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Potential Uses of Cocoa Bean Infested by *Conopomorpha cramerella* for Polyphenol Extraction

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Abstract: Cocoa pod borer *(Conopomorpha cramerella (*Snellen)*)* is the main fatal pest that destroys cocoa plantations in South East Asia, mainly in Indonesia and Malaysia. Infested cocoa beans stick to each other, the pulp become hard and normal fermentation process to produce flavour precursors cannot be done. This research aimed to utilize the infested cocoa beans as a source of phenolic compounds. Extraction of phenolic compounds was carried out for three infestation levels regarded as low, medium and heavy. Parameters of study were bean size, shell content, fat content, total polyphenol and antioxidant activity. Results of the study showed that the increase in cocoa pod borer infestation significantly decreased cocoa bean size, fat content and total polyphenol; but shell content was increased. Antioxidant activity of the extracted polyphenol was not significantly affected by the infestation. The results clearly indicate that cocoa beans infested by cocoa pod borer can potentially to be used as a source of phenolic compounds for natural antioxidant uses.

Keywords: Cocoa, *Conopomorpha cramerella*, polyphenol, antioxidant, extraction, cocoa butter, bean size, shell content

INTRODUCTION

Indonesia is the third largest producer of cocoa beans after Ivory Coast and Ghana, producing more than 450,000 MT annually. The occurrence of cocoa pod borer (CPB), *Conopomorpha cramerella* (Snellen) disease on cocoa plantations in Malaysia, Indonesia and Philippine, which started in the 1980's, has caused a serious problem in cocoa production. CPB infestation is the main limiting factor in improving the quality and quantity of production in Indonesia. Wiryadiputra (1993) and Prawoto *et al.* (1993) reported that CPB spoilage could cause a failure of the extension and intensification program launched by the government of Indonesia. Indonesian Cocoa Association (INCA) suggested that CPB has caused a difficulty in quality improvement, and the waste of cocoa bean from endemic areas gradually increases.

Larva of *C. cramarella,* aged over two months, enter cocoa pod and feed on the pods for 15 – 19 days. The young CPB larva enters the cocoa pod through a small hole and destroys the cocoa bean pulp, placenta and cotyledon. At the end of larva stadium, the young insect will then make another hole and exit the cocoa pod for their pupa stadium (Sulistyowati and Sulistyowati, 2003). Financial depression due to CPB attacks on Indonesian cocoa business during 1993 was calculated to reach Rp. 23.9 billion (USD2.4m) a year; in which the depression was suggested to strongly correlate with the quality and quantity injuries (Madry, 1994). An evaluation by the

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Indonesian Coffee and Cocoa Research Institute (ICCRI) through 2004 suggested that CPB had damaged most of the centre cocoa production areas in Indonesia. Wardoyo (1980) and Wiryadiputra *et al.* (1994) calculated the damaged cocoa beans by CPB to reach 80%.

Currently, no effective and economic method has been found to control the CPB infestation. Utilization of certain chemical, which is the last choice in the recommended integrated pest management (IPM), could effectively control the CPB infestation, although the young larva directly enters the cocoa pod and stays inside for around two weeks (Wiryadiputra, 1993; Prawoto *et al.*, 1993). Such insecticides kill eggs, adults and larvae which just out from the pod for pupation. Infested cocoa beans are stuck one to each other, their pulp become hard and contain less sugar. The bean size does not develop to the normal size. Fermentation stage needed for development of cocoa flavour precursors could not be carried out normally. The infested cocoa beans do not produce any cocoa aroma and chocolate flavour during roasting and other secondary processing; however flavour defects such as mouldy, rancid, hammy and earthy frequently occurred.

On the other hand, researches by Misnawi *et al.* (2002a, b) and Kim and Keeney (1984) found that cocoa beans are rich in polyphenols. Unfermented cocoa beans contain polyphenolic compounds of 120–180 g kg^{-1}. According to Wollgast and Anklam (2000a) three groups of polyphenol can be distinguished in cocoa beans which are. catechins or flavan-3-ols ca.37%, anthocyanins ca. 4% and proanthocyanidins ca. 58%.

Recently, cocoa bean polyphenols have gained much attention owing to their antioxidant activity and possible beneficial implications on human health, such as treatments and preventions of cancers, cardiovascular diseases and other pathologies (Kattenberg, 2000; Wollgast and Anklam, 2000b). Experiments using *in vitro* and animal models demonstrated that most of the cocoa bean polyphenol fractions were found to have antioxidant activities and of health benefits, such as in the inhibition of hydrogen peroxide and superoxide anion, protection against lipid peroxidation and deterioration, antiulceric, inhibition of oxidative stress and reduction of low density lipoprotein (LDL) oxidative cardiovascular disease, antimutagenic, inhibition of tumour development and carcinogenic, and antimicrobial (Kattenberg, 2000; Osakabe *et al.*, 2000, 1998a, 1998b; Sanbongi *et al.*, 1998). Ziegleder and Sandmeier (1983) stated that the strong antioxidant effects of cocoa polyphenols are due to its phenolic constituents, which differs from other classical antioxidants which are very low in fat solubility. Cocoa polyphenols with epicatechin base are effective in heterogeneous systems and even in oil-water emulsion. This research aimed to study possible utilization of CPB infested cocoa bean as the source of polyphenolic compounds.

MATERIALS AND METHODS

Cocoa Beans

Cocoa beans infested by CPB were collected from an estate in East Java for three levels of infestation according to the method of Sulistyowati and Sulistyowati (1993). Cocoa beans of low, medium and heavy infestation levels were collected from infested cocoa pods containing less than 10%, 11 – 50%, and greater–than 50%, stuck coffee beans respectively. Sticky bean is defined as beans which stick one to each other and could not be separated manually. Cocoa beans were then dried in an electric oven at 55°C up to moisture content of 7%.

Dried Bean per Pod

Dried cocoa beans per pod was measured by extracting, drying (at 55°C) and weighing the beans of healthy pods as control and CPB infested podsfor the three levels of infestation.

Shell and Cotyledon Contents

Shell and cotyledon contents were measured by peeling 100 g dried cocoa bean. Their fractions were then weighted and calculated in percentage (w/w).

Fat Content Analysis and Defatting

Fat content was analyzed using petroleum (40 – 60°C) extraction in soxhlet apparatus for 16 circulations. Fat content was calculated on a wet basis at 7% moisture content. The same extraction procedure was used for preparation of defatted cocoa powder for phenolic compounds and antioxidant activity analysis.

Total Polyphenol

Total polyphenol was determined spectrophotometrically using the method of (Shamsuddin and Dimmick, 1986). Five hundred milligram of defatted cocoa powder and 80 ml of 80% aqueous acetone were placed in a 125 ml conical flask and the mixture was sonicated in a Sonicor C-125 (Sonicor Inst., New York, USA) for 30 min. During sonication, the extraction mixture was kept cold by filling the sonicator vessel with ice water. Sonication was preferred over shearing as an aid in solubilizing polyphenol, since shearing promotes browning of the polyphenol extract by oxidation.

A clear extract was obtained by vacuum filtration of the mixture through Whatman no. 1 filter paper. The residue and all glass wares were washed with 80% aqueous acetone and total volume of the extract was made up to 100 ml in a volumetric flask. One milliliter of the extract was pipetted into a 100 ml volumetric flask and diluted with 70 ml of distilled water. The extracted polyphenol was then reacted with 5 ml of 2 N Folin-Ciocalteau's reagent for 2 min. Then 15 ml of saturated Na_2CO_3 solution was added to stabilize the color formed. The blue color was allowed to develop for at least 2 hr and its absorbance was measured at 765 nm. (–)-Epicatechin standard of nine known concentrations of 1 to 9 mg L^{-1} was used for calculation.

Antioxidant Capacity

Antioxidant capacity was measured according to DPPH (1,1 diphenyl 2-picrylhydrazyl) method (Molyneux, 2004). Dried cocoa phenolic compound from methanol extraction was diluted in 20 mL of absolute ethanol, stirred for 10 min and then centrifuged at 5,000 rpm for 3 min. One milliliter of supernatant was collected and added with 0.5 mL of 1 M DPPH, and then allowed to stand for 20 min. The solution was then brought to 5 mL by absolute ethanol dilution. The absorbance was measured at wavelength of 517 nm and compared with blank.

RESULTS AND DISCUSSION

Cocoa Yields

Cocoa pod borer (CPB) infestation significantly decreased extractable beans from the pod. A healthy cocoa pod produced an average of 50 g dried cocoa beans, while low, medium and heavy CPB infested cocoa pods produced 33, 20 and 16 g, respectively (Figure 1). Analysis of shell content (Figure 2) showed that the CPB infestation also decreased cotyledon content from 85% in cocoa bean extracted from healthy cocoa pods to 45% in that extracted from heavily infested pods. These results clearly indicate that larva of CPB feed on the placenta of the cocoa beans. As a result nutrient supply to the bean is interrupted and the bean does not develop normally. Sulistyowati and Sulistyowati (2003) stated that young larva of *C. cramarella* enter cocoa pod and stay inside for several days, destroy and eat the soft parts of pod, pulp and placenta. Sometimes, they also reach cotyledons and eat them before exiting the beans for their cocoon stage.

Losses in cocoa yield were confirmed by the increase in waste content in terms of shell and clamp bean arising from Indonesian cocoa beans. This condition has resulted in a significant decrease in quality of cocoa bean from main cocoa production areas in

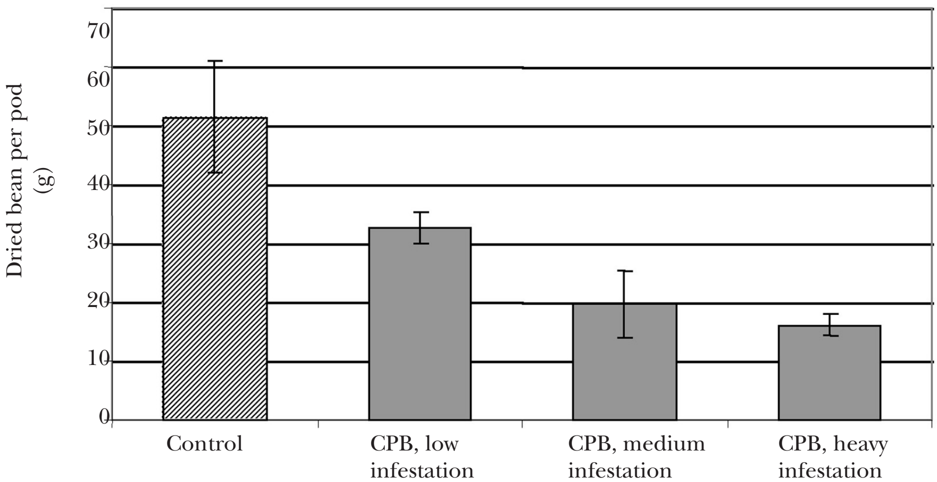

Figure 1: Dried cocoa bean in healthy and CPB infested pods

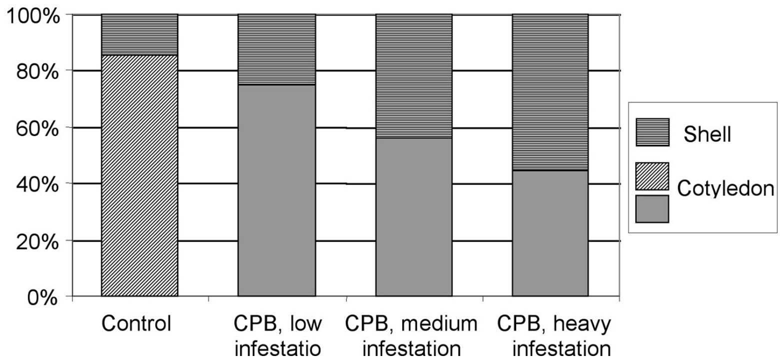

Figure 2: Shell and cotyledon contents of cocoa beans extracted from healthy and CPB infested pods

Indonesia such as in South and South East Sulawesi. According to Zulhefi (2005) the difficulty faced by smallholders to produce cocoa beans conforming to the National Standard (SNI 01-2323-2002) is due to the problem with high waste content. Normal fermentation could also not be applied to the CPB infested cocoa beans. Certain buyers of Indonesian smallholder cocoa bean discount the price. These facts indicate that the CPB infestation seriously diminishes the quality and quantity of cocoa bean as well as the profitability of the cocoa bean industry.

Polyphenol Content and Antioxidant Capacity

Extraction of phenolic compounds thought to be beneficial to humans can be derived from CPB infested cocoa beans. Researchers have shown that polyphenols of cocoa bean has beneficial effects on health (Wollgast and Anklam, 2000b). Result of this study showed that free fat unfermented cocoa bean powder extracted from healthy pods contains 174 g kg^{-1} phenolic compounds (Figure 3). This concentration is comparable to the concentration of other sources such as in tea and wine. Cocoa powder contains much higher levels of polyphenol and relative total

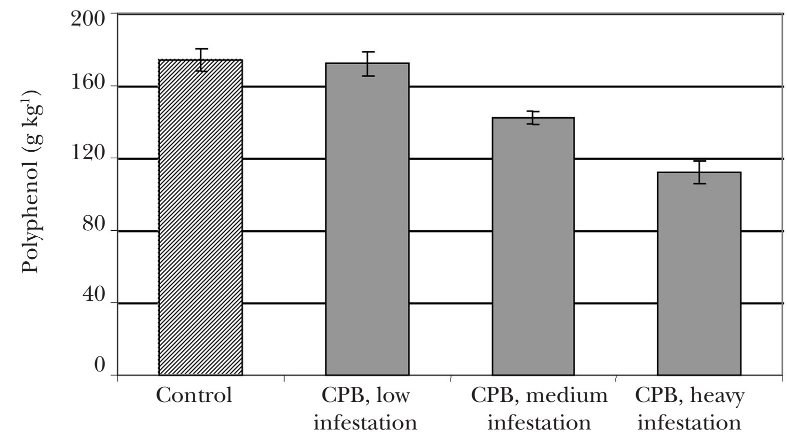

Figure 3: Polyphenol content of cocoa beans extracted from healthy and CPB infested pods

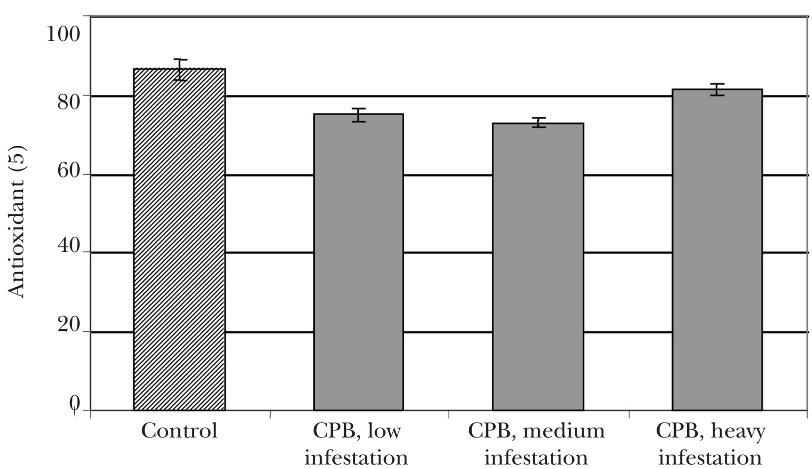

Figure 4: Antioxidant activity of phenolic compound extracted from cocoa beans of healthy and CPB infested pods

antioxidant capacities per in the order cocoa > red wine > green tea > black tea (Lee *et al.*, 2003).

CPB infestation significantly reduced polyphenol concentration. Nevertheless, the concentration in low CPB infested beans was still not significantly different from that of healthy beans. Decreasing plyphenol concentration in medium and heavy CPB infested beans were 18% and 36%, respectively. However, a concentration of 112 g kg^{-1} in heavy CPB infested beans is still considered a high and significant concentration of polyphenol extraction. Polyphenol concentration in white grapes, pink grapes and carrots were 7.9, 6.8 and 14.8 g kg^{-1} of dried mass, respectively (Cieslik *et al.*, 2006).

Antioxidant capacity of polyphenol extracted from cocoa beans of both healthy and CPB infested pods showed a level of more than 70% (Figure 4). Antioxidant capacity of phenolic compounds from the heavy CPB infested cocoa beans decreased by only 6% compared to that from healthy cocoa beans,

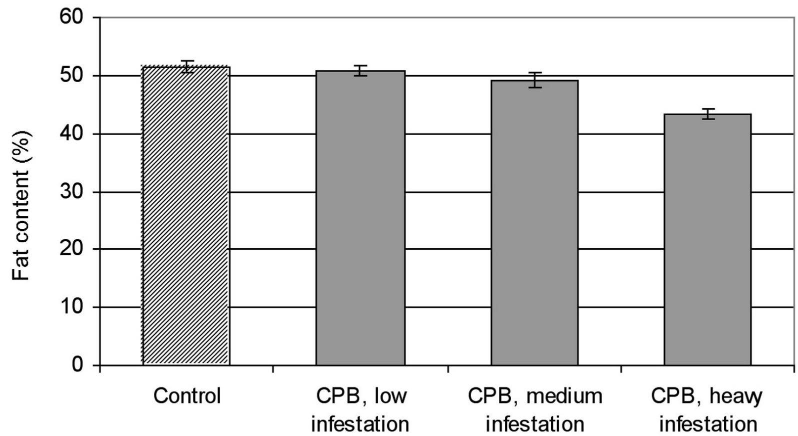

Figure 5: Extracted fat content of cocoa beans from healthy and CPB infested pods

meanwhile that from low and medium CPB infested cocoa beans decreased by 13% and 16%, respectively. Lower antioxidant capacity of phenolic compounds from cocoa beans infested by CPB at low and medium levels compared with that from heavily infested ones was suggested to relate with the level of oxidation occurring in their polyphenols. The low and medium infested beans are physiologically mature. Indigenous enzymes including polyphenol oxidase are suggested to occur in all the beans.

Damage to cotyledons by the action of *C. cramarella* larva causes polyphenol oxidase contacting that substrate and resulting in polyphenol oxidation. Misnawi *et al.* (2002a) found that cocoa bean polyphenol oxidase is very active even in dried cocoa beans and the oxidation of phenolic compounds would occur as soon as the enzyme and substrate come in contact. Due to the oxidation, cocoa polyphenol would lose its capacity to act as an antioxidant. Furthermore, a part of the oxidation product would interact and condense with protein to become less reactive compounds though a process called tanning. Heavy infestation of CPB occurs at an earlier stage. Compounds synthesis is thought to be incomplete at this stage. Polyphenol oxidase was in a very limited concentration and most probably in its precursor stage, thus phenolic compound oxidation was low. However, the incomplete phenolic compounds synthesis was also suggested to occur in these beans as shown by its low phenolic concentration (Figure 3).

Fat Content

Heavy CPB infested cocoa beans contained lower fat compared with the content in healthy beans and the other two infested levels (Figure 5). However, the results suggest that utilization of CPB infested cocoa beans for polyphenol extraction could produce high amounts of cocoa butter as a by-product. It is becoming an economic consideration to use CPB infested cocoa beans as a source of phenolic compounds. Fat content in cocoa beans extracted from healthy pods and low, medium and heavy CPB infested pods at moisture content of 7% were 51.5%, 50.8%, 49.2% and 43.4%, respectively. These concentrations are equivalent to 55.4%, 54.6%, 52.9% and 46.7%, respectively on a dry basis.

Cocoa butter fat is the most valuable of cocoa fractions. However further characterization of cocoa butter from CPB infested cocoa beans need to be carried out. Lower fat content in the heavily infested cocoa beans is suggested to be caused by the incomplete phase of fat syntheses. It is proposed that CPB infestation occurrs in the early stage of cocoa development.

CONCLUSION

Cocoa beans infested by *Conopomorpha cramerella* Snellen has a potential for use as a source of phenolic compounds due to their high phenolic compound content, antioxidant capacity and fat content. This effort will not only improve economic value of the infested cocoa beans, but can also be a way to cut down CPB life circle as a disease control measure.

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