

Antioxidant Activity and Phenolic Content of Different Pod Tissues of Five Selected Cocoa Hybrid Lines

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ABSTRACT

The antioxidant activities of the beans, bean husk and pod husk of five selected cocoa hybrid lines (*viz.* 1, 6, 14, 26 and 71) were determined using Ferric Reducing Ability of Plasma (FRAP) assay. The total phenolic contents were also examined by Folin-Ciocalteu method.

The FRAP values and total phenolic contents were significantly higher in bean husk and pod husk than in beans of all selected cocoa hybrid lines. Significantly higher FRAP values were observed in bean (38.43 ± 1.49 mg TE/g DW), bean husk (89.33 ± 6.77 mg TE/g DW) and pod husk (115.88 ± 5.25 mg TE/g DW) of hybrid line 71 than other selected hybrid lines. Bean of cocoa hybrid line 1 had the highest total phenolics (26.44 ± 0.35 mg GAE/g DW) whereas significantly higher total phenolic content in bean husk (71.20 ± 8.78 mg GAE/g DW) and pod husk (74.12 ± 2.09 mg GAE/g DW) of hybrid line 71 were observed than other selected hybrid lines. Linear relationships in bean ($R^2 = 0.87, p < 0.001$), bean husk ($R^2 = 0.88, p < 0.001$) and pod husk ($R^2 = 0.87, p < 0.001$) were attained between FRAP values and total phenolic contents.

The results indicated that, among all selected cocoa hybrid lines, the highest FRAP values and total phenolic contents were observed in all pod tissues (bean husk, pod husk and bean) of hybrid line 71 except total phenolic content in beans.

Keywords: Antioxidant activity, Different tissues of pods, FRAP assay, Phenolic content, *Theobroma cacao*.

INTRODUCTION

Cocoa (*Theobroma cacao* L.) is a native species of tropical humid forest on the lower eastern equatorial slopes of the Andes in South America (Cheesman, 1944), belonging to the genus *Theobroma* in the family Sterculiaceae. Cocoa is an industrially important crop since cocoa bean is the constituents of chocolate which is one of the most popular foods in the world. During the manufacturing process of chocolate, the nib portion of peeled-off cocoa bean is used as the raw material in making chocolate or cocoa powder, while its bean husk is generated as a waste (Kim *et al.*, 2004). Cocoa is cultivated on around 6.9 million ha worldwide. Ivory Coast is the largest producer of cocoa accounting for almost 37.4% of world production (Anon, 2011).

Cocoa cultivation gradually spread southward through the East Indies, and ultimately also to Sri Lanka in 1798 (Nair *et al.*, 2005). Cocoa is now becoming an important export agricultural crop and is widely grown as an intercrop under coconut in Sri Lanka and it has a great demand in the local and export market. In 2009, the total cultivated extent of cocoa was 2,521 ha. The annual production of cocoa was about 2,453 Mt in 2009 (Department of Export Agriculture, 2010). Cocoa fruit (cocoa pod) varies in size, shape, external color and appearance among its varieties. The mature pod consists of thick walled pod husk and beans that comprise an outer seed coat (bean husk) together with the mucilaginous whitish pulp surrounding it and an inner

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embryo and cotyledons known as nibs (Thompson *et al.*, 2001).

Polyphenols have received considerable attention because of their physiological functions, including antioxidant (Othman *et al.*, 2007), antimutagenic and antitumour activities (Kono *et al.*, 1995; Saliva *et al.*, 1991). Cocoa beans are known to be rich in polyphenols (Wollgast and Anklam, 2000). It is also one of the richest naturally occurring sources of antioxidants. Indeed cocoa products contain greater antioxidant capacity and greater amounts of flavonoids per serving than either tea or red wine (Lee *et al.*, 2003). Recently, it has been revealed that various biological and health-beneficial effects, such as anticaries (i.e. anti-tooth decay), antioxidant (Hatano *et al.*, 2002), and immunoregulatory activities (Sanbongi *et al.*, 1997), are possessed by polyphenols contained in cocoa.

Cocoa bean husks, a waste product from the chocolate industry, could offer a valuable source of dietary fibre for the low-calorie food segment. In addition to being rich in both soluble and insoluble fibre, the bean husks also contain antioxidant compounds that open up possibilities for health and preservatives. The antioxidant capacity of this cocoa fibre and its physico-chemical properties make it a suitable product to be used in the preparation of low-calorie, high-fibre foods like chocolate cookies, chocolate cakes, dietetic chocolate supplements, etc. where the colour and flavour of this cocoa fibre might be advantageous (Lecumberri *et al.*, 2007). Cocoa pod husk can be processed into potash fertilizer, animal feed or animal feed supplement, caustic potash for soft soap production and for production of alcohols (Sukha, 2003).

Sri Lanka is one of the cocoa producing countries with high quality. Currently most of cultivated varieties in Sri Lanka are traditional varieties (*viz.* Criollo,

Forastero and Trinitario) and their proclivity has not met with increasing demand. Therefore, aiming to improve yield, quality and flavor, new hybrid lines have been developed by the Department of Export Agriculture, Matale, Sri Lanka.

Though these new hybrid lines of cocoa are not currently in commercial use they are likely to be introduced in the near future. Besides yield, flavor and quality, information on contents of bioactive compounds and antioxidant activity of cocoa beans would be additional information to be considered when promoting the consumption of cocoa beans. However, there are no information on antioxidant activity and phenolic content in these hybrid lines. Therefore, the aim of this study was to determine the antioxidant activities and phenolic contents of five selected hybrid lines. In addition, the correlation between antioxidant activity and phenolic content was also determined.

MATERIALS AND METHODS

Location

The experiment was carried out in the laboratory of the Department of Plantation Management, Wayamba University of Sri Lanka, Makandura, Gonawila (NWP) from March to July 2011.

Sample Preparation

New hybrids of cocoa have been developed by the Department of Export Agriculture, Matale; and under their breeding program, open pollinated F1 generation of SCA6 × ICS6 crosses are maintained as hybrid lines at Betel Research Station, Narammala. Five of these cocoa hybrids lines (*viz.* 1, 6, 14, 26, and 71) were selected for this study. The well ripened cocoa pods of each line were harvested on May 02, 2011 and directly transported to the laboratory. Harvested pods were broken to extract the beans, which were then fermented for a period of

five days using the method of basket fermentation. After fermentation, washed beans were dried using an oven for 18 hr at 60°C. The pod husk was chopped into small pieces, which were dried using an oven for 18 hr at 60°C. Dried cocoa beans were manually de-shelled before grinding. Bean nibs, bean husk, and pod husk were separately ground into powder using a grinder. Ground powder were sieved using a 1 mm sieve and packed in polythene bags. Samples were stored in a refrigerator at 4°C. A complete randomized design (CRD) with three replicates was used in the experiment.

Chemicals and Reagents

Folin-ciocalteu reagent, Gallic acid, 2,4,6-tripyridyl-2-try-azine (TPTZ), 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (Trolox) and Ferric chloride ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) were purchased from Sigma Aldrich Chemical Co. (St. Louis, Mo). All other chemicals used were of analytical grade.

Preparation of Extracts

All cocoa samples were extracted and analyzed in triplicate. Cocoa bean samples were extracted by modifying previously published methods (Jonfia-Essien *et al.*, 2008). Ground cocoa beans (0.5 g) were weighed into 15 ml Teflon centrifuge tubes, and 5 ml of the extraction solvent (methanol/water; 80:20) was added. The samples were vortexed for 3 min and centrifuged at 5,000 rpm for 10 min. After clarification by centrifugation, supernatants were decanted into 15 ml test tubes and the cocoa bean sample pellets were reprocessed once with 5 ml of extraction solvent. Two supernatants were pooled and stored at -20°C until determination of total phenolics and antioxidant activity.

Phytochemicals of cocoa bean husk and pod husk samples were extracted according to the extraction method

described by Kim *et al.* (2004). Briefly, 0.5 g of ground cocoa samples were extracted with 5 ml of the extraction solvent (acetone/water; 50:50) and vortexed for 1 min. The extraction mixture was then placed in a water bath at 60°C for 4 hr. After centrifugation at 5,000 rpm for 10 min, the supernatant was decanted and stored at -20°C until analysis. The total extraction process was done two times.

Determination of Antioxidant Activity

The procedure described by Benzie and Strain (1996) was followed. The principle of this method is based on the reduction of a ferric-tripyridyltriazine complex to its ferrous, colored form in the presence of antioxidants. Briefly, the freshly prepared FRAP reagent contained 2.5 ml of a 10 mmol/l TPTZ solution in 40 mmol/l HCl plus 2.5 ml of 20 mmol/l FeCl_3 and 25 ml of 300 mol/l acetate buffer, pH 3.6. Aliquots of 100 μl sample supernatant were mixed with 900 μl of Ferric Reducing Ability of Plasma (FRAP) reagent and the absorbance of reaction mixture at 593 nm was measured spectrophotometrically after incubation for 4 min. The trolox was used as the standard solution. The final result was expressed as the concentration of antioxidants having a ferric reducing ability equivalent to that of trolox (TE) mg/g dry weight (DW) of cocoa samples. Adequate dilution was needed if the FRAP value measured was over the linear range of standard curve.

Determination of Total Phenolic Content

The total phenolic content of cocoa extracts was determined using the Folin-ciocalteu colorimetric method as modified by Abeysinghe *et al.* (2007). A 0.5 ml aliquot of the extract was mixed with 4 ml of distilled water and subsequently with 0.5 ml of Folin-ciocalteu reagent. After 3 min, 1ml of a saturated sodium carbonate solution was added and samples were incubated in a

water bath for 2 hr at 30 °C. The samples were then read at 760 nm against a prepared blank with a spectrophotometer (Shimadzu, UV Mini 1240, Japan) and compared with a known concentration range of gallic acid standards similarly prepared. All results were expressed as milligrams of gallic acid equivalents (GAE) per gram dry weight (DW) of cocoa.

Statistical Analysis

The results of this investigation are means of three measurements. To verify the statistical significance of all parameters the values of means \pm S.D. were calculated. To identify differences among means, analysis of variance (ANOVA) using the general linear model (GLM) procedure of SAS, followed by Duncan's multiple-range test (SAS Institute, 1999) were conducted. Linear regressions were also calculated. Correlation coefficient (R^2) and P-value were used to show correlations and their significance. The P values of < 0.05 were adopted as statistically significant.

RESULTS AND DISCUSSION

Antioxidant Activity

Antioxidant activities of cocoa extracts were determined by FRAP assay. The FRAP values varied significantly among different tissues of the selected five hybrid lines of cocoa pods (Table. 1).

The FRAP values were significantly higher in bean husk and pod husk than bean of all selected cocoa hybrid lines. As far as bean husk and pod husk were concerned, cocoa hybrid line 71 had significant high FRAP values, 89.33 ± 6.77 mg TE/g DW and 115.88 ± 5.25 mg TE/g DW, respectively. The lowest FRAP value of bean husk (59.8 ± 1.79 mg TE/g DW) was observed in cocoa hybrid line 14, whereas hybrid line 1 had the lowest FRAP value in pod husk (66.73 ± 3.83 mg TE/g DW) among all selected cocoa hybrid lines. In cocoa bean, FRAP values ranged from 23.00 ± 2.56 mg TE/g DW to 38.43 ± 1.49 mg TE/g DW with highest FRAP value in cocoa hybrid line 71.

Table 1. FRAP values of bean, bean husk and pod husk of five cocoa hybrid lines

Cocoa Hybrid line	FRAP value (mg TE/g DW)		
	Bean	Bean husk	Pod husk
1	37.55 ± 1.59^f	70.47 ± 5.89^{cd}	66.73 ± 3.83^{de}
6	23.71 ± 2.01^g	60.94 ± 4.51^e	73.11 ± 9.56^{cd}
14	23.00 ± 2.56^g	59.80 ± 1.79^e	76.59 ± 4.16^c
26	30.38 ± 0.90^{fg}	67.41 ± 2.71^{cde}	71.66 ± 9.54^{cd}
71	38.43 ± 1.49^f	89.33 ± 6.77^b	115.88 ± 5.25^a

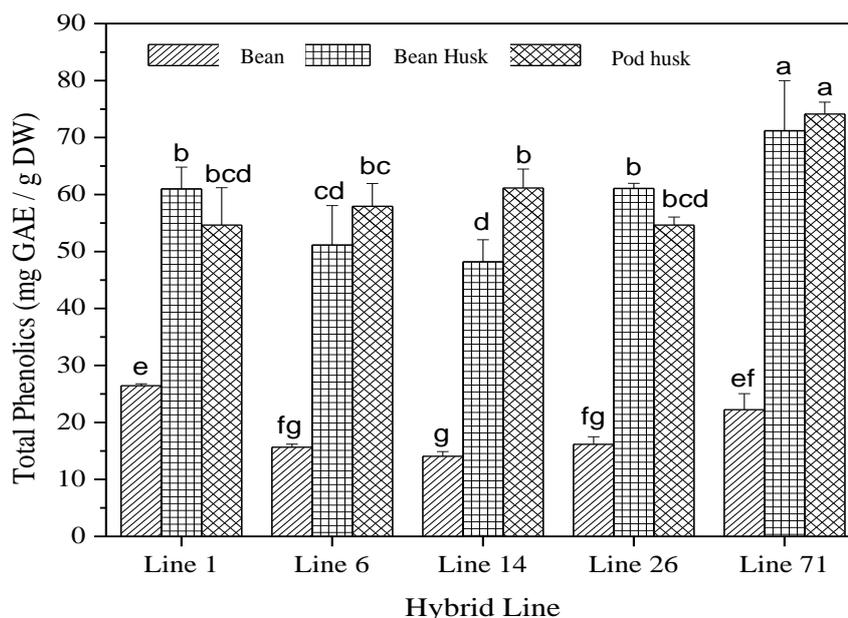
Means with the same letter represent non-significant differences ($p < 0.05$)

Total Phenolic Content

Phenolic compounds of cocoa (*T. cacao*) belong to many classes of molecules: catechins, epicatechins, anthocyanins, proanthocyanidins, phenolic acids, condensed tannins, other flavonoids and some minor compounds (Sanchez-Rabaneda *et al.*, 2003; Wollgast and Anklam, 2000). Significant differences in the contents of total phenolics among different tissues of selected cocoa pods were also observed in the present study (Figure 1). Of all selected cocoa hybrid lines, the total phenolic content was significantly higher in bean husk and pod husk than in beans. However, there were, no significant differences in the total phenolic content between bean husk and pod husk of all selected cocoa hybrid lines except hybrid line 14. Significantly higher phenolic contents were observed in bean husk (71.20 ± 8.78 mg GAE/g DW) and pod husk (74.12 ± 2.09 mg GAE/g DW) of hybrid line 71 than all selected hybrid

lines. The highest total phenolic content (26.44 ± 0.35 mg GAE/g DW) in cocoa beans was observed in hybrid line 1 whereas, hybrid line 14 showed lowest total phenolic content (14.08 ± 0.8 mg GAE/g DW). The total phenolic content of cocoa beans has been reported ranging between 70 and 80 mg/g for freshly harvested and 6 day fermented cocoa beans (Jonfia-Essien *et al.*, 2008). The lower values reported in this experiment could be attributed either due to the facts such as beans storing prior to analysis, extraction method, varietal differences or due to the country of origin.

FRAP values showed significant correlations with the total phenolic content in bean husk ($R^2 = 0.88, p < 0.001$), pod husk ($R^2 = 0.87, p < 0.001$) and bean ($R^2 = 0.87, p < 0.001$) (Figure 2). Similarly, Othman *et al.* (2007) have demonstrated a good correlation between total phenolic and antioxidant activity in cocoa beans and their products.



Means with the same letter represent non-significant differences ($p < 0.05$)

Figure 1. Contents of total phenolics in beans, bean husk and pod husk of five cocoa hybrid lines

In cocoa powder manufacturing, after fermentation and roasting processes of beans, bean husks are removed and only beans (nibs) are used for cocoa powder production. Our results revealed presence of significantly higher total phenolic and FRAP values in cocoa bean husk and pod husk than in cocoa bean. Since cocoa bean husk is an edible tissue, it is thus recommended to use cocoa beans with bean husk for cocoa powder production. However, further research is needed to

check the quality of cocoa powder with bean husk.

The FRAP values and total phenolics of cocoa pods in this study will be useful information for growers, producers and breeders. However, further research is required to compare total phenolic contents and antioxidant activity of hybrid varieties of cocoa with its traditional varieties (*viz.* Criollo, Forastero and Trinitario) in different agro ecological zones and to evaluate total phenolic contents and antioxidant activity in cocoa powder products.

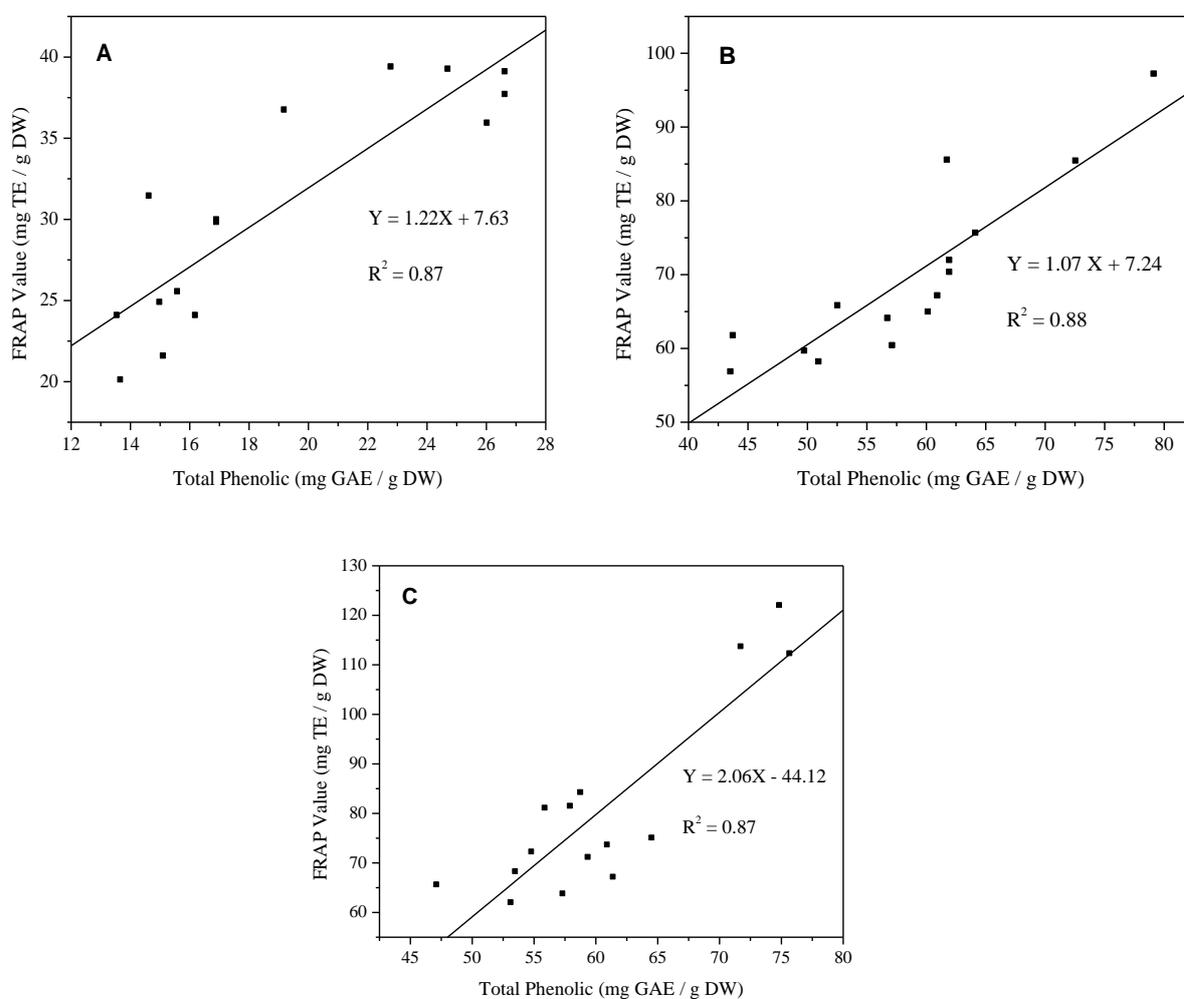


Figure 2. The correlation between FRAP values and total phenolic contents of (A) beans, (B) bean husks and (C) pod husks of cocoa

CONCLUSION

In conclusion, bean husk and pod husk of cocoa contained significantly higher antioxidant activity and total phenolics than cocoa beans. Among all selected cocoa hybrid lines, significantly high FRAP values were reported in all tissues (bean husk, pod husk and bean) of hybrid line 71. Bean husk and pod husk of hybrid line 71 contained significantly higher total phenolics whereas, bean of hybrid line 1 had the highest total phenolics.

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