Journal of Applied Horticulture, 11(2): 127-131, July-December, 2009

Caffeine, phenol and protein contents of thirty-seven clones of Nigerian robusta coffee (*Coffea canephora* Pierre ex. Froehner)

S.S. Omolaja

Plant Breeding Group, Cocoa Research Institute of Nigeria (CRIN), P.M.B. 5244, Ibadan, Nigeria.
E-mail: dolaomolaja@yahoo.com

Abstract

A study was carried out to characterise thirty-seven *Coffea canephora* clones using three biochemical characteristics, namely caffeine, phenol and protein content. The phenol and caffeine contents were determined by gravimetric method, while protein was assessed by polyacrylamide gel electrophoresis (PAGE) of floral bud. Caffeine content among the clones ranged from 1.1 to 1.5% on dry matter basis (dmb). C36 a high yielding clone, had relatively low caffeine content, hence it is a suitable clone that could be included in any breeding programme for low caffeine coffee in Nigeria. All the Niaollou (M) clones had high caffeine content. Phenol content in the berry pulp of the clones ranged from 2.6 to 15.6%. Averaged over clones, phenol content of berry pulp (9.5 %) was significantly ($P < 0.05$) higher than leaf phenol content (4.5 %). The coefficient of variation for pulp phenol was high (35.3), thus indicating that, rapid response to selection for favourable phenol percentage might be feasible. The high level of phenol found in some clones may be valuable in breeding for resistance to some major diseases and insect pests of coffee. There were differences in the mobility and intensity of protein bands in the clones. The variation in the protein banding patterns of the different *C. canephora* clones observed provides further information on the existing genetic diversity of the coffee clones in addition to that provided by agro-botanical characters.

Key words: Caffeine, phenol, protein, *Coffea canephora,* Nigeria

Introduction

Coffee, a beverage crop, is the second most important commodity in the international market after petroleum (ICO, 2008). Among the important chemical compounds that are found in coffee, three of interests to breeders are caffeine, phenol and protein (ICO, 1999).

Caffeine (1, 3, 7-trimethylxanthine) is a methylated purine alkaloid (Vasudeva *et al.,* 1981) that is widely distributed in the family Rubiaceae (Bonner, 1950). Its presence in different members of the genus *Coffea* is well documented (Raju and Gopal 1979; Raju *et al.,* 1981). Caffeine ($C_8 H_{10} O_2 N_4$) is also found in kola, cocoa and tea. Caffeine stimulates the synthesis or release of the catecholamine, epinephine and norpinephrine hormones (better known as adrenalin and nor-adrenalin, respectively) into the blood stream (Nguyen-van Tam and Smith, 2001). This increases alertness, but may lead to loss of sleep. The stimulant is also not beneficial during pregnancy (Borea *et al.,* 2001). Consequently many consumers of coffee prefer decaffeinated coffee. Hence, demand for decaffeinated coffee is increasing rapidly in the world market. There is, therefore, an urgent need to develop caffeine-free coffee cultivars (Sreenath, 1997) in order to protect the income of coffee farmers. Caffeine content is greater in robusta than in arabica coffee (Raju and Gopal, 1979). Given that the direction of international market on caffeine content in *C. canephora* is towards its reduction (Ashihara and Crozier, 2001; Montagnon *et al.,* 1998); one of the breeding objectives for improved coffee quality in the world today is low or no caffeine content (Sreenath, 1997).

Phenol, when present in significant quantity in coffee, has been observed to be associated with resistance to leaf rust and coffee berry disease (Walyaro, 1983). Ram *et al.* (1982) observed significant positive correlation between phenolic compounds in coffee varieties and their resistance to leaf rust disease. The polyphenol content of coffee leaves has also been reported as a good criterion for evaluating breeding material and selecting donors for resistance to the insect *Aphis humili* (Romanenko, 1985).

The protein band variation from different crop plants is based partly on differences in the molecular weight of protein. Crop traits are the expression of genes, and proteins are the primary products of genes. Agro-botanical variation reflects diversity in genetic structure. Therefore, genetic diversity in a crop germplasm can be illuminated through the analysis of protein. Gottlieb (1971) reported that variation in banding pattern could be equated to variation in genes coding for various proteins. Protein electrophoresis therefore offers an efficient means of identifying and quantifying genetic variation in crop germplasm (Carrens *et al.,* 1997; Davis, 1964). The advantage of biochemical markers such as protein, is that they are genotypic markers and hence, will reflect the actual genetic distances between accessions and their common ancestors more accurately than phenotypic markers (Gepts, 1995). Berthou and Trouslot (1979) used isozyme analysis to separate the Guinean group from the Congolese group of *C. canephora* populations. The objective of this study, therefore, was to carry out biochemical characterisation of Nigerian *C. canephora* germplasm.

Materials and methods

The study was carried out at the Cocoa Research Institute of Nigeria, Gambari, Ibadan. The *C. canephora* clones studied and their geographical origins were: A110, A116 (Ghana robusta), C36, C90, C96, C105, C107, C108, C111 (Quillou robusta from

Gabon), E1, E77, E92, E106, E1 (Java robusta), G87, G129 (Uganda robusta), M10, M31, M36, M53 (Niaollou robusta from Republic of Benin), T4, T24, T45, T93, T116, T164, T169, T204, T314, T365, T921, T1049, T197, T155, T176, T395 and T220 (Java-ex-Gambari from Zaire). The clones were planted in 1966. The plants were coppiced in 1997 and 2000. The morphological differences among the "A", "C", "E", "G", "M" and "T" clones are described by Omolaja and Fawole (2004a). Three biochemical compounds namely phenol, caffeine and protein contained in each of the thirty-seven clones were determined.

Determination of caffeine content

Sample preparation, caffeine extraction and determination: Fruit used for caffeine determination were harvested at maturity and depulped using the wet processing method (Omolaja and Williams, 1997). Caffeine content was determined by the modified extraction methods of Horwits (1970) and Barre *et al.* (1998). Caffeine content was evaluated using 40 randomly sampled coffee beans per clone from freshly harvested berries. The samples were milled to fine powder using Moulinex electronic grinder and stored in plastic containers in a dessicator at 4°C until used. Each batch of beans was crushed for eight minutes. One g of dried coffee powder was weighed into a conical flask. Then 50 mL of 0.5N boiling or hot H_2SO_4 was added. The mixture was then refluxed on water-bath for 30 -50 minutes. Afterwards it was allowed to cool, then centrifuged and filtered. Forty mL of the mixture was taken into a separating funnel and 30 mL chloroform was added, and vigorously shaken. Two layers were formed: organic layer at the top and chloroform layer at the bottom. The chloroform layer was collected. Another 30 mL chloroform was added to the organic content remaining in the funnel and the mixture properly shaken. The chloroform layer was again collected. Then 40 mL of the chloroform fraction was dispensed into a pre-weighed 100 mL flat-bottom flask. This was distilled off at between 60-80°C. The distillate was dried in the oven to obtain caffeine, and the weight of caffeine was determined. The percent caffeine for each clone was obtained by dividing the caffeine weight by the initial weight of sample, and multiplying by 100. The extraction for each clone was repeated three times. Analysis of variance was carried out and means separation was done using Duncan's Multiple Range Test.

Determination of phenolic content

Sample collection, sample preparation and determination of total phenol: The procedure of phenolic content determination used in this study was modified methods of Bate-Smith (1962) and Odebode (1995). Fresh leaves and the pulp of seven months old mature berry were investigated for their phenolic content. The plant parts of each of the thirty-seven clones were randomly sampled. Fresh leaves and berry pulp weighing 100g were separately washed and dried in an oven at 50-60°C. The samples were milled to powder using Moulinex electronic grinder and stored in a conical flask in a dessicator at 4°C until used. A sample of 2 g of each of oven dried and milled leaves as well as berry pulp was taken into different conical flask containing 100 mL of 80% ethanol and boiled for eight hours. The ethanol was changed by decanting three times (3 x 100 mL) during the extraction of the total phenol. The ethanol extractions were then concentrated to 30-40 mL in a vacuum evaporator. The residue was refluxed for 1 hour, filtered hot and diluted to 100 mL with distilled water. Then the filtrate was dried in the oven at a temperature of 80°C. The percent phenol for each clone was determined from the weight of the dried residue. The percent phenol was calculated by dividing the weight of the dried residue with the initial 2 g of oven dried sample and multiplying by 100. The phenol extraction for each clone was repeated three times. Data of percent phenol for the leaves and pulp were separately subjected to analysis of variance, while significant differences among means were established by Duncan's Multiple Range procedure.

Electrophoretic analysis of floral bud protein: Unopenned floral buds collected from each of the thirty-seven *C. canephora* clones were used for the analysis. Five g of floral bud was used per clone.

(i) Protein extraction: Floral bud protein was extracted by grinding 1 g of the floral bud with mortar and pestle. 7.5 mL of 0.9% sodium chloride (NaCl) was added during grinding. The mixture was allowed to settle inside the test tube immersed inside ice for one hour (Illoh, 1990), thereafter centrifuged at 4000 rpm for 15 minutes. The clear supernatant was then poured into the test tube and stored inside the refrigerator. On the day of use, sucrose crystals (5 grains), three drops of each of 2% mercapto-ethanol and sodium dodecyl sulphate (SDS) were added to 1 mL of the supernatant in the test tube and the mixture was boiled for 15 minutes in a water-bath. After cooling down to room temperature, a drop of 0.05% bromophenol blue was added to the sample as front marker (Akinwusi and Illoh, 1995).

Preparation of gels is as indicated in Table 1. The loading of gels, electrophoretic run, staining, destaining, and measurement of the gels were carried out according to Omolaja and Fawole (2004b). The positions of different bands were noted. Each protein band was considered as a qualitative character, with presence coded as 1 and absence coded as 0 (Carrens *et al.,* 1997). The data were analysed for significant differences among clones with separation of means carried out by Duncan's Multiple Range Test.

Table 1. Preparation of gels for the electrophoresis of floral bud, anther, pollinated and un-pollinated styles' protein of *Coffea canephora* clones

Stock solutions/ components	Upper gel	Lower gel
Floral bud protein		
40% Acrylamide	1.00 mL	10.00 mL
Upper gel buffer	2.50 mL (0.5M; pH 6.8)	-
Lower gel buffer	-	7.50 mL (1.5M; pH 8.8)
Distilled water	6.33 mL	19.50 mL
10% Sodium dodecyl sulphate	1.00 mL	0.40 mL
10% Ammonium persulphate	0.05 mL	0.10 mL
TEMED (N N N 'N'-Tetramethyl ethylenediamine), 'Electran'	20.00 µL	50.00 µL
Anther, pollinated and un-pollinated styles protein		
Acrylamide	1.35 mL	10.00 mL
Upper gel buffer	2.50 mL	-
Lower gel buffer	-	7.50 mL
Distilled water	6.00 mL	11.20 mL
10% Sodium dodecyl sulphate	0.10 mL	0.30 mL
Ammonium persulphate	0.01 mL	0.30 mL
TEMED	0.03 µL	0.10 µL

Results

Caffeine analysis of thirty-seven *C. canephora* clones: The caffeine content of the clones ranged between 1.1 and 1.5% on dry matter basis (Table 2). Clones A116, C36, C90, C111, E130, T24, T116, T204 and T314 had caffeine contents that were significantly lower than the other clones ($P < 0.5$). Clones E106, M36 and T176 recorded the highest caffeine content of 1.5% dry matter basis, respectively. Clone C36 was among the clones that recorded the lowest caffeine content of 1.1%. All the Niaollou (M) clones had high caffeine content.

Phenolic content of thirty-seven *C. canephora* clones: The percent phenolic content in the berry pulp ranged from 2.6 (E130 and T104) to 15.6 (in T4), among the clones studied (Table 2). The percent phenolics of clones A110, C36, E1, E106, E130, M10 and T204 were significantly lower than those of the other clones at $P < 0.5$. Clones A116, M31, T365, T1049 and T4 had the highest percent of phenol. The average percent phenol in berry pulp was 9.5, which was significantly ($P < 0.05$) higher than what was obtained in the leaves (4.5%). The range of phenolics in the leaves was between 3.0 and 6.3%. The coefficient of variation for pulp phenolic content was high (35.3%), thus indicating that, rapid response to selection for favourable phenolic content might be feasible.

Electrophoretic analysis of floral bud protein in *C. canephora*: The protein band variations among the thirty-seven clones of *C. canephora* are presented in Table 3. Marked differences were observed in the number, combination and intensity of bands among the clones. In terms of mobility of bands, there were three categories: the fast (6.0 - 9.0 cm), the intermediate (3.0 - 5.9 cm) and the slow bands (0.1 - 2.9 cm.) (Table 3). The protein bands at 0.1 to 2.9 cm and 6.0 to 9.0 cm were exhibited by Java (E) and Java-Ex. Gambari (T) varieties. The Ugandan robusta (G) showed protein bands in all the regions, while Ghana robusta (A) exhibited unit band at only the fast region. The Quillou (C) showed thick bands at the fast region, but the Niaollou robusta (M) exhibited varied protein bands. While M10 has bands in fast region, M31 had bands in all the regions. M36 had no band. Of the thirty-seven clones, one (clone T116) has five bands, three has four bands, eight has three bands, twenty has two bands, four has one band while one has no band. Twenty-two clones had bands at the slow and fast regions, one clone at the slow and intermediate regions, while ten clones showed protein bands at only the fast region. The three categories of bands occurred at four different levels of intensities: very thick, thick, thin and faint. Majority of the " T" clones showed thin and faint bands.

Table 2. Phenol and caffeine contents of thirty-seven clones of *Coffea canephora* clones

Clone	Phenol (%)		Caffeine (%)
	Pulp	Leaves	
A110	3.3d	3.2b	1.4a
A116	15.0a	3.2cb	1.2c
C36	3.3d	4.1b	1.1c
C90	10.8b	4.3b	1.2c
C96	9.9b	3.0cb	1.3b
C105	10.8b	6.3a	1.4a
C107	7.7cb	4.1b	1.4a
C108	6.6c	3.6b	1.3b
C111	11.4b	6.2a	1.2c
E1	3.4d	4.1b	1.4a
E77	7.4c	4.1b	1.3a
E92	8.3cb	5.0a	1.3a
E106	4.4d	5.1a	1.5a
E130	2.6d	3.1cb	1.1c
G87	9.6b	3.8b	1.4a
G129	9.5b	3.6b	1.4a
M10	4.0d	4.3b	1.4a
M31	12.5b	3.4b	1.3a
M36	11.4b	5.1a	1.5a
M53	10.5b	4.7b	1.4a
T4	15.6a	5.4a	1.3a
T24	11.6b	5.2a	1.2c
T45	11.6b	5.1a	1.3a
T93	10.4b	5.0a	1.3a
T116	11.7b	5.4a	1.2c
T164	11.6b	5.6a	1.3a
T169	10.5b	4.9a	1.4a
T204	2.6d	4.6b	1.2c
T314	9.6b	4.6b	1.2c
T365	12.6b	4.6b	1.3a
T921	10.7b	5.2a	1.2c
T1049	12.6b	3.5b	1.3a
T197	8.4cb	3.4b	1.4a
T155	9.2b	4.8a	1.4a
T176	10.4b	5.2a	1.5a
T395	10.1b	5.1a	1.3b
T220	10.5b	4.5b	1.4a
Mean	9.51	4.54	1.31
CV (%)	36.3	19.1	8.4

Means with similar letters are not significantly different ($P < 0.05$) by Duncan's Multiple Range Test.

Discussion

The coefficients of variation among many of the three biochemical characters were low, indicating that the genetic base of the Nigerian *C. canephora* germplasm is very narrow. Studies on intra-specific variation in caffeine content in many *Coffea* species including *C. canephora* showed that caffeine-free coffee was yet to be found (Barre *et al.,* 1998). Low caffeine coffee is, however, increasingly being demanded in the international market (Sreenath, 1997). Heritage (2006) noted that robusta coffee has average caffeine content of 2.2%, while arabica coffee contains an average of 1.2%. The caffeine content among the clones studied ranged between 1.10 and 1.53%. Clone C36 is regular bearing, high yielding and relatively low in caffeine content, hence it is a suitable clone that could be included in any breeding programme for low caffeine coffee in Nigeria. The thirty-seven clones studied showed varying levels of phenol content. According to Bate-Smith (1962) high level of phenol in plants are associated with their tolerance/ resistance to some diseases and insect pests. Romanenko (1985) who noted an average of 4.3% phenol in the leaves of Asian coffee clones, equally observed that clones with high phenolic contents were resistant to attack by the leaf sucking insect *Aphis humilis*. The high level of phenol, in the range of 4.8 to 6.3%, found in some of the clones studied, indicates their potential as parents in breeding for resistance to some major diseases and insect pests of coffee.

All Ghana robusta ("A" clones) had fast protein bands between 6.0 and 9.0 cm. All the "E" and "T" clones however, had their bands in the slow and fast ranges of relative mobility, indicating that they share genetic identity. This is possible, as they had a common ancestry in Java. The Niaollou ("M" clones) robusta

Table 3. Relative mobility of protein bands of thirty-seven clones of *C. canephora*

Clone	Slow bands (0.1-2.9 cm)	Intermediate bands (3.0-5.9 cm)	Fast bands (6.0-9.0 cm)	Total number of bands
A110	-	-	1	1
A116	-	-	1	1
C36	-	-	2	2
C90	1	-	2	3
C96	-	-	3	3
C105	1	1	1	3
C107	1	-	2	3
C108	-	-	1	1
C111	-	3	3	7
E1	1	-	1	2
E77	1	-	2	3
E92	1	-	1	2
E106	1	-	1	2
E130	1	-	2	3
G87	2	1	1	4
G129	2	1	1	4
M10	-	-	2	2
M31	1	2	1	4
M36	-	-	-	-
M53	1	1	-	2
T4	2	-	1	3
T24	1	-	1	2
T45	2	-	-	2
T93	2	-	-	2
T116	1	-	2	3
T164	1	-	2	3
T169	1	-	1	2
T204	1	-	1	2
T314	1	-	1	2
T365	1	-	1	2
T921	1	-	1	2
T1049	1	-	1	2
T197	1	-	1	2
T155	1	-	1	2
T176	1	-	1	2
T395	1	-	1	2
T220	1	-	1	2

- Band absent

haddistinct protein bands in that their bands were spread in the slow, intermediate and fast mobility ranges. A previous report (Omolaja and Fawole, 2004a) on morphological traits corroborates the finding of the present study on the distinct characteristics of Niaollou robusta. Niaollou robusta is characterised by purple young leaves, purple immature berry, multi-petalloid corrola and with few tri-forked stigma, as against other clones. The high yielding clones most of which are found among the Quillou ("C") (Omolaja and Fawole, 2004a) have thick protein bands that are located in the fast range of mobility. Ugandan (G) clones are equally distributed in all the ranges: slow, intermediate and fast, but did not have thick bands.

The variation in the protein banding patterns of the different *C. canephora* clones observed in this study provides more information on the existing genetic diversity of the coffee clones. In the choice of parents for hybridization in robusta coffee in Nigeria, the clones that have the widest of variability in terms of bands' location are Niaollou (M) and Quillou (C), hence the two would be good candidates in *C. canephora* (robusta) improvement programme.

Acknowledgement

The contribution of Prof. Iyiola Fawole, Department of Crop Protection and Environmental Biology, University of Ibadan, during this study is highly appreciated. I thank the Executive Director, Cocoa Research Institute of Nigeria, for his permission to publish this paper.

References

Akinwusi, O. and H.C. Illoh, 1995. Crude protein electrophoresis of seeds of some species of *Hibiscus. Nigerian J. Bot.,* 8: 71-76.

Ashihara, H. and A. Crozier, 2001. Biosynthesis and catabolism of caffeine in low caffeine-containing species of *Coffea*. In: *Book of Abstracts, XIX Colloque International sur la Science du Café (International Coffee Science Association, ASIC)*, Trieste, Italy. May 14th-18th 2001, PB 702.

Barre, P., S. Akaffou, T. Louarn, A. Charrier, S. Hamon and M. Niorot, 1998. Inheritance of caffeine and heteroside contents in an interspecific cross between a cultivated coffee species *Coffea liberica* var. dewevrei and a wild species caffeine-free *C. pseudozanguebariae. Theoretical Appl. Genet.,* 96: 306-311.

Bate-Smith, E.C. 1962. The phenolic constituents of plants and their taxonomic significance. I. Dicotyledons. *Bot. J. Linn. Soc.,* 58: 95-173.

Berthou, F. and P. Trouslot, 1979. L'analyse du polymorphism enzymatique dans in genre *Coffea*: adaptation d'une method d'electrophorese en series: premieres resultants. *Proceeding 5th Colloque International Scientifique sur la Science du Cafe, ASIC*. Abidjan, 28 November – 3 December 1977, pp 373-384

Bonner, J. 1950. *Plant Biochemistry*. Academic Press Inc., New York. 539p.

Borea, P.A., K. Varani, D. Rigamonti, S. Sipione, A. Camurri, F. Cattabeni, M.P. Abbrachio and E. Cattaneo, 2001. Adenosine A_{2A} Antagonists and Huntington" s disease. In: *Book of Abstracts, XIX Colloque International sur la Science du Café (International Coffee Science Association, ASIC)*, Trieste, Italy. May 14th-18th 2001, p503.

Carrens, M.E., E. Fuentes and E.F.Merino, 1997. Seed protein patterns of nine species of *Cactaceae. Biochem. Systematics Ecol.,* 25: 43-49.

Davis, D.J. 1964. Disc electrophoresis II. Method and application to human serum proteins. *Ann. New York Acad. Sci.,* 2 (4): 121-125.

Gepts, P. 1995. Genetic markers and core collections. In: *Core Collection of Plant Genetic Resources*. T. Hodgkin, A.H.D. Brown, Th. J. L. van Hintum, and E.A.V. Morales (eds). John Wiley and Sons, Chichester, U.K. p127-146.

Gottlieb, L.D. 1971. Gel electrophoresis. New approaches to the study of evolution. *Bioscience,* 21(18): 939-944.

Heritage, 2006. Coffee and caffeine in the news. *Newsletter*-The Heritage Coffee Company Limited, London, 15pp.

Horwits, W. 1970. *Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC)*. Published by AOAC, Washington DC, U.S.A., p 236-239.

IIIoh, H.C. 1990. An electrophoretic study of crude protein diversity in the seeds of the genus *Amaranthus. Nigerian Journal of Botany,* 3: 151-158.

International Coffee Organisation (ICO), 1999. Information Bulletin., 15p.

International Coffee Organisation (ICO), 2008. Information Bulletin., 12p.

Montagnon, C., T. Leroy and A.B. Eskes, 1998. Varietal improvement of *Coffea canephora* II. Breeding programmes and their results. *Plantations Recherche Developpement,* 2: 89-98.

Nguyen-van-Tam, D.P. and A.P. Smith, 2001. Caffeine and human memory: A literature review and some data. In: *Book of Abstracts XIX ASIC*, Trieste, Italy. May 14th-18th, 2001. P501.

Odebode, A.C., 1995. Phenolic compounds in the kola nut (*Cola nitida* and *Cola acuminata*) in Africa. *Review of Biology Tropicale,* 44 (2): 513-515.

Omolaja, S.S. and I. Fawole, 2004a. Characterisation of Nigerian Robusta coffee (*Coffea canephora* Pierre ex. Froehner) germplasm. In: *Proc. International Conference on Coffee Science,* Bangalore, India, 2004: 680-683.

Omolaja, S.S. and I. Fawole, 2004b. Determination of the number of Self-Incompatibility alleles (SIA) in *Coffea canephora* and the role of pollen-stylar protein in the expression of SIA. In: *Proc. International Conference on Coffee Science,* Bangalore, India, 2004: 684-687.

Omolaja, S.S. and J.A. Williams, 1997. Harvesting, processing and storage of coffee. In: *Coffee Production Technology Transfer Workshop*. Filani, G.A. and C.R. Obatolu (eds). Publication of Cocoa Research Institute of Nigeria, p 47-49.

Ram, A. S., M. S. Sreenivasan and S. Vishveshwara, 1982. Chemotaxonomy of *Mozambicoffea. J. Coffee Res.,* 12(2): 23-30.

Raju, K.I. and H.H. Gopal, 1979. Distribution of caffeine in arabica and robusta coffee plants. *J. Coffee Res.,* 9: 83-90.

Raju, K.L., M.C. Ratageri, D. Venkataramanan and H.H. Gopa, 1981. Progressive quantitative changes of caffeine and total nitrogen in the reproductive parts of arabica and robusta coffee. *Journal of Coffee Research,* 11(3): 70-75.

Romanenko, T.T. 1985. Polyphenol contents as factors of coffee resistance to *Aphis humili*. *J. Coffee Res.,* 11(5): 11-19.

Sreenath, H.L. 1997. Development of caffeine-free coffee varieties. *Indian Coffee,* 5(2): 13-14.

Vasudeva, N., M.C. Ratageri, D. Venkataramanan and K.I. Raju, 1981. Caffeine, carbohydrates and protein in spent coffee from commercial blends. *J. Coffee Res.,* 11(2): 49-51.

Walyaro, D.J.A. 1983. *Consideration in breeding for improved yield and quality in arabica coffee (Coffea arabica* L.). PhD. Diss., Wageningen Agricultural University. The Netherlands., 1983. 156pp.