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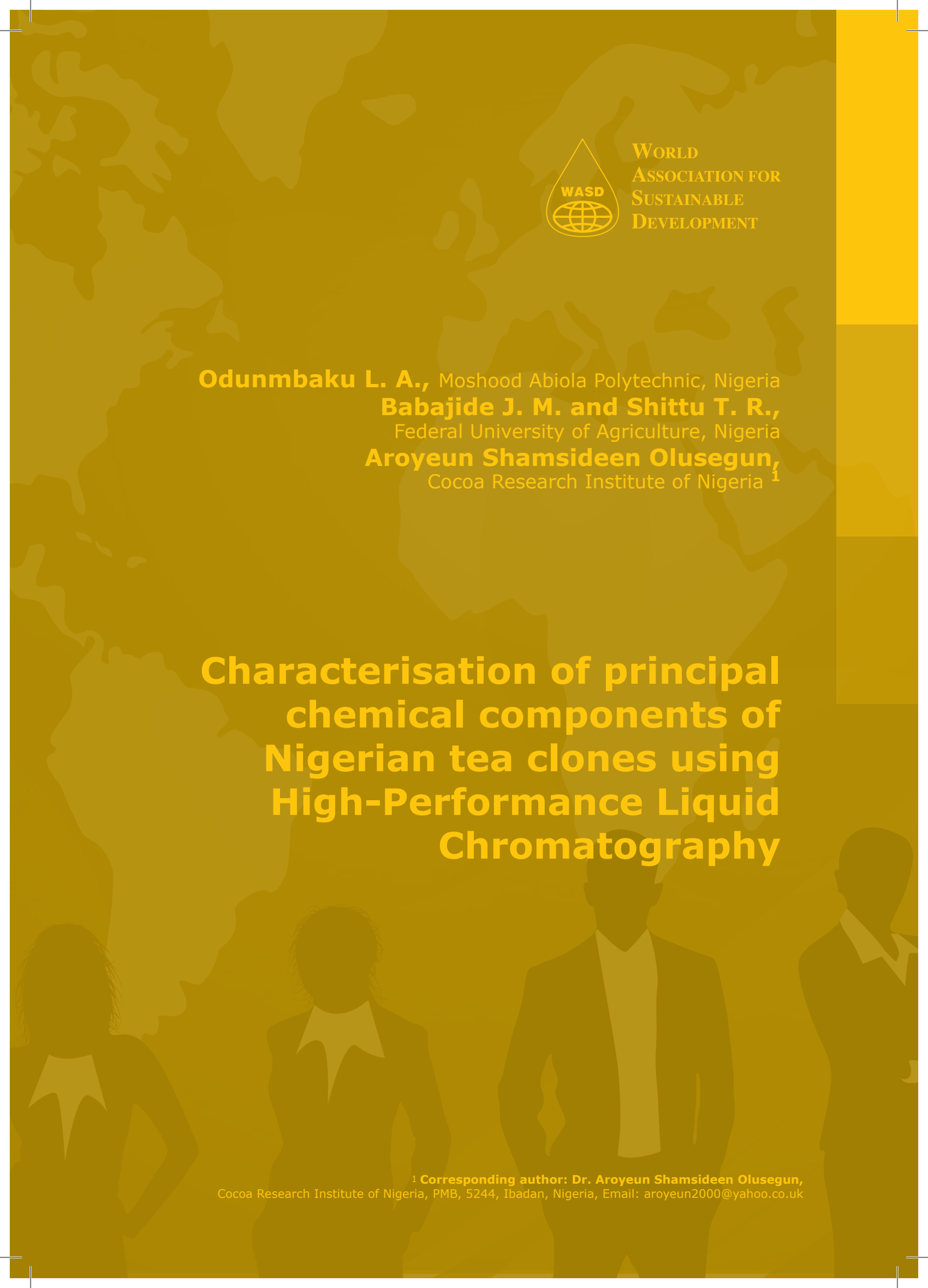
Odunmbaku L. A., Moshood Abiola Polytechnic, Nigeria
Babajide J. M. and Shittu T. R.,
Federal University of Agriculture, Nigeria
Aroyeun Shamsideen Olusegun,[1]
Cocoa Research Institute of Nigeria

Characterisation of principal chemical components of Nigerian tea clones using High-Performance Liquid Chromatography



[1] **Corresponding author: Dr. Aroyeun Shamsideen Olusegun,**
Cocoa Research Institute of Nigeria, PMB, 5244, Ibadan, Nigeria, Email: aroyeun2000@yahoo.co.uk

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Abstract

Purpose To characterise tea clones obtained from Mambilla Highland Nigeria for polyphenols including catechins, EGC, EGCG, EC, ECG and caffeine.

Methodology Ten (10) clonal genotypes of the tea plant, Camellia sinensis (L.) O. Kuntze grown in Nigeria were analysed for epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechingallate (ECG), epicatechin (EC) and caffeine using High-Performance Liquid Chromatography. Fresh apical buds and a leaf were harvested, oven dried at 60oC to %5 moisture, finely size reduced and extracted using water-acetonitrile (1:1, v/v) for 40 min at room temperature. HPLC analysis of catechins was carried out using Agilent Technologies 1120LC compact series, German and Japan. The system comprises a U.V-Vis detector and Hp computer system. Concentrations of catechins of different clonal tea were determined. Data obtained were analysed using SPSS 17.0 version.

Findings There were significant differences ($p < 0.05$) in all five components, among the 10 clones analysed. The range of values obtained for the chemical components EGCG, EGC, EC, ECG and caffeine content were: ,0.16 ± 64.75 - 1.00 ± 11.78 ± 0.61 ,0.22 ± 1.25 - 0.08 ± 0.09 4.66 - 0.30 ± 1.06 ,0.04 ± 6.67 -0.02 0.16 ± and 0.02 ± 2.17 - 0.02 ± 0.60 (mg/g) respectively.

Practical implications This study confirmed the quality characteristics of tea clones available in Nigeria for green tea production.

Originality/value The results revealed that tea clonal materials can be good raw materials for green tea production because the values obtained were within the ISO standard chemical qualities of tea clones meant for green tea production.

Keywords Tea, Catechins, Polyphenols, EGCG, EC, EGC, Caffeine

Introduction

Tea's beneficial health effects are thought to stem from polyphenols with antioxidant properties. Green tea contains polyphenols which include flavanols, flavandiols, and phenolic acids (up to 30% of dry weight). The most important flavonoids are catechins, which are present at about 10% of the dry weight basis (Yamamoto et al., 1997). Six major catechins known to display biological activity are (+)-catechin (C), (_)-epicatechin (EC), (_)-epigallocatechin (EGC), (_)-gallocatechin gallate (GCG), (_)-epigallocatechin gallate (EGCG) and (_)-epicatechin gallate (ECG). These native phenolic compounds are strong antioxidants with antimutagenic, anticarcinogenic, hypocholesterolemic, antibacterial, antiallergic and other clinically relevant activities. Kao et al. (2000) found that among green tea polyphenols, (_)-epigallocatechin gallate (EGCG) especially significantly reduced food intake, body weight, blood levels of testosterone, estradiol, leptin, insulin, insulin-like growth factor I, LH, glucose, cholesterol and triglyceride, as well as growth of the prostate, uterus and ovary.

Green tea also contains methylxanthines: caffeine (1,3,5-trimethylxanthine) and two minor isomeric dimethylxanthines, theobromine and theophylline, which are responsible for the mildly stimulant effects of the tea (Anaya et al., 2006; Cloughley, 1981). Besides being very important factors in tea quality, these compounds are also reported to exhibit beneficial health effects. Selected studies describe the use of caffeine to enhance mental activity and running performance, to treat apnoea and migraine headaches, and implementation of theophylline and theobromine in asthma and bradycardia treatment (Friedman et al., 2006). Tea is grown in at least 30 countries on five continents. In the past two decades the most significant change in tea production has been the development of tea plantations in Africa and South America (International Tea Committee, 1998).

Tea production is highly centralised. In 1993, five countries – India, China, Sri Lanka, Indonesia and Kenya – accounted for 75% of the world production. Most countries produce tea mainly for export, but in India, China, Japan and Turkey about 70% of the tea produced is consumed within the country. Tea is grown on about 2.5 million hectares of land in Asia (89% of global tea cultivated areas) and Africa (8%) (International Tea Committee, 1998). The main tea-producing countries are Asia (Bangladesh, China, India, Indonesia, Sri Lanka and Taiwan), Africa (Burundi, Kenya, Malawi, Mauritius, Rwanda, South Africa, Tanzania, Uganda, Zaire and Zimbabwe), and South America (Argentina and Brazil). In addition, Russia and a number of CIS countries also produce a large quantity of tea.

Table 1 presents the world tea production from 1961 to 2004. In Nigeria, tea is exclusively grown in the humid, high altitude regions of Mambilla Plateau, Taraba State and Obudu in Cross River State (Esan, 1982). This plateau is Nigeria's northern continuation of the Bamenda Highlands of Cameroon. It has an average elevation of about 1,524 metres (5,000 ft) above sea level, making it the highest plateau in Nigeria, since no other plateau in Nigeria rivals it in elevation. Some of its villages are situated on hills that

must be at least 1,828 metres (5,997 ft) above sea level. The Nigerian Beverages Production Company (NBPC), Mambilla Plateau, introduced tea clones from Kenya into Nigeria for commercial planting in 1972 (Hainsworth, 1981). The tea clones were later acquired by the Cocoa Research Institute of Nigeria (CRIN) for research purposes in 1982 (Esan, 1982). In spite of the insufficient/inadequate resources, and given the low level of funding of research in Nigeria, the Institute in the last 41 years of its existence has been able to assess thirty-three (33) highland clonal tea genotypes based on growth performance and yield. Ten of these have been recommended for commercial cultivation. There is therefore a dearth of information on the chemical constituents of Nigerian tea clones.

The main objective of this study was to characterise the catechin constituent of Nigerian tea clones.

Table1. *Theory of Planned Behaviour is widely used to explain environmental behaviours. Source: Adapted from Ajzen (1991)*

	YEAR						
	1961	**1971**	**1981**	**1991**	**2001**	**2003**	**2004**
World	983,785	1,308,424	1,885,907	2,561,050	3,065,927	3,209,831	3,341,827
China	97,064	179,984	368,223	562,961	721,536	788,815	855,192
India	354,397	435,468	559,583	720,300	848,400	846,000	850,500
Sri Lanka	206,488	217,773	210,148	240,747	295,090	303,230	308,090
Kenya	12,641	36,290	90,941	203,588	294,620	293,670	295,000
Turkey	5,450	33,585	42,606	136,887	142,900	153,800	201,663
Indonesia	77,100	60,922	109,135	139,520	163,068	169,818	164,817
Vietnam	7,500	15,500	21,178	33,100	75,700	99,750	108,422
Japan	81,527	93,111	102,300	87,800	85,000	92,000	101,000
Argentina	6,486	29,900	22,785	46,075	62,775	63,775	64,000
Bangladesh	26,542	12,007	38,772	45,012	52,000	56,833	55,627
Iran	10,922	16,000	33,100	42,091	51,160	52,000	52,000
Malawi	14,288	18,597	31,965	40,501	36,800	41,693	50,090
Uganda	5,100	18,000	1,700	8,877	8,877	36,895	36,000

Source: FAOSTATDatabase

MATERIALS AND METHODS

Materials

Tea shoots sampling

Tea shoots comprising apical bud and a leaf were harvested from ten (10) established commercial clones from the Cocoa Research Institute of Nigeria (CRIN) experimental plots, Mambilla plateau, Taraba State, Nigeria. The plucked shoots were dried in the oven at 60OC to constant weight, while the dried samples were ground and sieved prior to further infusion and instrumental analysis.

Chemical Standard Epigallocatechingallate, epigallo catechin, epicatechin gallate, epicatechin, and caffeine standards were sourced from Sigma Aldrich Chemical Co. (USA). HPLC grade acetonitrile, ethyl acetate and methanol were obtained from Merck, Germany.

Characterisation of constituents

High-Performance Liquid Chromatography (HPLC) methods were used to determine catechins present in the fresh tea leaves. These are: catechins, epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechingallate (ECG) and epicatechin (EC) (Del Rio et al., 2004; Friedman et al., 2005). Ground tea leaf samples were extracted using methanol and distilled water (95:5%) for 40 min at room temperature (Agilent, 2012). Solutions were filtered using a Millipore filter of 0.45um size. Filtered samples were poured into vial bottles and 20µl each were programmed for injection twice per sample for HPLC auto sampler analysis. Stock solutions of caffeine, catechin, epicatechin and epigallocatechingallate were prepared by dissolving reference standards into a mobile phase. Less concentrated solutions were prepared, as needed, by dilution in the same mobile phase.

Results and discussion

Table 2 shows the mean values and the standard error of means for the five major antioxidants analysed in the Nigerian tea clone. There were significant differences ($p \leq 0.05$) among the ten clones. Saravanan et al. (2005), Owuor and Obanda (2007) reported that the content of individual catechins in fresh tea leaves varies with the tea clones. Caffin et al. (2004) also reported that the distribution pattern of polyphenols varied a great deal among tea clones, whereas the patterns appeared to be more or less fixed within a clone. Similarly, total polyphenol content in green leaves showed minimal variations within the same clone but significant differences among clones (Obanda and Owuor, 1997). Standard calibration plots for EGCG, EGC, EC, ECG and caffeine gave a coefficient of multiple determination (r2) values of 0.9486, 0.9694, 0.9963, 0.9963 and 0.9986 respectively (see Figures 1–5). The range of values obtained for the chemical components EGCG, EGC, EC, ECG and caffeine content, based on the standard equation from the calibration plots, were: 11.78 ± 1.00 - 64.75 ± 0.16, 0.09 ± 0.08 - 1.25 ± 0.22, 0.61 ± 0.02- 6.67 ± 0.04, 1.06 ± 0.30 - 4.66 ± 0.16 and 0.60 ± 0.02 - 2.17 ± 0.02 (mg/g) respectively.

Table 2. *Mean antioxidant content of selected tea clones (mg/g)*

CLONE	EGCG	EGC	EC	ECG	Caffeine
23c	11.78 ± 1.00^{a}	0.03 ± 0.00^{a}	1.01 ± 0.02^{a}	3.82 ± 0.12^{d}	1.02 ± 0.26ab
68	27.29 ± 0.60^{b}	0.08 ± 0.04^{a}	0.61 ± 0.02^{a}	1.11 ± 0.01^{a}	0.60 ± 0.02a
25	31.69 ± 1.20^{c}	0.34 ± 0.08^{ab}	1.72 ± 0.02^{b}	2.37 ± 0.06^{b}	1.89 ± 0.00b
228	32.10 ± 0.68^{c}	0.09 ± 0.08^{a}	0.87 ± 0.05^{a}	2.35 ± 0.13^{b}	1.25 ± 0.09b
108	39.96 ± 0.36^{d}	0.64 ± 0.40^{abc}	1.75 ± 0.03^{b}	3.06 ± 0.05^{c}	2.01 ±0.11c
35	41.21 ± 0.49^{d}	0.92 ± 0.41^{bcd}	1.13 ± 0.05^{a}	4.50 ± 0.04^{e}	1.85 ± 0.01c
148	43.13 ± 0.39^{e}	1.02 ± 0.50^{cd}	1.08 ± 0.52^{a}	1.06 ± 0.30^{a}	1.38 ± 0.31b
357	44.64 ± 0.76^{f}	0.55 ± 0.00^{abc}	6.67 ± 0.04^{c}	2.06 ± 0.08^{b}	2.17 ± 0.02cd
238	44.91 ± 0.16^{f}	0.27 ± 0.04^{a}	1.89 ± 0.64^{b}	3.15 ± 0.24^{c}	2.09± 0.03cd
318	64.75 ± 0.16^{g}	1.25 ± 0.22^{d}	1.91 ± 0.03^{b}	4.66 ± 0.16^{e}	2.51 ± 0.44e

(P≤0.05)

Figure 1. *Standard calibration plot for EGCG*

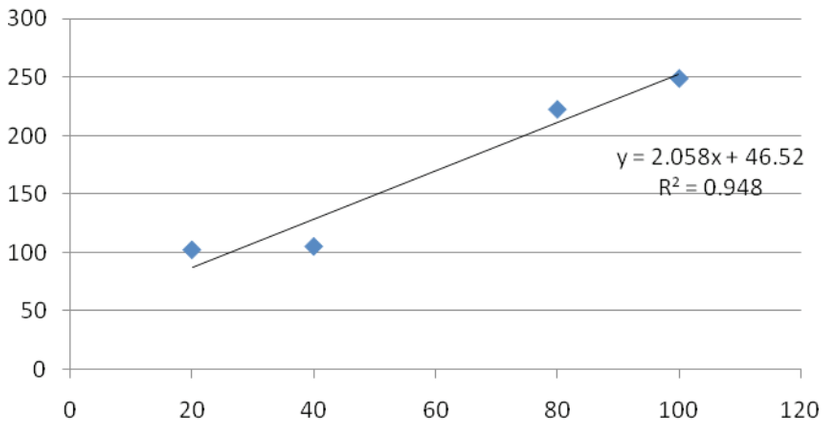


Figure 2. *Standard calibration plot for EGC*

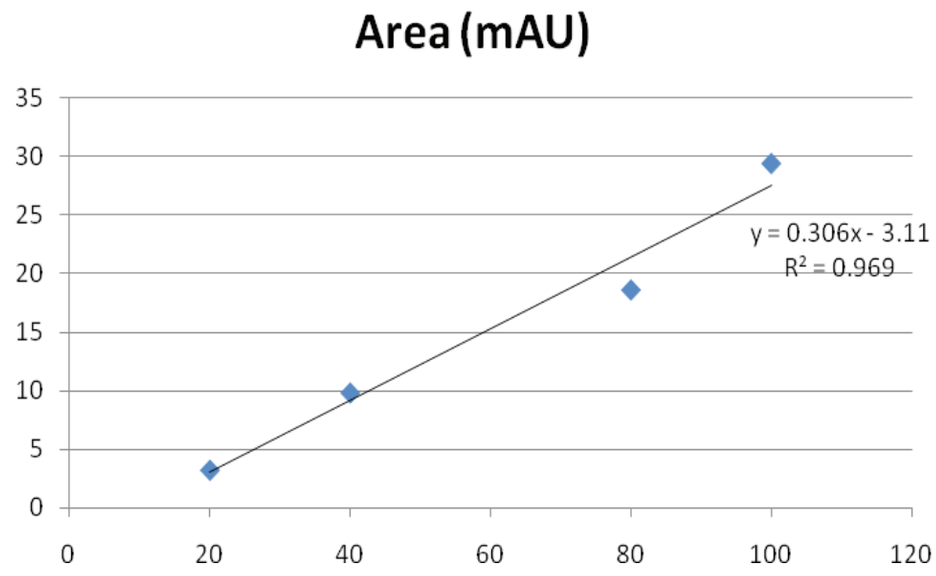


Figure 3. *Standard calibration plot for EC*

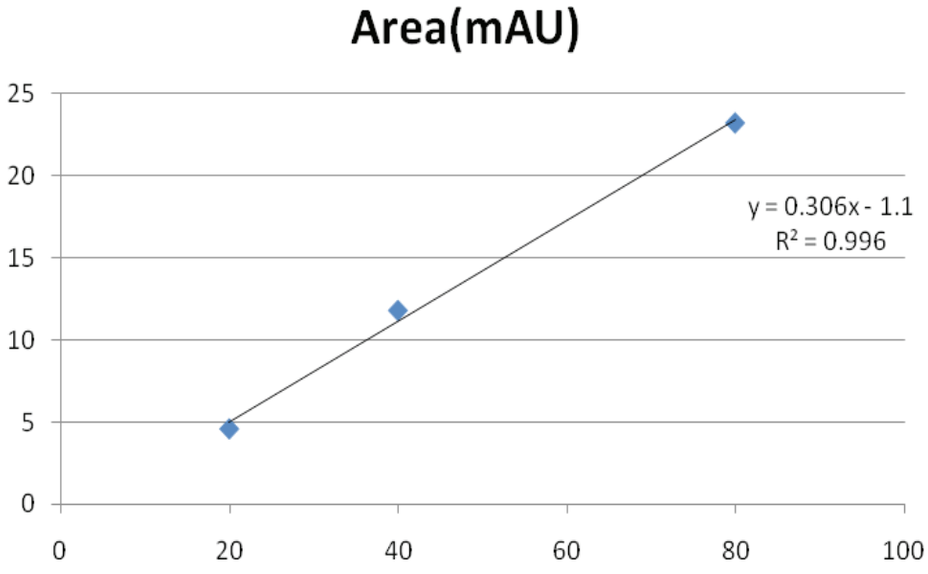


Figure 4. *Standard calibration plot for ECG*

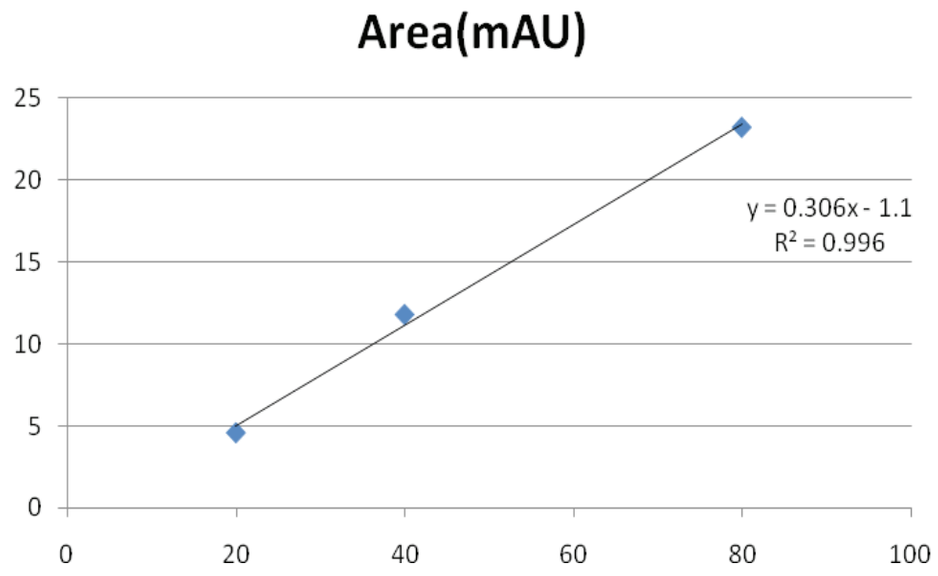
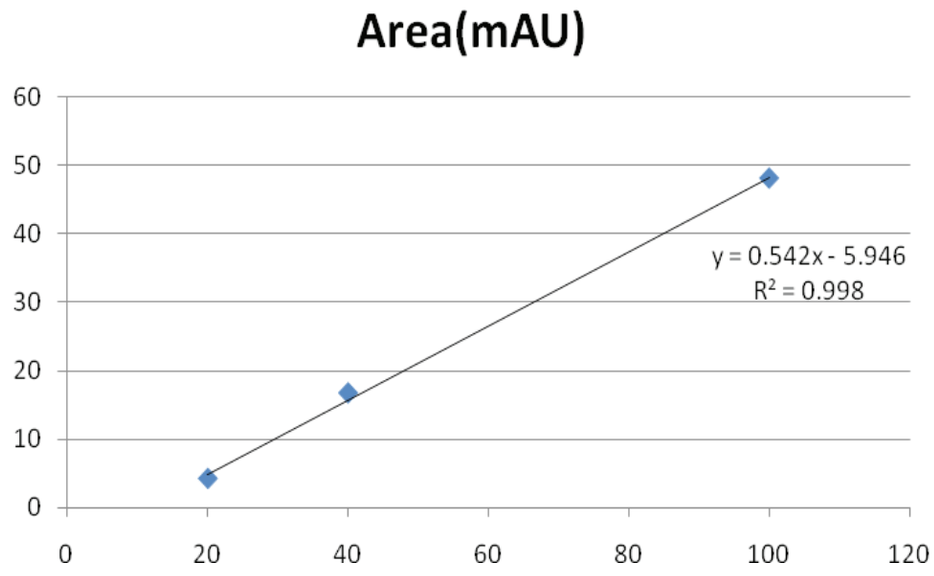


Figure 5. *Standard calibration plot for caffeine*



References

Agilent, (2012) *The Essential Chromatography & Spectroscopy, 2011/2012 edition. Agilent Technologies.*

Anaya, A.L., Cruz-Ortega, R. and Waller, G.R. (2006) *"Metabolism and ecology of purine alkaloids", Frontiers in Bioscience, 11, pp. 2354-2370.*

Cloughley, J.B. (1981) *"Storage deterioration in Central African tea: The effect of some production variables on theaflavin degradation", Journal of the Science of Food and Agriculture, 32, pp. 1229-1234.*

Friedman, M., Levin, C.E., Choi, S-H., Kozukue, E. and Kozukue, N. (2006) *"HPLC analysis of catechins, theaflavins, and alkaloids in commercial teas and green tea dietary supplements: Comparison of water and 80% ethanol/water extracts". Journal of Food Science, 71, C328–C337.*

Caffin, N., D'Arcy, B., Yao, L. and Rintoul, G. (2004) *"Developing an index of quality for Australian tea". RIRDC Publication No. 04/033, Project No. UQ- 88A, Publication of Rural Industries Research and Development Corporation, p. 192, Australia.*

Del Rio, D., Stewart, A.J., Mullen, W., Burns, J., Lean, M.E.J. and Brighenti, F. (2004) *"HPLC-MSn analysis of phenolic compounds and purine alkaloids in green and black tea". Journal of Agricultural and Food Chemistry, 52, pp. 2807-2815.*

Esan E.B, (1982) *"Acquisition of tea germplasm". In: Annual Report of the Cocoa Research Institute of Nigeria (CRIN). p. 9.*

FAO (Food and Agric. Organisation) (2009) *http://faostat.fao.org/agricultural production.*

Friedman, M., Kim, S.Y., Lee, S.J., Han, G.P., Han, J.S. and Lee, K.R (2005) *"Distribution of catechins, theaflavins, caffeine, and theobromine in 77 teas consumed in the United States", Journal of Food Chemistry and Toxicology, 70(9), C550–C559.*

Hainsworth E. (1981) *"Tea production on the Mambilla Plateau, Gongola state (now Taraba State), Nigeria". A report on the project by the consultant for the Nigerian Beverages Production Company Limited. p. 26.*

Kao, Y.H., Hiipakka, R.A. and Liao, S. (2000) *"Modulation of endocrine systems and food intake by green tea epigallocatechin gallate". Endocrinology, 141, pp. 980-987.*

Obanda M. and Owuor P.O. (1997) *"Flavanol composition and caffeine content of green leaf as quality potential indicators of Kenyan black teas". Journal of the Science of Food & Agriculture, 74, pp. 209-215.*

Owuor, P.O. and Obanda, M. (2007) *"The use of greentea (Camellia sinensis) leaf flavan-3-ol composition in predicting plain black tea quality potential", Food Chemistry, 100, pp. 873-884.*

Saravanan, M., Maria John, K.M., Raj Kumar, R., Pius, P.K. and Sasikumar, R. (2005) *"Genetic diversityof UPASI tea clones (Camellia sinensis (L.) O.Kuntze) on the basis of total catechins and theirfractions", Phytochemistry, 66, pp. 561-565.*

About the authors

Dr. Aroyeun Shamsideen Olusegun is a Chief Research Officer at the Cocoa Research Institute of Nigeria, Ibadan. As a member of WASD, Dr Aroyeun has attended WASD conferences held in New Jersey (US), and Abu Dahbi, United Arab Emirates. A prolific writer, Aroyeun has publications in many scientific journals and conference proceedings to his credit.

Babajde J. M. is a senior lecturer in d department of food science and Technology of University of Agriculture Abeikuyta. She specialised in Food Processing and preservation. She has many scholarly publications to her credit.

Dr Shitui T. R. is a brilliant lecturer whose statistical knowledge and applications of data mining in experimental projects has earned him significant recognition in Food Technology. He is a brilliant scientist with track records in Food engineering, Food processing and. Preservation. He is a highly prolific writer with many international journal publications to his credit. etc.

Mr Odumbaku L. A. is a lecturer at Moshood Abiola Polytechnic, Abeokuta. He is. a doctoral student in the Food Science and Technology department of the University of Agriculture. He is married with children