

Journal of Food Processing & Technology

Open Access

Effect of Process Variables on the Chemical Constituents and Sensory Characteristics of Nigerian Green Tea

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Abstract

Green tea possesses functional properties with attendant health benefits, Nigerian tea leaves has only been commercially processed into black tea. This study therefore evaluated the effect of Steaming Time (ST), Drying Temperature (DT) and Drying time (Dt) on the chemical and sensory properties of Nigerian Green Tea (NGT). Epical bud and two leaves from agronomic proven commercially viable clone were harvested from the Cocoa Research Institute of Nigeria experimental tea plots, Taraba State. Response surface methodology (Central Composite Design) was used to combine the three processing variables: ST (60, 90 and 120 s), DT (60, 65 and 70°C) and Dt (90, 120 and 150 min). Epigallocatechin gallate (EGCG), Epigallocatechin (EGC), Epicatechin gallate (ECG) and Epicatechin (EC) contents of NGT were determined using High Performance Liquid Chromatography while descriptive sensory evaluation of NGT samples was carried out using semi trained panellist. Data generated were subjected to ANOVA and regression analysis. Results showed that NGT contain EGCG, EGC, EC and ECG contents that ranged from 46.90 to 178, 0.30 to 4.24, 1.03 to 8.83, and 8.05 to 33.96 (mg/g), respectively. Greenness, sweetness, bitterness, and astringency score of NGT extracts were 4.00-6.00, 1.00-2.23, 5.07-7.97 and 1.00-2.23 respectively on a 1-9 intensity scale. This study revealed that acceptable green tea can be obtained from Nigerian tea leaves in terms of chemical constituents, especially for the high EGCG content. The optimum process conditions for NGT were steaming for 60 s and drying at 70°C for 150 min for high EGCG content and sensory acceptability.

Keywords: Camellia; Catechin; Optimization; Polyphenol; Tea

Introduction

Teas are classified into three major types depending on the manufacturing process: 'non-fermented' green tea; 'semi-fermented' oolong tea and 'fermented' black tea [1,2]. Green tea has long been exclusively consumed by the Chinese and Japanese, with cultural ties dating to the first millennium A.D. and, in particular, to tea ceremonies from the twelfth century. Gradually, green tea has received global acceptance due in part to the special health characteristics that have become more widely known through extensive scientific study.

Green tea has been considered a medicine and a healthful beverage since the olden days. The traditional Chinese medicine do recommend tea plant for headaches, body aches and pains, digestion, depression, detoxification, as an energizer and in general, longevity of life.

Several studies have established that, within each category of tea, differences in characteristics exist due to factors such as differences in the processing methods, stage of maturity of tea leaves at harvest, type of tree species, and the region where the tea was cultivated [3-5].

Tea importation to Nigeria has continued to increase steadily since the year 2003. The growth according to the Food and Agricultural Organisation was not unconnected with the perceived health benefit of tea consumption and as such, there is a need to develop the indigenous tea industry. It is equally important to translate the agronomical efforts on tea to more viable and economical ends. The objective of this study therefore is to determine the effect of steaming time, drying temperature and drying time on the chemical and sensory qualities of Nigerian Green tea.

Materials and Methods

Tea shoots sampling

Tea shoots comprising of apical bud and two leaves from

agronomic proven commercially viable tea clone were harvest from the Cocoa Research Institute of Nigeria experimental tea plots, Taraba State, Nigeria. The plucked shoots were steamed, rolled and oven dried to make green tea.

Product optimization

Surface response methodology using Central Composite Design (CCD) at three levels, three variables was adopted in optimizing the process variables to obtain 15 experimental runs. The three independent variables experimented were; the steaming time, (60, 90 and 120 s), drying temperature (60, 65 and 70°C) and drying time (90, 120 and 150 min). The first eight treatment combinations form a 2^3 factorial design. The next six treatment combinations are referred to the axial runs, because they lie on the axes defined by the design variables. The last treatment combination represents the centre run and this arrangement of CCD as shown in Table 1 is in such a way that allows the development of the appropriate empirical equations (i.e., the second-order polynomial multiple regression equation) [6]. The model for predicting the quality of the green tea was expressed as:

 $Y_{i} = \beta_{0} + \beta_{1} X_{1} + \beta_{2} X_{2} + \beta_{3} X_{3} + \beta_{12} X_{12} + \beta_{13} X_{13} + \beta_{23} X_{23} + \beta_{1}^{2} X_{1}^{2} + \beta_{2}^{2} X_{2}^{2} + \beta_{3}^{2} X_{3}^{2} + \boldsymbol{s}_{i}$

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Received September 14, 2015; Accepted September 29, 2015; Published October 07, 2015

Citation: Odunmbaku LA, Babajide JM, Shittu TA, Aroyeun SO, Eromosele CO, et al. (2015) Effect of Process Variables on the Chemical Constituents and Sensory Characteristics of Nigerian Green Tea. J Food Process Technol 6: 510. doi:10.4172/2157-7110.1000510

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Chemical standard

Epigallocatechin gallate, epigallocatechin, epicatechin gallate and epicatechin standards were sourced from Sigma Aldrich Chemical Co. (USA); HPLC grade acetonitrile, ethyl acetate and methanol (Merck, Germany).

Equipment

Chromatography Acrodisc Syringe membrane Filters (0.45 μ m, 30 mm diameter), Agilent (Germany); Water Distiller, AC-L4 Model, Optic Ivymen System, Europe; Vortex Mixer; KMC-1300 v Model, Vision scientific Co. Ltd., Korea; Syringes (1, 2 and 5 mL), Agary; Micro-pipette (200 and 1000 μ L) Gilson, France; 5 mL Plain sample Bottles (Polypropylene); Porcelain Mortar and Pestle. Volumetric Flasks (10, 500 and 1000 mL), Borosilicate, Technico, England. Beakers (250, 500 and 1000 mL), Borosilicate, NAFCO, Nigeria; Digital Analytical Weighing Balance (Metler Toledo Instrument Company), North and South America; High Pressure Liquid Chromatography (HPLC), Agilent Technologies 1120 LC Compact series (Agilent), Germany and Japan. The system comprises a UV-Vis detector and an HP computer system. The HPLC column used was a ZORBAX SB C8

	ST (sec)	DT (°C)	Dt (min)
1	-1	-1	-1
2	+1	-1	-1
3	-1	+1	-1
4	+1	+1	-1
5	-1	-1	+1
6	+1	-1	+1
7	-1	+1	+1
8	+1	+1	+1
9	-1	0	0
10	+1	0	0
11	0	-1	0
12	0	+1	0
13	0	0	-1
14	0	0	+1
15	0	0	0

ST; steaming time, DT; Drying temperature, Dt; Drying time

 Table 1: Experimental Runs of Optimized green tea sample.

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 $(75\times4.6$ mm, 3.5 $\mu m)$ from (Hewlet Packard, HP). Data acquisition was done with Chemstation Software.

Determination of chemical constituents

High-Performance Liquid Chromatography (HPLC) methods was used to determine the amount of epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG) and epicatechin (EC) present in the green tea [7,8]. Grounded green tea samples were extracted using Methanol and distilled water (95:5%) for 40 min at room temperature [9]. Solutions were filtered using a Millipore filter of 0.45 μ m size. Filtered samples were filled into a vial bottles and 20 μ l each programmed for injection twice per sample for HPLC auto sampler analysis. Detection was carried out by measurement of UV absorbance at 270 nm. Stock solutions of EGCG, EGC, ECG and EC were prepared by dissolving reference standards into mobile phase. Less concentrated solutions were prepared, as required, by dilution in the same mobile phase.

Sensory evaluation

Sensory attributes of the green tea infusion (Colour, Clearness, Dry Leaf Aroma, Green Tea Aroma, Sweetness, Bitterness, Green Tea Flavour and Astringency) were evaluated by semi trained panel (n=25) using descriptive analysis [10]. Attribute intensities were rated on a scale of 1-9 with 1 representing lowest intensity value and 9 representing highest intensity value. The samples were presented to the panellists in random order as coded samples out of tea cups covered by lids. This was prepared 30 min before evaluation. Water was provided for cleansing the palate between the samples.

Data analysis

The results were subjected to analysis of variance using SPSS 16b statistical package. The means were used to calculate linear regressions between individual Catechin and sensory evaluations responses using MATLAB R2012a.

Results

As presented in Table 2, the EGCG content of green tea samples ranged from 46.90-178 mg/g, EGC, 0.30-4.24 mg/g; EC, 1.03-8.83 mg/g and ECG, 8.05-33.96 mg/g. Table 3 showed the regression model result of the expected response against the observed responses. The

Sample	ST time (Sec)	D T (°C)	D time (Min)	EGCG	EGC	EC	ECG
621	60	60	90	106.75 ± 0.00 ^d	0.95 ± 0.00^{de}	3.08 ± 0.40 ^{ef}	11.29 ± 0.50 ^h
738	60	70	90	112.45 ± 1.20°	0.62 ± 0.30 ^e	2.21 ± 0.18 ^f	10.64 ± 0.64 ^h
926	60	60	150	145.67 ± 0.68 ^b	0.30 ± 0.06 ^e	2.10 ± 0.17 ^f	8.60 ± 0.24 ⁱ
194	60	70	150	178.06 ± 0.17ª	1.03 ± 0.13 ^{de}	1.03 ± 0.41 ^g	8.05 ± 0.19 ⁱ
531	60	65	120	112.05 ± 0.49°	0.79 ± 0.00 ^{de}	2.61 ± 0.94 ^{ef}	11.11 ± 0.16 ^h
756	90	60	120	77.25 ± 0.24 ⁱ	2.82 ± 0.93 ^{bc}	6.10 ± 0.49°	17.99 ± 1.01 d
573	90	70	120	91.94 ± 1.01 ^f	1.88 ± 1.01 ^{cd}	3.36 ± 0.07 ^e	14.15 ± 0.31 ^f
980	90	65	90	96.95 ± 0.82°	0.95 ±0.02 ^{de}	3.27 ± 0.76 °	12.77 ± 0.50 ^g
292	90	65	150	90.74 ± 0.499	2.46 ±0.36°	4.49 ± 0.31 ^d	14.26 ± 0.76 ^f
430	90	65	120	80.07 ± 0.14 ^h	2.50 ±0.50°	4.71 ± 0.17 d	16.13 ± 0.11°
658	120	60	90	46.90 ± 0.17 ^m	3.88 ± 1.06 ^{ab}	1.04 ± 0.05 ª	33.96 ± 0.09ª
271	120	70	90	72.51 ± 0.59 ⁱ	3.68 ±0.16 ^{ab}	6.11 ± 0.68 °	19.72 ± 1.26°
321	120	60	150	50.68 ± 0.00^{1}	4.24 ±0.17 ^a	8.83 ± 0.00 ^b	24.32 ± 0.13b
564	120	70	150	72.22 ± 0.16 ⁱ	3.72 ±0.24 ^{ab}	6.39 ± 0.50°	23.55 ± 0.44 ^b
250	120	65	120	70.05 ± 0.03 ^k	3.99 ±0.38ª	8.40 ± 0.33 ^b	23.78 ± 0.34 ^b

Values in the same column with same superscript are not significantly different at P≤ 0.05.

 Table 2: Green tea Polyphenol content (mg/g).

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ST (Sec)	DT (°C)	Dt (min)	EGCG Measured	EGCG Predicted	EGC Measured	EGC Predicted	EC Measured	EC Predicted	ECG Measured	ECG Predicted	MSE
90	60	120	77.25	73.75	2.82	2.56	6.10	5.90	18.00	18.30	2.52
120	60	90	46.90	58.53	3.88	3.98	10.37	9.97	33.96	31.37	28.45
60	60	90	106.75	103.94	0.95	0.70	3.08	3.18	11.29	12.73	2.06
60	70	90	112.45	115.76	0.62	0.54	2.21	1.68	10.64	8.82	3.11
120	70	90	72.51	74.83	3.68	3.25	6.11	6.09	19.72	20.57	1.29
60	60	150	145.67	143.84	0.30	0.68	2.10	2.11	8.60	7.63	0.92
120	60	150	50.68	47.86	4.24	4.29	8.83	9.34	24.32	26.00	2.39
90	65	150	90.74	102.91	2.46	1.96	4.49	3.69	14.26	12.85	30.21
60	65	120	112.05	124.19	0.79	0.88	2.61	2.63	11.11	10.16	29.67
120	70	150	72.22	75.53	3.72	3.93	6.39	6.27	23.55	21.98	2.74
90	70	120	178.06	167.02	1.03	0.90	1.03	1.42	8.05	10.49	25.64
120	65	120	70.05	55.74	3.99	4.04	8.40	8.44	23.78	25.22	41.40
90	65	90	96.95	82.61	0.95	1.63	3.27	4.13	12.79	14.70	42.15
60	70	150	91.94	93.49	1.88	2.30	3.36	3.62	14.15	14.33	0.57
90	65	120	80.07	83.96	2.50	2.20	4.71	4.60	16.13	15.17	3.25

Table 3: Polyphenol Regression model (mg/g).

Sample Code	ST	DT	Dt	Greenness	Sweetness	Bitterness	Astringency	Clearness	DL Aroma	GT Aroma	GT Flavour
756	90	60	120	5.77 ± 0.57 ^{bc}	1.17 ± 0.38 ^{bc}	5.07 ± 0.87°	2.20 ± 0.41 ^b	5.80 ± 0.48 ^b	2.83 ± 0.97 ^d	2.27 ± 0.45 ^b	6.87 ± 0.35 ^b
658	120	60	90	8.10 ± 0.48ª	1.00 ± 0.00°	8.03 ± 0.46 ^a	1.00 ± 0.00 ^a	3.90 ± 0.55°	1.87 ± 0.35 ^e	1.07 ± 0.25 ^d	8.07 ± 0.25 ^a
621	60	60	90	3.90 ± 0.40 ^d	2.10 ± 0.31ª	4.10 ± 0.40 ^d	4.10 ± 0.48°	7.83 ± 0.53 ª	5.97 ± 0.56ª	4.17 ± 0.38 ^a	4.77 ± 0.50°
738	60	70	90	3.97 ± 0.41 ^d	1.03 ± 0.18 ^{bc}	4.17 ± 0.46 ^d	4.00 ± 0.46^{a}	7.97 ± 0.49 ª	2.03 ± 0.49°	1.07 ± 0.25 ^d	8.13 ± 0.43 ^a
321	120	60	150	8.03 ± 0.41ª	1.00 ± 0.00°	4.20 ± 0.41 ^d	1.20 ± 0.55 ^a	3.93 ± 0.52°	2.13 ± 0.57°	1.20 ± 0.41 ^d	8.13 ± 0.43 ^a
926	60	60	150	4.03 ± 0.49 ^d	1.20 ± 0.41 ^b	6.90 ± 0.31 ^b	4.20 ± 0.41 ^b	7.90 ± 0.61 ª	1.93 ± 0.37°	2.10 ± 0.31 ^{bc}	6.80 ± 0.48 ^b
271	120	70	90	8.03 ± 0.41ª	2.20 ± 0.41ª	8.00 ± 0.37ª	1.10 ± 0.31°	3.93 ± 0.45°	5.67 ± 0.71 ^b	4.17 ± 0.38 ^a	4.77 ± 0.50°
292	90	65	150	6.00 ± 0.26 ^b	1.13 ± 0.35 ^{bc}	7.10 ± 0.31 ^b	2.10 ± 0.40 ^b	5.80 ± 0.55 ^b	2.77 ± 0.50^{d}	2.17 ± 0.38 ^{bc}	6.77 ± 0.43 ^b
531	60	65	120	3.97 ± 0.49 ^d	1.00 ± 0.00°	4.17 ± 0.38 ^d	4.13 ± 0.43 ª	7.83 ± 0.59 ª	2.13 ± 0.43 ^e	1.07 ± 0.25 ^d	8.07 ± 0.37 ^a
564	120	70	150	8.03 ± 0.49ª	2.13 ± 0.35ª	8.00 ± 0.37ª	1.17 ± 0.46 [°]	3.90 ± 0.40°	5.93 ± 0.37^{ab}	4.20 ± 0.48^{a}	4.93 ± 0.45°
430	90	65	120	5.87 ± 0.43 ^{bc}	2.23 ± 0.43ª	7.97 ± 0.18ª	2.23 ± 0.68°	6.00 ± 0.37 ^b	5.90 ± 0.40^{ab}	4.17 ± 0.46 ^a	4.83 ± 0.46°
250	120	65	120	8.00 ± 0.52ª	1.00 ± 0.00°	8.00 ± 0.41ª	1.27 ± 0.58 ª	3.87 ± 0.51°	2.13 ± 0.35°	1.20 ± 0.48 ^d	8.27 ± 0.45 ^a
980	90	65	90	5.70 ± 0.47°	1.13 ± 0.35 ^{bc}	6.87 ± 0.35 ^b	2.20 ± 0.41 ^b	5.77 ± 0.63 ^b	2.93 ± 0.52^{cd}	2.23 ± 0.57 ^{bc}	6.83 ± 0.38 ^b
194	60	70	150	4.00 ± 0.64 ^d	2.17 ± 0.38ª	4.13 ± 0.35 ^d	4.23 ± 0.43°	7.97 ± 0.49 ª	5.73 ± 0.52^{ab}	4.17 ± 0.38 ^a	4.77 ± 0.57°
573	90	70	120	5.87 ± 0.43 ^{bc}	1.17 ± 0.38 bc	7.00 ± 0.37 ^b	2.37 ± 1.03 ^b	5.83 ± 0.53 ^b	3.13 ± 0.43°	2.03 ± 0.18°	6.87 ± 0.51 ^b

Values in the same column with same superscript are not significantly different at P≤ 0.05.

Table 4: Descriptive sensory evaluation mean scores of green tea samples.

regression coefficients (r²) for EGCG, EGC, EC and ECG were 0.931, 0.939, 0.976 and 0.943 respectively. Descriptive sensory attributes of the green tea samples as affected by steaming time, drying temperature and drying time were presented in Tables 4 and 5. The result shows that green colour attribute ranged between 3.90 and 8.10, sweetness ranged between 1.00 and 2.23, bitterness ranged between 4.0 and 8.23 while astringency ranged between1.00 and 4.23. Clearness ranged between 3.87 and 7.97, dried leaf aroma ranged between 1.87 and 5.97 while GT aroma and flavour ranged from 1.07 to 4.20 and 4.77 to 8.27 respectively. Chromatogram of NGT chemical constituent and the effect of the steaming time, drying temperature and drying time on the EGCG content and sensory characteristics of optimized green teas were presented in Figures 1 and 2 respectively. The predicted models for the optimization of chemical constituents and sensory attributes of Nigerian green tea are presented in Tables 6 and 7.

Discussion

From the results, there were significant differences (P \leq 0.05) in the chemical constituents and sensory attributes of the optimized Green tea samples. It was observed that, steaming for shorter period of time resulted in higher EGCG content compared with longer steaming

duration. Xu and Chen [11] reported that gallated catechins usually convert into non-gallated catechins through hydrolysis under humid and heating conditions. Gulati [12] also reported that the chemical content and the composition of green tea catechins may vary with the conditions of processing. Reduction in the EGCG content at longer steaming duration therefore could be as a result of the release of EGC from EGCG.

The predictive model result for epigallocatechin gallate (EGCG) revealed that, increase in the steaming time, drying temperature, drying time and the product effect of the "steaming time and drying time" brings about a reduction in the amount of EGCG content. Increase in the product effect of the "steaming time and drying temperature", "drying temperature and drying time", quadratic effect of the steaming time as well as the quadratic effect of drying time positively enhances the EGCG content. The correlation coefficient R value was estimates to be 0.965. This indicates that there is a strong positive relationship between the EGCG and the independent variables.

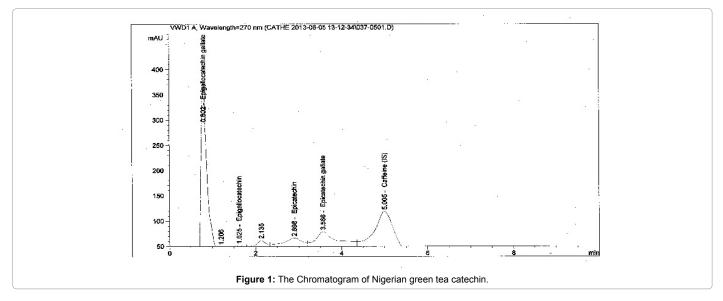
The epigallocatechin (EGC) content increases with unit increase the in steaming time, drying time, product effect of the "steaming time and drying time", "drying temperature and drying time", quadratic effect of the steaming time and drying temperature while increase in

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Parameters	Predicted model	RMSE	R ²	P-value
EGCG	$y_i = 193.61 - 1.1407x_1 + 0.8295x_2 - 1.9733x_3 + 0.0074685(x_1x_2) - 0.014046\beta_{13}(x_1x_3) + 0.018927(x_2x_3) + 0.006666x_1^2 - 0.014046\beta_{13}(x_1x_3) + 0.006666x_1^2 - 0.00666x_1^2 - 0.00666x_1^2 - 0.00666x_1^2 - 0.00666x_1^2 - 0.006666x_1^2 - 0.006666x_1^2 - 0.006666x_1^2 - 0.006666x_1^2 - 0.006666x_1^2 - 0.006666x_1^2 - 0.00666x_1^2 - 0.00666x_1^2 - 0.00666x_1^2 - 0.00666x_1^2 - 0.00666x_1^2 - 0.0066x_1^2 - 0.006x_1^2 - 0.006x_1^2 - 0.006x_1^2 - 0.006x_1^2 - 0.006x_1^2 - 0.006x_1^2 - 0.006x_$	15.2	0.931	0.0198
EGC	$y_i = 33101 + 005159x_1 - 11996x_2 + 0065013x_3 - 000095174(x_1x_2) + 89843e^{(-45)}?_1 3(x_1x_3) + 000062478(x_2x_3) + 000028963x_1^2 + 00091122x_2^2 - 000045081x_3^2 - 0000$	0.585	0.939	0.0148
EC	$y_i = 24593 + 015247x_1 - 0861x_2 + 0077131x_3 - 00039647(x_ix_2) + 000012372?_13(x_ix_3) + 00013472(x_2x_3) + 00010407x_1^2 + 00063686x_2^2 - 000076346x_3^2 - 00007636x_3^2 - 000076x_3^2 - 000076x_3^2 - 000076x_3^2 - 000076x_3^2 - 000076x_3^2 - 000076x_3^2 - 00007$	0.719	0.976	0.0016
ECG	$y_i = 23657 + 050076x_1 - 66859x_2 - 0386611x_3 - 0011475(x_1x_2) - 72518e^{(-05)}?_1 \cdot 3(x_1x_3) + 0011283(x_2x_3) + 00028049x_1^2 + 0045906x_2^2 - 00015459x_3^2 - 00015458x_3^2 - 0001545x_3^2 - 0001545x_3^2 - 0001545x_3^2 - 000154x_3^2 - 00000000000000000000000000000000000$	2.87	0.943	0.0123
Colour	$y_i = -1.1278 + 0.046081x_i + 0.053789x_2 - 0.00049034x_3 - 9.1084e^{-45}(x_i,x_2) - 3.1885e^{-45}\beta_{13}(x_i,x_3) - 2.5386e^{-45}(x_2,x_3) + 0.00017436x_i^2 - 0.00031591x_2^2 + 2.6196e^{-45}x_3^2 - 0.00049034x_3 - 9.1084e^{-45}(x_i,x_2) - 3.1885e^{-45}\beta_{13}(x_i,x_3) - 2.5386e^{-45}(x_2,x_3) + 0.00017436x_i^2 - 0.00031591x_2^2 + 2.6196e^{-45}x_3^2 - 0.00049034x_3 - 9.1084e^{-45}(x_i,x_2) - 3.1885e^{-45}\beta_{13}(x_i,x_3) - 2.5386e^{-45}(x_i,x_3) - 0.00017436x_i^2 - 0.00031591x_2^2 + 2.6196e^{-45}x_3^2 - 0.00049034x_3 - 9.1084e^{-45}(x_i,x_3) - 3.1885e^{-45}\beta_{13}(x_i,x_3) - 2.5386e^{-45}(x_i,x_3) - 0.00017436x_i^2 - 0.00031591x_2^2 + 2.6196e^{-45}x_3^2 - 0.00017436x_1^2 - 0.00031591x_2^2 + 2.6196e^{-45}x_3^2 - 0.00017436x_1^2 - 0.00031591x_2^2 + 0.00017436x_1^2 - 0.0001746x_1^2 - 0.000174x_1^2 - 0.000174x_1^2 - 0.000174x_1^2 - 0.0000174x_1^2 - 0.0000174x_$	0.094	0.999	7.06e-07
clearness	$y_{1} = 0.5076 + 0.063381x_{1} - 0.049555x_{2} + 0.017627x_{3} - 0.000134(x_{1}x_{2}) - 1.3921e^{-45}\beta_{13}(x_{1}x_{3}) - 0.00019836(x_{2}x_{3}) + 7.5381e^{-45}x_{1}^{2} + 0.00066464x_{2}^{2} - 1.464e^{-45}x_{3}^{2} + 0.00066464x_{2}^{2} - 1.464e^{-45}x_{3}^{2} - 0.00019836(x_{2}x_{3}) + 7.5381e^{-45}x_{1}^{2} + 0.00066464x_{2}^{2} - 1.464e^{-45}x_{3}^{2} - 0.00019836(x_{2}x_{3}) + 7.5381e^{-45}x_{1}^{2} + 0.00066464x_{2}^{2} - 1.464e^{-45}x_{3}^{2} - 0.00019846(x_{2}x_{3}) + 7.5381e^{-45}x_{1}^{2} + 0.00066464x_{2}^{2} - 1.464e^{-45}x_{3}^{2} - 0.00019846(x_{1}x_{2}) - 1.464e^{-45}x_{3}^{2} - 0.00019846(x_{1}x_{2}) - 0.0001984(x_{1}x_{2}) - 0.000198(x_{1}x_{2}) - 0.000198(x_{1}x_{2}) - 0.000$	0.094	0.999	7.54e ⁻⁰⁷
Dried Leaf Aroma	$y_{i} = 32.308 - 0.22121x_{1} - 0.56524x_{2} + 0.02394x_{3} - 0.00035837(x_{i}x_{2}) - 5.1348e^{-65}\beta_{i,1}(x_{i}x_{3}) - 0.00024031(x_{i}x_{3}) + 0.0010424x_{i}^{2} + 0.0048981x_{2}^{2} - 1.5324e^{-65}x_{3}^{2} - 1.532$	0.178	0.996	1.92e ⁻⁰⁵
Green Tea Aroma	$y_i = 12.56 - 0.1406x_1 - 0.044293x_2 - 0.0024466x_3 - 2.526e^{-65}(x_i x_2) - 3.1978e^{-65}\beta_{11}(x_i x_3) - 2.526e^{-65}(x_2 x_3) + 0.00052871x_1^2 + 0.00035264x_1^2 + 2.3157e^{-65}x_3^2 - 2.526e^{-65}(x_1 x_2 x_3) - 2.526$	0.078	0.999	1.03e ⁻⁰⁶
Sweetness	$y_i = 9.125 - 0.11677x_1 - 0.055922x_2 + 0.0010414x_3 + 0.00019096(x_ix_2) + 4.0746e^{-66}\beta_{13}(x_ix_3) - 2.4558e^{-65}(x_2x_3) + 0.00046961x_1^2 + 0.0002948x_2^2 + 2.1918e^{-66}x_3^2 - 2.1918e^{-6}x_3^2 - 2.1918e^{-6}$	0.032	0.999	1.01e ⁻⁰⁶
Bitterness	$y_i = 4.1628 + 0.23717x_1 - 0.37469x_2 + 0.026199x_3 + 8.341e^{-05}(x_ix_2) + 2.2227e^{-45}\beta_{13}(x_ix_3) - 8.3168e^{-05}(x_2x_3) - 0.0010064x_1^2 + 0.0028732x_2^2 - 9.5125e^{-45}x_3^2 - 9.5125e^{$	0.567	0.961	0.00495
Green Tea Flavour	$y_i = 8.5731 + 0.14363x_1 - 0.32556x_2 - 0.0015971x_3 - 0.00015029(x_1x_2) - 1.3888e^{-45}\beta_{13}(x_1x_3) - 1.6506e^{-45}(x_2x_3) - 0.00042705x_1^2 + 0.0026267x_2^2 + 1.1808e^{-45}x_3^2 - 0.0015971x_3 - 0.00015029(x_1x_2) - 1.3888e^{-45}\beta_{13}(x_1x_3) - 1.6506e^{-45}(x_2x_3) - 0.00042705x_1^2 + 0.0026267x_2^2 + 1.1808e^{-45}x_3^2 - 0.0015971x_3 - 0.00015029(x_1x_2) - 1.3888e^{-45}\beta_{13}(x_1x_3) - 1.6506e^{-45}(x_2x_3) - 0.00042705x_1^2 + 0.0026267x_2^2 + 1.1808e^{-45}x_3^2 - 0.0015971x_3 - 0.00015029(x_1x_2) - 1.3888e^{-45}\beta_{13}(x_1x_3) - 1.6506e^{-45}(x_2x_3) - 0.00042705x_1^2 + 0.0026267x_2^2 + 1.1808e^{-45}x_3^2 - 0.0015971x_3 - 0.0001592(x_1x_2) - 1.3888e^{-45}\beta_{13}(x_1x_3) - 0.00042705x_1^2 + 0.0026267x_2^2 + 1.1808e^{-45}x_3^2 - 0.0015971x_3 - 0.0001592(x_1x_2) - 0.00042705x_1^2 + 0.00042705x_1^2 - 0.0004x_1^2 - 0$	0.075	0.999	5.76e ⁻⁰⁷
Astringency	$y_i = 5.0908 - 0.15651x_1 + 0.16684x_2 + 0.015398x_3 + 0.00011728(x_1x_2) - 8.2575e^{-46}\beta_{13}(x_1x_3) - 4.8924e^{-47}(x_2x_3) + 0.00055648x_1^2 - 0.0013763x_2^2 - 5.4016e^{-45}x_3^2 - 0.001x_3^2 - 0.001x_3^2 - 5.4016e^{-45}x_3^2 - 0.001x_3^2 - 0.001x_3^2 - 5.4016e^{-45}x_3^2 - 0.001x_3^2 - 5.4016e^{-45}x_3^2 - 0.001x_3^2 - 5.4016e^{-45}x_3^2 - 0.001x_3^2 - 0.001$	0.101	0.998	4.23e-06

 x_1 , Steaming time; x_2 , Drying temperature; x_3 Drying time.





drying temperature, product effect of the "steaming time and drying temperature" and quadratic effect of the drying time brings about a reduction in the amount of EGC content. The correlation coefficient value was estimated to be 0.969. This indicates that there is a strong positive relationship between EGC content and the independent variables.

The Epicatechin (EC) content, increases with unit increase in steaming time, drying time, product effect of the "steaming time and drying time", "drying temperature and drying time", quadratic effect of the steaming time as well as the quadratic effect of drying temperature. Increase in drying temperature, the product effect of the "steaming time and drying temperature" and quadratic effect of the drying time brings about a reduction in the amount of EC content. Strong positive relationship exists between the EC content and the independent variables.

Epicatechin gallate (ECG) increases with unit increase steaming time, product effect of the "drying temperature and drying time", quadratic effect of the steaming time and quadratic effect of the drying temperature while increase in drying temperature, drying time, product effect of the "steaming time and drying temperature", "steaming time and drying time" and quadratic effect of the drying time brings about a reduction in the amount of ECG content. The estimated correlation coefficient value of 0.971 indicates that there is a strong positive relationship between the amount of ECG content and the independent variables.

Somkiat [13] reported that colour is an important indicator for the quality of processed tea. The predictive model for the green colour intensity of green tea revealed that increase in steaming time and drying temperature brings about an increase in the green colour intensity of the tea extract while unit increase in drying time reduces the green colour intensity. The product effect of the "steaming time

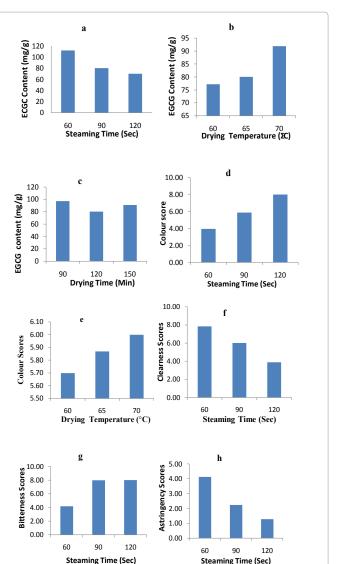


Figure 2: Effect of process variables on EGCG and sensory characteristic of NGT.

and drying temperature", "steaming time and drying time" and "drying temperature and drying time" also brings about a reduction in the green colour intensity. The quadratic effect of the steaming and drying time brings about an increase in the colour intensity of the green tea extract while the quadratic effect of the drying temperature leads to a reduction in the colour intensity. There exist a strong positive relationship between the green colour intensity and the independent variables. However, for the nine explanatory variables studied, only the quadratic effect of the steaming time exerts significant influence (r= 0.99, p=0.044629) on the green colour intensity of the tea extract.

Sample steamed for 120 sec, dried at 60°C for 90 min. was the most preferable based on green colour intensity. Sample 658 has the highest colour score (8.10) which was not significantly different ($P \le 0.05$) from samples 564 (8.03), 321 (8.03), 271 (8.03) and 250 (8.00). Green colour intensity of this samples were significant different ($P \le 0.05$) from those of all other samples, however, the least scores were recorded in samples 926 (4.03), 194 (4.00), 531 (3.97), 738 (3.97) and 621(3.90) in that order.

Unit increase in the steaming time, drying temperature as well as the product effect of "drying temperature and drying time" brings about a reduction in the sweetness of the green tea extract while increase in the drying duration as well as the product effect of the "steaming time and drying temperature", "steaming time and drying time", quadratic effect of the steaming time, drying temperature and drying time enhances the sweetness. A strong positive relationship exists between the sweetness of the green tea extract and the independent variables. However, for the nine explanatory variables studied, only the steaming time, product effect of the "steaming time, exert significant influence on the sweetness (r=0.999, p=9.1011e⁻⁰⁶, 0.049795 and 4.0076e⁻⁰⁶). All samples taste attributes for sweetness were weak and therefore recorded low mean scores as recorded in sample 531, 250, 658 and 321 (1.00). Sample 430 had the highest sweetness mean score (2.23).

The predictive model for Bitterness indicates that, every unit increase in the steaming time as well as the drying duration, product effect of the "steaming time and drying temperature", "steaming time and drying time" and quadratic effect of the drying temperature brings about an increase in the bitterness of the tea extract while the drying temperature, product effect of the "drying temperature and drying time", quadratic effect of the steaming time and quadratic effect of

ST sec	DT ℃	Dt min	Colour (Measured)	Colour (Predicted)	Sweetness (Measured)	Sweetness (Predicted)	Bitterness (Measured)	Bitterness (Predicted)	Astringency (Measured)	Astringency (Predicted)	MSE
90	60	120	5.77	6.12	1.17	1.01	4.13	5.94	2.20	1.86	0.91
120	65	120	8.10	7.98	1.00	1.02	7.97	8.01	1.00	1.13	0.01
60	60	90	3.90	3.91	2.20	2.22	4.20	3.85	4.10	4.15	0.03
60	65	120	3.97	4.32	2.13	2.00	4.17	4.73	4.00	3.69	0.16
120	60	150	8.03	8.39	1.00	0.84	8.00	8.20	1.10	0.75	0.10
60	70	90	4.03	3.60	2.23	2.42	4.17	3.82	4.20	4.60	0.15
120	60	90	8.03	7.60	1.00	1.21	8.03	7.10	1.20	1.62	0.36
60	60	150	6.00	5.70	1.20	1.30	6.90	6.28	2.10	2.34	0.16
60	70	150	3.97	4.33	2.17	1.99	4.13	4.89	4.13	3.76	0.25
120	70	150	8.03	7.97	1.03	1.04	8.00	8.20	1.17	1.17	0.01
90	70	120	5.87	5.75	1.17	1.22	7.00	5.93	2.13	2.29	0.32
120	70	90	8.00	8.24	1.00	0.93	8.00	8.46	1.27	1.08	0.10
90	65	150	5.70	5.35	1.13	1.35	6.87	6.35	2.20	2.68	0.20
90	65	90	4.00	4.59	2.10	1.75	4.10	5.26	4.23	3.55	0.63
90	65	120	5.87	5.42	1.13	1.34	7.10	5.88	2.23	2.58	0.47

 Table 6: Regression results for the sensory attributes of Nigerian Green tea.

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S T sec	DT °C	Dt min	Clearness (Measured)	Clearness (Predicted)	DLA (Measured)	DLA (Predicted)	GT A (Measured)	GT A (Predicted)	GT F (Measured)	GT F (Predicted)	M SE
90	60	120	5.80	6.16	5.93	4.10	2.27	1.86	6.87	7.15	0.91
120	65	120	7.90	7.86	2.13	2.27	1.20	1.26	8.27	8.14	0.01
60	60	90	3.87	3.84	5.67	5.98	4.17	4.19	4.77	4.75	0.03
60	65	120	3.93	4.24	5.93	5.24	4.20	3.90	4.93	5.23	0.16
120	60	150	7.97	8.30	2.13	1.83	1.20	0.84	8.13	8.53	0.10
60	70	90	3.90	3.50	5.90	6.34	4.17	4.59	4.83	4.39	0.15
120	60	90	7.97	7.54	2.13	3.11	1.07	1.56	8.07	7.63	0.36
60	60	150	5.80	5.56	1.93	2.68	2.10	2.38	6.80	6.57	0.16
60	70	150	3.90	4.25	5.73	4.91	4.17	3.75	4.77	5.16	0.25
120	70	150	7.83	7.79	2.03	1.85	1.07	1.11	8.13	8.11	0.01
90	70	120	5.83	5.74	3.13	4.29	2.03	2.20	6.87	6.76	0.32
120	70	90	7.83	8.00	1.87	1.27	1.07	0.85	8.07	8.26	0.10
90	65	150	5.77	5.38	2.93	3.48	2.23	2.68	6.83	6.31	0.20
90	65	90	3.93	4.62	5.97	4.84	4.17	3.46	4.77	5.48	0.63
90	65	120	6.00	5.45	3.20	4.31	2.17	2.64	6.77	6.43	0.47

Table 7: Regression for the sensory attributes of Nigerian Green tea.

the drying time, brings about a reduction in the bitterness. The result also indicated that there is a strong positive relationship between the bitter taste intensity and the independent variables. Sample 658 was rated highest in bitterness, followed by samples 250, 564, 271 and 430. Sample 621 (4.10) had the lowest score for bitterness. This could be as a result of the short steaming period.

From the predictive model result for astringency, every unit increase in the steaming time, drying temperature, drying time, product effects of the "steaming time and drying temperature", "drying temperature and drying time" as well as the quadratic effect of the drying temperature and drying time brings about a reduction in the astringency of the tea extract. Increase in the quadratic effect of the steaming time brings about an increase in the astringency of the green tea extract. The correlation coefficient value was estimated to be 0.999. This indicates that there is a strong positive relationship between astringency and the independent variables. However, for the nine explanatory variables studied, only the steaming time and quadratic effect of the steaming time exert significant influence on the astringency with p-Value of 0.0061525 for steaming time, and 0.00050398 for the quadratic effect of steaming time. Sample 194 (4.23) was the most preferred in astringency followed by 926 (4.20), 531 (4.13) in that order. This finding agrees with Wismer [14] that astringency is an important and often appealing characteristic of brewed tea. Sample 658 had the lowest scores for astringency which was significantly different ($p \le 0.05$) from those of all other samples.

Unit increase in the steaming time, quadratic effects of the steaming time as well as drying temperature and drying duration brings about an increase in the clearness of the green tea extract while increase in drying temperature, product effect of the "steaming time and drying temperature", "steaming time and drying time", "drying temperature and drying time" and the quadratic effect of the drying time reduces the clarity of the green tea extract. A strong positive relationship exists between the clarity of the tea extract and the independent variables. However, for the nine explanatory variables studied, only the steaming time exerts significant influence on the clearness (r = 0.999, p=0.0211). The highest clearness score was found in samples 738 (7.97), 194 (7.90), 531 (7.83) which had a better appearance attribute in terms of clarity. Steaming for a shorter period (60 s) could be attributed to the clarity of these samples. Sample 250 (3.87) was rate the least and significantly different ($P \le 0.05$) from other samples.

Increase in steaming time, Drying time, quadratic effects of the steaming time and drying temperature, brings about a rise in the dried leaf aroma of the tea extract while increase in drying temperature, the product effect of the "steaming time and drying temperature", "steaming time and drying time" as well as "drying temperature and drying time" and the quadratic effect of the drying time brings about a reduction in the dried leaf aroma of the tea extract. The correlation coefficient *R* value was estimated to be 0.998. This indicates that there is a strong positive relationship between dried leaf aroma and the independent variables. However, for the nine explanatory variables studied, only the steaming time and the quadratic effect of the steaming time exert significant influence (*p*=0.0017056 and 0.00038171) on the dried leaf aroma.

Steaming for a shorter period (60 s), drying at 60°C for 90 mins enhances the aroma of the green tea sample. The duration of the steaming process was said to be a key determinant in green tea flavour, aroma, and colour. Steamed leaves left at high temperature will lose their bright colour and their flavour and aroma will be negatively affected [15]. From the result for Green tea aroma (GTA), every unit increase in the steaming time, drying temperature, drying time, product effect of the "steaming time and drying temperature", "steaming time and drying time" as well as "drying temperature and drying time" brings about a reduction in the GTA while the quadratic effect of steaming time, drying temperature and time positively enhance the green tea aroma of the tea extract. Strong positive relationship exists between GTA and the independent variables however, for the nine explanatory variables studied, only the steaming time and the quadratic effect of the steaming time exerts significant influence (r=0.999, P=0.00029991, 0.00018735) on the GTA.

Unit increase in the steaming time while the drying temperature, drying time, product effect of the steaming time and drying temperature; steaming time and drying time; drying temperature and drying time, and the quadratic effect of the steaming time; drying temperature; and drying time are kept constant brings about an increase in the green tea flavour while increase in drying temperature brings about a reduction in the green tea flavour. There is equally a negative relationship between the drying time and the green tea flavour. This indicates that increase in drying time will bring about a reduction in the green tea flavour. Furthermore, a negative relationship exists between the product effect of the "steaming time and drying temperature" as well as "steaming time

and drying time" and the green tea flavour; this indicates that increase in this variable brings about a reduction in the green tea flavour. Increase in the quadratic effect of drying temperature and drying time however brings about an increase in the flavour of the tea extract. The correlation coefficient *R* value for the green tea flavour was estimated to be 0.999. This indicates that there is a strong positive relationship exists between the green tea flavour and the independent variables, however, for the nine explanatory variables studied, only the steaming time and quadratic effect of the steaming time exerts significant influence (*p*=0.00022153, 0.00042252) on the green tea flavour.

Conclusion

There were significant differences ($P \le 0.05$) among the optimized Green tea samples as influenced by the processing variables (steaming time, drying temperature and drying time). This study revealed that, steaming for shorter period of time resulted in higher EGCG content compared with longer steaming duration. Significant difference ($P \le 0.05$) was also recorded in the EGC content of the optimized green tea samples. Steaming for longer period (120 s) positively enhanced the green colouration in green tea while shorter steaming period (90 s) gives better aroma as the intensity of green tea aroma reduces with elongation of the steaming period. Astringency reduces with steaming time and this correlates with the Epigallocatechingalate (EGCG) content which is higher at minimum (60 s) steaming time.

Drying at higher temperature (70°C) enhances the colour intensity of green tea while an average drying temperature of 60°C gives better clarity of the extract. More EGCG is recorded in green tea dried at 70°C than at lower temperature, this could possibly be as a result of higher drying rate at higher temperature. The intensity of green tea flavour gets more pronounced as the drying temperature increases.

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