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CRUDE FIBRE, WATER EXTRACTS, TOTAL ASH, CAFFEINE AND MOISTURE CONTENTS AS DIAGNOSTIC FACTORS IN EVALUATING GREEN TEA QUALITY

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Abstract: *Purpose* – This experiment was carried out to diagnose the qualities of green tea form Nigeria using quality markers including crude fibre, water extracts, total ash, caffeine and moisture content respectively.
Design/methodology/approach – Green tea was processed from seven different tea clones from Mambilla Highland located 1840m above mean sea level. The clonal materials used for green tea followed standard Chinese methods viz: plucking, fixing (pan firing), rolling and drying. The green teas produced were analyzed for moisture content, MC; crude fibre, CF; water extracts, WE; total ash, TA; water-soluble ash, WSA; alkaline-insoluble ash, ALIA and acid-insoluble ash, AIA. The quality of the green tea produced was compared to the recommended ISO standard for tea.
Findings – Our findings showed that there were significant differences ($p<0.05$) in the chemical components studied as a result of clonal variations, which made the quality characteristics of the green tea vary accordingly. Of all the tea clones examined except clone 236, WE were within values recommended for good quality green tea as speculated by ISO 3720, and percentage MC was between 4.11–12.5 per cent, with an average of 10.2 per cent. The high MC was due to the high humidity of the processing environment, where sunlight was inadequate and farmers were not patient enough to dry the tea samples to the recommended moisture level of 6.5 per cent. The percentage CF ranged between 4.37–20.8 per cent; the ALIA 0.90–1.30 per cent; TA, 4.2–6.5 per cent; WSA, 50–67 per cent; WE 21.7–43.6 per cent and the caffeine content was within 1.29–2.56 per cent.
Originality/value – The materials used in this study were obtained locally.
Practical implications – The study showed that the determination of quality markers for green tea can be done routinely with reduced costs.

Keywords: *Tea clones, Green tea, Black tea, Crude fibre, Water extracts, Caffeine*

Paper type Research paper

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INTRODUCTION

Crude fibre in plants originates from certain structural units: cellular walls, sclerenchyma, collenchymas and transporting tissues. Young cells have thin cellular walls that become hard as the plant grows and provide the plants with protection from ants, excess transpiration and the influence of other undesirable factors (Strasburger, 1962). Fibre is composed of many different compounds, in particular cellulose, hemicelluloses and wood-wool. Most of these are polysaccharides with the exception of wood-wool, which is a hydrocarbon. The content of crude fibre in young tea leaves is much less than in older leaves. The fundamental (and one of the oldest) definitions of crude fibre states that it is the residue after treating with boiling 0.255N sulfuric acid and 0.313N sodium hydroxide (Bartnikowska, 1997). Crude fibre is the main part of the insoluble matter of black tea. The fibre content can vary and ranges between 7 and 20 per cent in tea products. In general, when the fibre content of tea increases, most of the quality characteristics components decrease (Fermuz *et al.*, 1993). Coarse plucking and aged leaves result in higher levels of fibre. Coarse leaves and stalks are mixed with the finer parts of the shoots during the different rolling processes and it is very difficult to remove all of them from tea in later processes.

Tea is one of the oldest beverages in the world and is the second most popular beverage after water (Smiechowska and Dmowski, 2006). Its quality is determined by aroma and taste properties, among other things. Much attention has been drawn to the fact that crude fibre is an important parameter in the quality evaluation of tea and influences its sensory properties. Another very important parameter that determines tea quality, according to Fermuz *et al.* (1993), is water extract (WE). A tea shoot of two leaves and a bud contains about 25 per cent solid matter. About half of the solid matter is soluble in water and this is termed “extract”. Many components of the liquor influence tea quality. The amounts of these components generally increase in proportion to the amount of tea extract. Thus, the percentage of extract is limited by the International Standard Organization (ISO). The amount of extracts decreases as plucking interval lengthens and also decreases due to coarse plucking and the ageing of the leaf (Smiechowska and Dmowski, 2006). Caffeine is an alkaloid that can constitute approximately 5 per cent of the dry matter in fine tea shoots. Tea is widely consumed all over the world and caffeine plays a very important role in explaining why tea is used as a beverage, because it has a stimulating effect on the human body.

A high content of caffeine contributes to briskness, which is one of the factors indicating quality tea. The caffeine content of green tea increases after harvesting, particularly during the withering process. Some authors have reported that different rolling processes do not affect the caffeine content of processed black tea. Green tea (unfermented tea) is a major beverage in Asian countries such as China and Japan, whereas black tea (fermented tea) is more popular in other parts of the world. Although green tea and black tea are made from the leaves of the same plant, *Camellia sinensis*, differences in the processing of the leaves results in differing chemical components. This study is the first report on green tea production in Nigeria—there are no previous reports in the literature—and was, designed with the objective of diagnosing the quality of green tea processed from Nigerian tea clonal materials using quality markers such as crude fibre, caffeine, moisture content, water extracts and total ash according to the international quality standards for tea.

MATERIALS AND METHODS

Green tea samples used in this study were processed on the Mambilla Highland from seven different tea clones: 35, 68, 228, 318, 363, 61 and 236 respectively. The fresh tea leaves used in the green tea production were plucked from vegetative propagation (VP) tea fields at the Cocoa Research Institute of Nigeria (CRIN) substation, Kusuku, Mambilla, located around 1840m above mean sea level, latitude 6^{o} 24´ N and longitude 10^{o} 35´E. The plants were grown under recommended agronomic conditions. The tea clones were plucked at the tea plantation and processed by the miniature green tea processing facilities. The plucked tea leaves were withered, and this process was shortly followed by fixing to inactivate the activities of the polyphenol oxidase enzyme, using the Chinese method of pan fixing. The pan-fixed leaves were later rolled and dried using an oven. The green tea produced was then packaged in paper board boxes and reserved for analysis.

PREPARATION OF THE TEA SOLUTION

The tea preparation consisted of addition of boiling water (200ml) to leaf tea (2g); the tea solution was then filtered through cotton wool and the residue was washed with distilled water (30x10ml). The tea solution was cooled to room temperature and washings were diluted 250ml with distilled water. The samples were analyzed in triplicate.

CAFFEINE AND WATER EXTRACTS

The methods used for the analyses of caffeine and water extracts of the tea solution were based on international standards (ISO 1839), 1980; (ISO 9768, 1994); (Yao *et al.*, 1992), as stated below:

CAFFEINE

Lead acetate solution $(CH_3COO)_2Pb$100g was dissolved and diluted to 200ml with distilled water. Hydrochloric acid solution, HCl36%, specific gravity, 1.18, 0.9ml was diluted to 100ml with distilled water.

SULPHURIC ACID SOLUTION

Sulphuric acid ([98%] H_2SO_4, specific gravity, 1.84, 16.7ml) was diluted to 100ml with distilled water.

Measurements: Tea solution (10ml), HCl (5ml) and lead acetate solution (1ml) were mixed in a 100ml volumetric flask and diluted with distilled water. The solution was then filtered through Whatman No1 quantitative filter paper. The filtrate (25ml) and sulphuric acid solution (0.3ml) were placed in a volumetric flask and diluted to 50ml with distilled water. The solution was filtered using the same type of filter paper. The absorbance of the filtrate was measured using Ultraspec III UV/visible spectrophotometer at 274nm. The measurement was performed in triplicate.

STANDARD CURVE

Caffeine stock solution (10ml), 1mg/ml, w/v in distilled water was diluted to 200ml with distilled water. Next, 0, 10, 20, 30, 40 or 50 ml of the diluted caffeine solution were separately mixed, each with hydrochloric acid solution (4ml) in a volumetric flask and diluted to 100ml with distilled water. Thereafter, the remaining steps were repeated as described earlier. The readings of the standard solution against the concentrations were used to prepare the standard curve.

CALCULATION

Caffeine (%) = $E/1000 \times V_0 \times (100/V_1) \times (50/25)/W = 0.2EV_0/V_1/W$

where E is 'mg' of caffeine from the standard curve against the reading of the spectrophotometer and E/1000 is to convert 'mg' into 'g'. V_0 is the total volume of the tea solution (250ml); V_1 is the volume used for the measurements (10ml), and 100/V1 indicates 10ml tea solutions that were diluted to 100ml, while 50/25 shows that another dilution from 25ml tea filtrate was made to 50ml in the measurement. W is the dry weight of the tea sample.

WATER EXTRACTS

Measurements: Tea solution (50ml) was placed in a weighed evaporating dish and was then evaporated to dry over a water bath. The residue (tea extracts) in the dish was fully dried in a vacuum oven at 75^{O}C with a negative pressure of 65kPa for 4h until the weight of the dish with extract was constant.

CALCULATION

Water extract (%) = $(D_1\text{-}D_0) \times V_0 \times 100/V_{1/}W$

Where D_1 is the weight of the dry tea extracts with the dish; $D_{0,}$ is the weight of the dish, V_0 is the total volume of the tea solution (250ml), V_1 is the volume used for the analysis (50ml); W is the dry weight of the tea sample moisture content.

The tea moisture was measured using a vacuum oven based on an international standard method: ISO 1573, BS 6049-2, 1984.

RESULTS AND DISCUSSION

One of the parameters described in quality norms is crude fibre content. According to Table 1, the averages of the crude fibre contents ranged between 4.3–20.8 per cent dry matter for the tea clones from Nigeria. The crude fibre contents were generally similar in value to those determined in Turkish black tea samples as reported by Fermuz *et al.* (1993). The crude fibre of tea has been linked to shooting period. Black tea from the first shooting period has been reported to have the lowest amount of fibre whereas the fibre contents were found to be higher in tea from the third shooting period. This result indicates that the seven tea clones used in this study differed significantly in crude fibre, suggesting large generic

	Tea clonal varieties							
Chemical parameters	228	318	68	35	61	363	236	ISO standard
% water extracts	42	42	42	43.46	42	21.7	42	Minimum 32
% total ash	6.5	6.2	6.3	4.3	4.2	6.3	6.3	Maximum 8 Minimum 4
% water-soluble ash	66.6	67	66.6	50	50	67	67	Minimum 45
% acid-insoluble ash	0	0	0	0	0	0	0	Maximum 1.0
alkaline-insoluble ash	1.1	1.1	1.00	0.90	0.70	1.30	0.90	Maximum 3.0 Minimum 1.0
% crude fibre	8.35	8.35	12.5	8.6	20.8	20.8	4.37	Maximum 16.5
% moisture content	8.94	6.72	7.11	5.64	6.22	7.20	4.11	Maximum 6.5
% caffeine	2.18	2.56	1.29	1.68	1.37	1.96	2.39	

Table 1 Chemical composition of green tea processed from seven tea clones

Countries	n	Range	x ±SD
China	10	10.96-12.92	11.49±0.89
India	12	9.56-27.89	15.24±7.91
Malawi	12	5.83-43.27	21.08±15.70
Nigeria	27	4.3-20.8%	10.3±0.12

n = no of samples; x = mean value; SD = standard deviation

Table 2 Crude fibre (g/100g) of tea from different countries

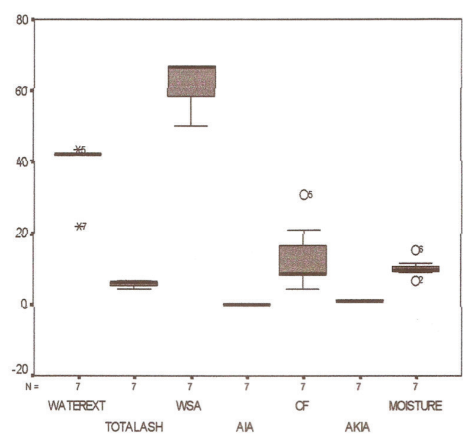

Figure 1 Box plots of the physicochemical characteristics of different tea clonal materials used in this study

variations (Magoma *et al.*, 2000). Indeed, the significant differences ($p<0.05$) in crude fibre levels suggest that the quality potential of these clones could be different. It is noteworthy that the quality requirements for black tea states that crude fibre content should not exceed 16.5 per cent (ISO 3720, 1986). From the data collected in our own studies, it appears that on average, the least crude fibre was found in clone 236, whereas the highest crude fibre was recorded only for both clones 61 and 363. In all the seven tea clones analyzed, only these two clones deviated from the ISO standard 3720–1986 for crude fibre. From our data, it was obvious that minimum crude fibre from clone 236 was still better than the minimum from China, and the maximum was recorded for samples from Malawi. Moreover, the extent of crude fibre in tea from Malawi ranged very drastically from 5.83 to 43.27 per cent. Smiechowska and Dmowski, 2006) reported that crude fibre can be an accurate parameter for the description of tea age. The younger the tea leaves used to process the green tea, the lower the fibre content.

In so-called leafy teas, a significant content of fibre in the products may show that lower quality material has been used in the production (5th to 6th leaf) due to high contents of careless mechanical harvest of tea when the yield contained not only leaves but also stems. This factor may not be significant in this study, as leaves plucked were strictly one leaf plus a bud for green tea. It could be suspected that the maturity of the tea used in the experiment might not have been consistent. The genetic nature of the tea clones might also be responsible.

Data on water extract, caffeine, moisture content and, crude fibre provide very important physical and chemical parameters as well as economic in screening tea clones for green tea and black tea processing. The water extracts found in this study ranged between 21.7–43.4 per cent, giving a mean of 39.3 per cent. This is in agreement with previous studies (Fermuz *et al.*, 1993). According to the international standard, the water extract of a tea is the soluble matter extracted from a test portion by boiling water under conditions specified in the international standard expressed by mass on a dry basis (ISO 9768, 1994).

In this study, the water extracts of all the tea clones examined except clone 236 conformed to the ISO 3720, 1986 standard, which stipulates that the water extract in tea should be more than or equal to 32 per cent of the dry mass. The water extracts of green tea produced from other countries are as follows: China: 36.79 per cent; India, 36.89–41.95 per cent. However, the teas in this study had water extract values lower than those of tea from Sri Lanka (36.72–46.9 per cent) and Kenya (44.12 per cent). It is, however, established that the mean contents of water extract in green tea from Nigerian tea clones as evidenced in this study is relatively high and compared favourably with the water extracts from leading tea-producing countries of the world. The mean content of water extracts in all the tea clones were within those reported by Yao *et al.* (2006), who reported 39.18 per cent in six green tea bags from Australian supermarkets. The similarity in the values obtained in our experiment is not unexpected, as water extracts from black and green tea cannot be the same, green being reportedly higher than black tea due to differences in manufacturing conditions leading to decomposition of tea components to different degrees (Hara *et al.*, 1995; Harbowy and Balentine, 1997).

MOISTURE CONTENT OF GREEN TEA

Moisture content is an important quality parameter of tea (Roberts and Smith, 1963) and is usually neglected by researchers, but not by the industries or tea traders. Tea researchers (Othieno and Owuor, 1984; Robinson and Owuor, 1992) have suggested that the moisture content of tea should be controlled to remain under 6.5 per cent for marketing teas, whereas Millin (1987) noted that teas had a moisture content of 7–85 per cent during retailing.

In this study, the mean moisture content obtained was 10.17 per cent, which was above 6.5 per cent and deviated from the recommended standard for good quality green tea. The high level of moisture in our studied samples was due to the high humidity of the processing environment, as dried tea picked up moisture during the holding time between production and analysis. In this study, the variation in tea clones did not have any significant effects on the moisture contents. The most important factor for the recorded moisture was the high humidity of the processing environment.

The main implications of these findings are that high moisture content will produce negative effects on the shelf life of the green tea produced. There is, however, a need for improved quality control for the drying and packaging of green tea produced in Nigeria. The dried tea leaves must be packaged in a moisture barrier material to prevent moisture pick-up after drying. As for water-soluble extracts (WSE), the ISO 9768 standard is minimum = 32. All the tea clones have a higher level than this except clone 363, which had only 2.5 per cent WSE. The total ash of the clones varied according to variations in clones and ranged between 4.2–6.65, falling within the recommended ISO 1575 standard for total ash of good quality green tea (minimum 4, maximum, 8). The water-soluble ash (WSA) of the clones were all within the recommended ISO 1576 standard, minimum = 45 per cent. The acid-insoluble ash (AIA) levels of all the tea clones were also appropriate when compared to ISO 5998, apart from clone 61 and clone 363, which had high values of 20.8 per cent each when compared to recommended levels.

CAFFEINE

The caffeine contents varied significantly according to the tea clones. The green tea caffeine for clones 228, 318 and 236 were within the standard level of 2–5 per cent. Other clonal varieties, such as clone 61, 35, 68 and 363 were relatively low in caffeine. Caffeine is an important component of tea. Green tea contains abundant caffeine, which is an alkaloid that can have negative effects on the human body depending on the level of intake. Less than 300mg intake per day is not harmful for adults, but an intake of more than 500mg has been shown to cause excessive excitation in the central nervous system and cause arrhythmia and vertigo (Paspa and Vassalle, 1984; Seale *et al.*, 1984).

Since not everybody can withstand strong caffeine in the system, clones 61, 35, 68 and 363 can be considered good material for producing green tea, with clone 61 being the best. It is noteworthy therefore that close attention should be paid to caffeine intake by children and pregnant women, because it is slowly metabolized and can remain in the body for a prolonged period (Giannelli *et al.,* 2003; Rasch, 2003). Accordingly, the removal or marked reduction of the caffeine content of green tea has been actively attempted by the establishment of a new technique called decaffeination in the manufacturing process. Methods using organic solvents, supercritical carbon IV oxide and hot water have been reported for the decaffeination of green tea (Lee *et al.,* 2007; Liang *et al.*, 2007; Park *et al.*, 2008; Park *et al.*, 2007).

In conclusion, the combination of good yields coupled with the processing potential of the clone should be used as a basis for plant breeders to develop a clone that can be recommended for use in the processing of green tea with minimum caffeine contents.

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