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RESEARCH PAPER

Polyphenols in cocoa beans: A potential antioxidant

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Abstract : Cocoa (*Theobroma cacao* L.) is a potent source of polyphenol. There are reports that polyphenol and antioxidant activity is positively correlated. The present study investigated the total fat content, polyphenol content and antioxidant activity of cocoa beans obtained from twenty different cocoa hybrids. Folin – Ciocalteau (FC) reagent method was used to determine total polyphenol content in cocoa beans. Antioxidant activity was expressed as per cent radical scavenging activity and it was found out using DppH assay. Hybrid PIV 45.4 was found to be superior with respect to polyphenol content and antioxidant activity. Correlation studies revealed that total polyphenol content and antioxidant activity is positively correlated (r=0.613).

Key Words : Cocoa, *Theobroma cacao* L., Hybrids, Polyphenol content, Radical scavenging activity, Correlation

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INTRODUCTION

Cocoa (*Theobroma cacao* L.) a beverage crop and source of fashionable delicacy chocolate is native to Amazon region of South America. Chocolate and cocoa products are one among the very few delicacies which is equally loved by every part of globe. Cocoa was first cultivated as a crop by Aztecs and Mayans who were the indigenous populations of South America. They used cocoa beans for the preparation of a bitter drink known as 'Cacahuatl' which they served during their religious ceremonies and from this, the word chocolate originated. In indigenous cultures cocoa beans were so important that it was used as currency in trade, given as post-battle reward and was one of the main items in royal feasts (Amma *et al*., 2009).

More than 50 per cent of cocoa beans are fat and 20-25 per cent is sugar (Nehling, 2013). Apart from fat and sugar, cocoa beans also contains theobromine, caffiene and many other polyphenols such as flavonoids. Cocoa beans have high phenolic content of about 12–18 per cent in unfermented dried beans (Kim and Keeney, 1984). Polyphenols are considered important now a days due to their antioxidant, antimutagenic and antitumour activities (Saliva *et al*., 1991). The place of origin as well as the method of processing was found to influence the polyphenol content of cocoa products (Jalil and Ismail, 2008). Phenol extracts from unfermented dry cocoa beans have higher polyphenol content and stronger antioxidant capacity when compared to partially fermented cocoa beans (Prayoga *et al*., 2013).

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An antioxidant can significantly delay or prevent oxidation of substrates present in the body (Gutteridge and Halliwell, 2000). There are reports that polyphenols are having antioxidant capacity (Rice-Evans *et al*., 1997). The polyphenols present in the cocoa beans, especially the flavanoids are responsible for the antioxidant activity of cocoa beans (Steinberg *et al*., 2002; Sun and Ho, 2005; Aikpokpodion and Dongo, 2010 and Martinez *et al*., 2012). According to Lee *et al.* (2003) and Steinberg *et al.* (2003) cocoa products contains greater antioxidant capacity and flavonoids content than tea or red wine.

In the present study we estimated fat, total polyphenol content and antioxidant activity of unfermented cocoa beans and made an attempt to correlate polyphenol content and antioxidant property of cocoa beans.

MATERIAL AND METHODS

Collection of sample:

The experiment was conducted at the research farm of Cocoa Research Centre, Kerala Agricultural University during the period 2017 to 2019. Twenty cocoa hybrids were used as material of the study. Five mature pods each, were collected from cocoa hybrids. They were cut open to collect beans. The beans collected from each hybrid were mixed together and forty beans were collected randomly. These beans were peeled and dried. Dried beans were powdered and this powder was subjected for fat extraction.

Estimation of fat content:

Soxhlet apparatus method proposed by Sadasivam and Manickam (1996) was used for estimating fat content of cocoa beans. Fat present in cocoa beans get dissolved in organic solvents like petroleum ether and can be collected by evaporating the solvent. The procedure is explained in detail below.

In soxhlet apparatus cocoa beans powder was defatted using petroleum ether as solvent. Cocoa beans powder of 10g was wrapped in blotting paper and tied using a twine. It was placed inside the extraction tube of the apparatus and sufficient amount of solvent was added. Fat got settled at the bottom of round bottom flask due to the siphoning of petroleum ether in soxhlet apparatus. It took almost 6 hours to complete whole extraction process. When extraction processes were complete, the fat settled was transferred to a pre-weighed beaker and kept open, which allows the remaining solvent to get evaporated. After that weight of fat was taken and expressed as per cent.

Estimation of total polyphenol content and antioxidant activity:

Defatted cocoa powder of weight 500 mg was grinded with 80 per cent ethanol in a mortar and pestle. This was transferred into a centrifuge tube and centrifuged at 10,000 rpm for 20 minutes. After that supernatant were transferred to an evaporating dish. This procedure was repeated 2-3 times in order to collect all the phenol present in the sample. To remove the excess ethanol, evaporating dishes were kept over a hot water bath for one hour. To the left-over residue 40 ml of distilled water was added. From that 0.2 ml aliquot was taken to a test tube and 13 ml distilled water was added followed by, addition of 0.5 ml Folin – Ciocalteau (FC) reagent. The test tubes with reaction mixture were kept for three minutes incubation. After that 2 ml, 20 per cent Na_2CO_3 solution was added. These test tubes were kept for one minute over boiling water bath and incubated at room temperature for 60 minutes. Using spectrophotometer absorbance was read at 650 nm against reagent blank. Catechin was taken as standard for estimating total phenols in cocoa powder.

$$\text{Total phenol} = \frac{\text{OD of sample}}{\text{OD of standard}} \text{ x } \frac{\text{Concentration of standard}}{\text{Volume of sample}} \text{ x } 100$$

Antioxidant activity was estimated by DppH assay method, following the procedure given by Schinella *et al*. (2002) and the result was expressed as radical scavenging activity (%). EC_{50} value was also estimated by plotting graph of radical scavenging activity against the concentration of cocoa extracts. EC_{50} value is the cocoa sample concentration required to reduce the DppH radical concentration by 50 per cent.

Correlation analysis between mean values of per cent polyphenol and radical scavenging activity was carried out using Karl Pearson's correlation co-efficient test with the help of SPSS software.

RESULTS AND DISCUSSION

Fat content of beans varies between cocoa genotypes (Rossini *et al*., 2011). In the hybrids used for the present study fat content varied from 48.2 per cent in the hybrid VSDI 23.21 to 62.53 per cent in the hybrid PIV 31.9 (Table 1). Hybrid SIV 1.10 has the second highest (61.2 %) fat content. Studies of Mossu (1992)

revealed the role of fat content in enhancing flavour and aroma of chocolates. Hybrids with high fat content can act as a genetic stock for improving quality in future breeding purposes (Monteiro *et al*., 2009). Genotypes having fat content more than 45per cent is considered superior (Afoakwa *et al*., 2013). All the hybrids included in the present study exhibited more than forty five per cent fat content in their beans. Eighty five per cent of the hybrids used in the study recorded more than fifty per cent fat content.

Polyphenols plays a role in imparting flavour and colour to chocolate (Kim and Keeney, 1984). Total polyphenol content in the twenty cocoa hybrids included in the study ranged from 3.71 per cent in PIV 31.9 to 9.36 per cent in PII 12.11 (Table 1). Second highest polyphenol content (9.14 %) was recorded in hybrid PIV 45.4. Zumbe (1998) reported that polyphenol content in cocoa beans is about 6-8 per cent and it can reach upto a value of 10 per cent. Present study is on line with the findings of Zumbe (1998). Studies of Asna (2013) revealed that total polyphenol content in the unfermented cocoa beans ranged from 2.25 per cent to 9.09 per cent. Rubeena (2015) estimated total polyphenol content in unfermented samples of cocoa and reported that it ranged from 1.51 per cent to 5.64 per cent. These results reveal that polyphenol content of cocoa beans is genotype dependent character. The minimum level for polyphenol considered desirable in cocoa is 4.5 per cent (Veeresh, 2017). In the present study most of the cocoa hybrids exhibited more than 4.5 per cent polyphenol content which shows their genetic superiority with respect to polyphenol content.

Free radicals produced inside the body have the capacity to destroy cells and antioxidants are substances used to scavenge these radicals. Radical scavenging activity is an indirect measure of antioxidant property of a substance. Radical scavenging activity ranged from 49.95 per cent in the hybrid PIV31.9 to 90.75 per cent in the hybrid PIV 45.4 (Table 1). Out of the twenty hybrids, ten exhibited more than 80 per cent radical scavenging activity. Central Plantation Crops Research Institute (CPCRI) evaluated antioxidant capacity of beans harvested from different cocoa varieties and found out that they differ significantly and it varied from 77 per cent to 98 per cent (Malhotra and Apshara, 2017).

EC_{50} value indicates the actual potential of an

Table 1 : Biochemical characters of cocoa beans obtained from twenty cocoa hybrids

Hybrids	Total polyphenol (%)	Radical scavenging activity (%)	EC_{50} value (mg/ ml)	Fat content (%)
PIV 45.4	9.14	90.75	1.31	55.66
PIII 2.3	7.36	77.13	1.85	52.66
PIV 59.8	6.33	75.62	2.02	53.00
SIV 10.11	6.48	73.79	2.44	52.56
VSDI 10.13	7.01	85.06	1.59	52.40
SIV 1.10	6.41	72.50	2.43	61.20
PIV 60.9	6.54	82.61	2.68	55.43
PII 12.11	9.34	84.81	2.61	51.63
SIV 5.15	4.74	68.86	2.95	50.90
VSDI 33.4	6.34	80.48	1.74	54.73
VSDI 23.21	6.16	78.20	1.89	48.20
PIV 58.6	6.48	70.83	2.57	55.73
PIV 56.9	7.61	83.89	1.65	53.76
VSDI 30.8	7.21	82.98	1.71	49.93
VSDI 11.11	7.86	84.68	1.69	55.26
SIV 1.6	7.73	77.60	1.92	49.33
PIV 19.9	7.99	87.01	1.42	56.80
PIV 26.8	5.72	49.95	3.77	53.56
PIV 31.9	3.71	74.51	2.41	62.53
VSDI 29.9	4.50	74.25	2.53	56.53

antioxidant to scavenge the free radicals. It ranged from 1.31 mg/ ml in hybrid PIV 45.4 to 3.77 mg/ ml in PIV 26.8 (Table 1). Lowest EC_{50} value indicates high antioxidant activity. Othman *et al*. (2007) conducted a study to estimate antioxidant capacity of cocoa beans from different countries and reported that EC_{50} value varied from 0.625 mg/ ml to 2.5 mg/ ml. Ibriae and Eavar (2014) estimated the EC_{50} value of cocoa powder and different chocolates and the result was in the range of 1.968 ± 0.076 mg/ml to 42.200 ± 1.737 mg/ml.

Total polyphenol content and radical scavenging activity of cocoa bean powder obtained from cocoa hybrids showed significant positive correlation (0.613) (Fig. 1). There are reports that phenols are having antioxidant capacity (Rice- Evans *et al*., 1997). The phenols present in the cocoa beans, especially the flavanoids may be responsible for the antioxidant activity of cocoa beans. Othman *et al*. (2007) investigated antioxidant capacity and total phenol content of cocoa beans from different countries and concluded that they are positively correlated (r = 0.78). Thus, selecting cocoa genotypes having high polyphenol content in cocoa beans, results in indirect selection of genotypes that can contribute to high antioxidant activity. Similar observations are reported by many scientists like Steinberg *et al*. (2002); Sun and Ho (2005); Aikpokpodion and Dongo (2010) and Martinez *et al*. (2012).

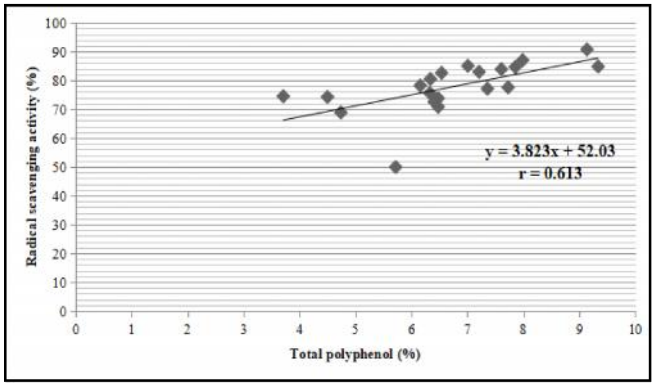

Fig. 1 : Correlation between total polyphenol content and antioxidant activity

The present study revealed that the polyphenol content has a strong correlation with antioxidant property. Genotype with high polyphenol content is a major thrust area focused by many chocolate industries. Hence, the identified hybrids can be popularized among the farmers for getting better price for their product.

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