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Evaluating the processed beans of different cocoa (*Theobroma cacao* L.) accessions for quality parameters

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ABSTRACT

The objective of this study was to evaluate the variability in quality parameters of different identified cocoa plus trees from Coimbatore district in Tamil Nadu. Thirty five (35) plus trees in two different farmers field were evaluated for their quality traits. The quality parameters like fat content (%), carbohydrate content (mg/g), protein content (mg/g), theobromine content (mg/g), caffeine content (mg/g), catechin content (mg/g) and caffeic acid content (mg/g). A rapid method like high performance liquid chromatography (HPLC) method is used for the determination of theobromine, caffeine, catechin and caffeic acid in cocoa beans. Among the 35 plus trees evaluated for bean quality, highest fat content was recorded in Tc (Vedapatti) 94, while highest carbohydrate content was recorded in Tc (Vedapatti) 64 and highest protein content was recorded in Tc (Vedapatti) 76. The highest theobromine was recorded in Tc (Vedapatti) 63, caffeine was recorded in Tc (Vedapatti) 91, while the amount of caffeic acid and catechin was the highest in Tc (Vedapatti) 76. This evaluation study showed wide variation for quality parameters. These variability may be used in further breeding program enhance the quality traits and improve flavours of the final product.

KEYWORDS: Cocoa, plus trees, quality, HPLC

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INTRODUCTION

Cocoa (*Theobroma cacao* L.) belonging to the family Malvaceae is one of the important beverage crops after tea and coffee. *Theobroma* means "Food of God". Amazon valley of South America is native of cocoa and at present cocoa cultivation is spread across many countries [8]. The economic part is cocoa beans which is a rich source of fat, protein, carbohydrate, theobromine, caffeine, caffeic acid, catechin and epicatechin with antioxidant properties. These beans are mainly used in chocolate industry as source of cocoa butter and cocoa mass. The post harvest practices in cocoa is important operation to decide the product quality which reflects in market price [11]. methylxanthine alkaloids like theobromine and caffeine in cocoa beans have stimulatory activity on central nervous system, prevents cardio vascular diseases and are also used in pharmaceutical industry to prepare bronchodilators drug [1]. However, high doses affect the central nervous system and may induce addiction and anxiety [9].

The polyphenol compounds like catechin and caffeic acid along with methylxanthines contribute to the bitterness and astringency which in turn determines cocoa flavour [2, 6, 10]. The present study aims to evaluate the quality of processed beans from 35 plus trees identified for higher yield.

MATERIALS AND METHODS

The seven year old cocoa trees at Thondamuthur and Vedapatti region of Coimbatore district were evaluated on the basis of yield and yield contributing characters for several seasons 35 plus trees were selected. The present study was undertaken during June- December, 2018 to evaluate the quality traits of these 35 plus trees. Pods from 35 plus trees were collected and the beans were extracted and was subjected to fermentation by box method for 6 to 7 days. After fermentation, beans were dried under sun for one week. The fermented and dried beans of each tree were collected and used for analysis.

The quality parameters like fat content, total carbohydrate, protein content were estimated. Fat content was estimated and expressed in percentage [3].

$$\text{Percentage of fat content on moisture free basis} = \frac{(W_2 - W_1)}{\text{Weight of the sample} \times (100 - M)} \times 100$$

Where,
W1 – Weight of the receiver (Bottom flask)
W2 – Weight of the flask + ether extractives
M – Moisture content of the sample

Total carbohydrate was estimated by using anthrone method [12]. The amount of carbohydrate present in the sample was calculated by using the following formula and expressed in mg/g.

$$\text{Total carbohydrates } (mg/g) = \frac{\text{'Y'} \times 100 \times 0.9}{\text{Volume of test sample} \times 1000}$$

The sample OD at 630 nm was plotted in the standard graph and the corresponding concentration is 'Y' mg.

The soluble protein content of cocoa beans were estimated and expressed as mg g^{1} [7].

$$\text{Amount of Soluble protein} = \frac{X}{0.5} \times \frac{25}{500} \times 1000$$

The sample OD at 660 nm was plotted in the standard graph and the corresponding concentration is *x* μg.

Further, among the 35 plus trees, first 10 best performing trees were screened and theobromine, caffeine, caffeic acid and catechin content were determined by high performance liquid chromatography (HPLC).

The determination of compounds such as theobromine, caffeine, catechin and caffeic acid was performed according to the method described by Elwers et al. [4]. Twenty microliters of each sample solution was analyzed by HPLC system (Star pro Variant Model) equipped with C18 column (100 mm × 4.6 mm O.D.S -2,3 μm) and using the eluent comprising of 87% water: 8% methanol: 5% acetic acid, flowing at 1.0 ml/min. The compounds were monitored by UV detection at 280 nm wavelength. The total run time was 30 min and the temperature was 26°C. Quantitative analysis was carried out by using the individual standard curves for each type of compounds. Data were collected using Young Lin Autochro – 3000 Chromatograph Data System software.

Standards were dissolved in methanol (HPLC grade) and were injected separately to find out the retention time of each standard. Retention time identified for each standard and after finding out the retention time of each standard, standards solution were mixed. These different concentration of mixed standard solutions were prepared to get calibration curve for the analysis. Chromatograms of mix standards are given in Figure 1. The retention time are furnished in Table 1.

RESULTS AND DISCUSSION

Quality parameters like fat content, total carbohydrate and soluble protein content of 35 cocoa plus trees are furnished in Table 2. Among the 35 identified plus trees evaluated for quality parameters, highest fat content (56.00 %) was recorded in Tc (Vedapatti) 94 and lowest was recorded in Tc (Vedapatti) 9. The average fat content of 35 plus trees was 37 %. The highest carbohydrate content 120.23 mg/g was recorded in Tc (Vedapatti) 64 and lowest of 29.78 mg/g recorded in Tc

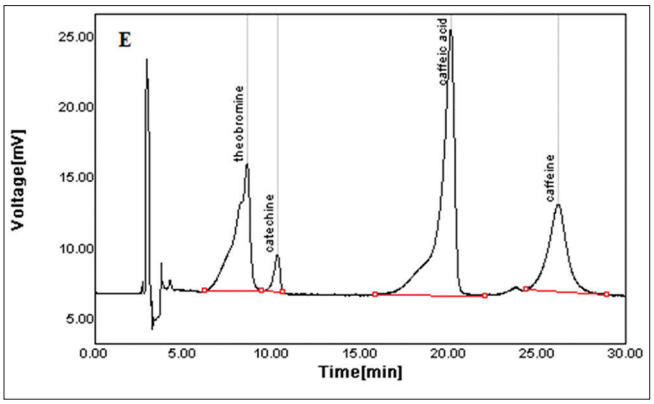

Figure 1: Chromatograms of mix standards for theobromine, catechin, caffeic acid and caffeine

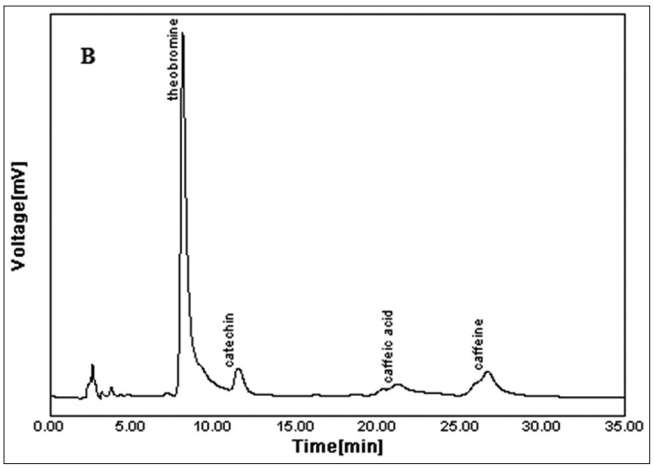

Figure 2: Chromarographic profiles of Tc (Vedapatti) 76

Table 1: Retention time of different compounds of cocoa

Compounds	Retention time (Minute)
Theobromine	8.61
Catechin	11.31
Caffeic acid	20.08
Caffeine	26.15

(Vedapatti) 1. The average total carbohydrate content was 84.38 mg/g. Among the 35 plus trees maximum protein content of 50.82 mg/g was reported in Tc (Vedapatti) 76 and minimum (7.88 mg/g) was recorded in Tc (Vedapatti) 66. The average soluble protein content of the plus trees was 28.03 mg/g.

In the present study, quality parameters like fat content, carbohydrate content, and protein content showed greater variation. These variability due to both genetic and environmental factors including soil moisture, age of the tree, spacing, nutrient and moisture availability. Maximum carbohydrate content in the plus trees may be based on efficiency of plants in partitioning assimilates. Cocoa beans containing high fat content have higher marketability as they play a major role in chocolate industry [5].

The bean samples from the first 10 best performing plus trees (Tc (Vedapatti) 1, 63, 66, 72, 76, 78, 88, 91, 94 and 110)

Table 2: Evaluation of identified plus trees for quality characters

S. No	Plus trees (Accessions)	Fat content (%)	Carbohydrate (mg/g)	Protein (mg/g)
1	Tc (Vedapatti) 1	34.00	29.78	22.54
2	Tc (Vedapatti) 2	37.67	90.54	44.12
3	Tc (Vedapatti) 9	22.67	32.12	22.78
4	Tc (Vedapatti) 15	47.00	36.78	18.67
5	Tc (Vedapatti) 18	26.33	82.18	18.05
6	Tc (Vedapatti) 29	29.33	110.12	9.54
7	Tc (Vedapatti) 31	29.33	108.98	27.18
8	Tc (Vedapatti) 33	26.67	115.84	20.54
9	Tc (Vedapatti) 37	33.67	52.89	26.47
10	Tc (Vedapatti) 40	36.00	108.12	7.89
11	Tc (Vedapatti) 41	30.67	89.72	46.74
12	Tc (Vedapatti) 42	40.00	115.92	24.76
13	Tc (Vedapatti) 45	30.67	67.78	16.58
14	Tc (Vedapatti) 48	35.00	99.72	43.54
15	Tc (Vedapatti) 55	33.00	58.28	29.32
16	Tc (Vedapatti) 61	33.00	118.72	21.68
17	Tc (Vedapatti) 63	46.33	70.68	13.72
18	Tc (Vedapatti) 64	49.67	120.23	50.75
19	Tc (Vedapatti) 66	32.67	118.95	7.88
20	Tc (Vedapatti) 67	41.67	101.25	33.72
21	Tc (Vedapatti) 68	49.33	54.42	29.48
22	Tc (Vedapatti) 72	45.00	62.48	43.54
23	Tc (Vedapatti) 75	37.00	82.12	37.51
24	Tc (Vedapatti) 76	34.67	92.78	50.82
25	Tc (Vedapatti) 78	26.00	69.54	23.54
26	Tc (Vedapatti) 85	39.33	119.54	38.38
27	Tc (Vedapatti) 86	27.67	60.84	35.78
28	Tc (Vedapatti) 88	47.33	75.72	30.85
29	Tc (Vedapatti) 90	37.67	61.58	29.54
30	Tc (Vedapatti) 91	27.00	98.72	8.51
31	Tc (Vedapatti) 94	56.00	115.84	20.97
32	Tc (Vedapatti) 99	50.67	68.75	40.75
33	Tc (Vedapatti) 110	47.33	82.78	46.98
34	Tc (Vedapatti) 111	43.67	60.84	19.88
35	Tc (Thondamuthur) 121	31.00	118.72	17.92
	Mean	37.00	84.38	28.03
	SE (d)	0.77	1.92	0.72
	CD (P=0.05)	1.54	3.82	1.43

Table 3: Determination of phenolic compounds and methylxanthines in screened cocoa beans

S.No	Plus trees (Accessions)	Theobromine content (mg/g)	Caffeine content (mg/g)	Catechin content (mg/g)	Caffeic acid content (mg/g)
1	Tc (Vedapatti) 1	8.66	1.75	0.76	0.43
2	Tc (Vedapatti) 63	9.12	2.08	0.95	0.38
3	Tc (Vedapatti) 66	8.31	1.53	0.85	0.11
4	Tc (Vedapatti) 72	8.48	1.87	0.80	0.27
5	Tc (Vedapatti) 76	7.57	1.92	1.32	0.54
6	Tc (Vedapatti) 78	8.11	1.32	0.95	0.18
7	Tc (Vedapatti) 88	7.97	1.78	1.28	0.21
8	Tc (Vedapatti) 91	8.95	2.11	0.69	0.16
9	Tc (Vedapatti) 94	8.86	1.64	0.72	0.32
10	Tc (Vedapatti) 110	8.74	1.54	0.79	0.09
	Mean	8.48	1.76	0.91	0.27
	SE (d)	0.19	0.04	0.02	0.01
	CD (P=0.05)	0.37	0.07	0.05	0.01

were evaluated for quality parameters such as theobromine, caffeine, catechin and caffeic acid by HPLC method. The data on theobromine, caffeine, catechin and caffeic acid contents observed in 10 plus trees are furnished in Table 3. Among the 10 cocoa samples, lowest theobromine content of 7.57 mg/g was recorded in Tc (Vedapatti) 76. Chromarographic profile of Tc (Vedapatti) 76 presented in Figure 2. The average theobromine content in 10 cocoa samples was 8.78 mg/g. Tc (Vedapatti) 91 recorded highest caffeine content (2.11 mg/g) and lowest of 1.32 mg/g was recorded in Tc (Vedapatti) 78. The average caffeine was 1.76 mg/g. The maximum catechin content (1.32 mg/g) was noted in Tc (Vedapatti) 76 and minimum (0.69 mg/g) was noted in Tc (Vedapatti) 91. Mean value of catechin was 0.91 mg/g. The highest caffeic acid content of

0.54 mg/g was recorded in Tc (Vedapatti) 76 and the lowest of 0.09 mg/g was recorded in Tc (Vedapatti) 110. The average caffeic acid among the 10 cocoa sample was 0.27 mg/g.

As cocoa is highly heterogeneous crop, wider variation was observed for theobromine, caffeine, catechin, caffeic acid content. These variability is characterized by both genetic and environmental factors [13].

CONCLUSION

Among the 35 plus trees, wide variation was observed for quality parameters. Since, cocoa is a cross pollinated crop, the seedling population showed wide variability for quality parameters. Among the plus trees, Tc (Vedapatti) 94 recorded the highest fat content and out of best 10 genotypes screened for its flavour compound, minimum theobromine content was recorded in Tc (Vedapatti) 76. Low theobromine content results in low bitterness in cocoa based products manufactured in chocolate industries. Hence, the identified plus trees may be continuously evaluated for its stability in producing high quality beans and can be utilized in future breeding program for quality improvement in cocoa.

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