, pentaphene and pentacene as shown by their benzene basic structure. PAHs also occur in charbroiled and dried foods, and may form in edible oils by pyrolytic processes, such as incomplete combustion of organic substances (DIONIX, 1998).

Cocoa bean is rich in polyphenols. Cocoa and its derived products (cocoa powder, cocoa liquor and chocolates) contain varied polyphenol concentration. The polyphenols in cocoa beans contribute to about 12-18% of the dry weight of the whole bean. Main classes of polyphenolic compounds identified are such as simple phenols, benzoquinones, phenolic acids, acetophenones, phenylacetic acids, hydroxycinnamic acids, phenylpropenes, coumarines, chromones, naphtoquinones, xanthones, stilbenes, anthraquinones, flavonoids, lignans and lignins (Hii *et al*., 2009; Misnawi *et al*., 2002, 2003; Kim and Keeney, 1984). Three groups of polyphenol can be distinguished in cocoa beans i.e. catechins or flavan-3-ols ca. 37%, anthocyanins ca. 4% and proanthocyanidins ca. 58%. Oxidation of these compounds produced reactive compounds which in turn are hypnotized to produce numbers of derivate and possibly contribute to the rise in PAH concentration in dried cocoa beans (Wollgast and Anklam, 2000). Enzymatic oxidation of cocoa bean polyphenol by polyphenol oxidase in the presence of oxygen produces quinone where is very reactive agents and can react further with amino acids and proteins into dark colored covalent bond complex (Kattenberg and Kemmink, 1993).

The occurrence of defective smoke smell in over fermented and or/ abnormal fermented cocoa bean due to the excessive content of mono-phenolic compounds supported the suggestion that PAH compounds could be produced during drying and fermentation. Data analysis of this research obtained PAH compounds was found in all cocoa bean samples including those of dried by using electric oven i.e. at 0.26 ppb.

### *Effect of artificial drying*

Artificial drying with fuel of fossil oil and wood (firewood and vegetation waste) showed high risk for PAH contamination in cocoa beans, especially when the drying uses direct heating system. Smoke from incomplete firing can contact cocoa bean surface and diffuse into the beans. It was suggested that PAHs contaminate foods during their heating and drying process, in which the firing gas products directly contact with the material being heated or dried (Wollgast and Anklam , 2000).

Data Analysis in Table 2 showed that cocoa beans dried by using combination of sundrying and artificial drying contain BaP at lower concentration compared with those of from full artificial drying. BaP concentration in cocoa butter extracted from the drying combination ranged from 0.22 to 0.81 ppb while that of from full artificial drying ranged from 0.25 to 1.18 ppb. BaP concentration of control treatment ranged from 0.15 to 0.42 ppb. Ghanaian cocoa bean which was dried fully under sun produced cocoa butter with BaP concentration of 0.28 ppb. All the BaP concentration in cocoa butter was still below the maximum limit of 2 ppb. These findings clearly indicated that artificial drying shows a higher risk in PAHs contamination compared with sundrying.

Considering that the occurrence of PAHs contamination most are due to contact with incomplete combustion (smoke) and petroleum such as kerosene and diesel oil, it was suggested that highest risk of PAHs contamination was in the beans dried on direct heating dryer type. Nevertheless, there was no significant different of BaP concentration in these two heating methods obtained in this research. Observation during experiment obtained that in the direct firing method in which kerosene or IDO was used, the drying was started when the combustion had run perfect, indicated by high temperature and limited smoke.

On the other hand, although drying were using indirect firing method with woods as fuel, the cocoa beans produced could reach high BaP concentration in its cocoa butter (1.13 and 1.18 ppb). Leaking of the wall separating firing gas and cocoa bean was suggested to facilitate the contamination, and the deficient burning of woods was suggested resulting intense smoke which in turn contacted cocoa bean shell and penetrated into bean cotyledon. These findings clearly showed that the uses of artificial dryer with wood as fuel showed a higher risk of contamination compared with uses of kerosene and IDO. The highest risk of contamination during artificial drying was obtained when there was a leak of wall facilitating contact of firing gas with cocoa beans.

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