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Sub-theme: Innovative technologies in Tea production product diversification and value

EFFECTS OF PROCESSING TECHNOLOGIES ON CHLOROGENIC ACID, GALLIC ACID AND THEANINE IN SELECTED KENYAN TEA CULTIVARS

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ABSTRACT

Tea (*Camellia sinensis*) is popular for its high levels of polyphenols which are attributed to its antioxidant properties. However, other associated biomolecules are also potent antioxidants and contribute to health effects. These include theanine which increases gamma-Aminobutyric acid (GABA) levels, serotonin and dopamine, thus reducing stress, relaxes the body and improves mood. Chlorogenic acid (CGA) which has antioxidant, anti-obesity, anti-atherosclerotic and anti-cancer properties; and Gallic acid (GA) has antioxidant and antimicrobial properties. A study was conducted to determine the variation of theanine, CGA, GA and with processing technologies including aeration and non-aeration coupled with Cut, Tear and Curl (CTC) and Orthodox maceration. Six (6) tea varieties including TRFK 6/8, TRFK 11/4, TRFK 12/2, TRFK 31/8, TRFK 54/40 and TRFK 306/1 were used. Profiling of theanine, CGA and GA was done using High Performance Liquid Chromatography and data analysis was done using SAS software (P=0.05). Chlorogenic acid ranged from 15.16 to 32.87% for green teas and 22.82 to 39.14% for black teas with the variety TRFK 12/2 registering least values and TRFK 306/1 the highest mean value. Galic acid values ranged from 0.28 to 0.51% for green teas and 0.28 - 0.55% for black teas with TRFK 6/8 registering the least content and TRFK 306/1 registering the highest amounts. Theanine range was highest in non-aerated teas at 0.97-1.82% and 0.79-1.47% for black teas with clone TRFK 6/8 registering the lowest level and TRFK11/4 the highest content. The research shows that theanine values in teas are best preserved through non-aeration processing, while CGA and GA values are enhanced by aeration processing.

Key Words: Black tea, Green tea, Theanine, Chlorogenic acid, Gallic acid

INTRODUCTION

Tea (*Camellia sinensis*) originated in China during the Shang dynasty as a medical drink [23, 39] and afterwards, it spread to the rest of the world and is commonly used as a beverage [10, 13]. The quality of the tea is pegged on the variety, the region where grown and processing method [4, 19]. Tea processing is done through aeration, non-aeration, semi-aeration and microbial fermentation [38]. It is shaped/macerated by Cut, Tear and Curl (CTC) or orthodox methods. Flavor is determined by the cultivar and processing technique [8, 11, 33, 41, 40].

In recent years, tea consumption has been on the increase for its health benefits resulting from tea biomolecules such as enzymes, carbohydrates, proteins, lipids, polyphenols, methyl xanthine (caffeine), purines, chlorogenic acids, gallic acids, theanine and other amino acids as well as carotenoids among others [1, 5, 24]. These constituents helps in protection against arteriosclerosis and cardiovascular diseases, neural and obesity, kidney and liver disease, pulmonary disease, ailments, flu, AIDS, anti-inflammatory, antimicrobial, anti-diabetic properties, anti-cancer properties etc. [8, 12, 31, 34, 35]. However the biomolecules are usually affected by processing.

Chlorogenic acid is the ester formed between Caffeic acid and quinic acid (Figure 1a). Isomers of Chlorogenic acid include the caffeoyl ester: 4-*O*-caffeoylquinic acid (cryptochlorogenic acid or 4-CQA) and 5-*O*-caffeoylquinic acid (neochlorogenic acid or 5-CQA) among others [5, 25]. Health effects encompass regulation of blood sugar, exertion of anti-diabetic effects weight loss and an anti-obesity effect. In addition, it reduces blood pressure and cell inflammation [12].

Gallic acid is an organic acid known as 3,4,5-trihydroxybenzoic acid (Figure 1b) which exists as a free acid and as a part of tannins [18]. Gallic acid selectively kills hepatic stellate cells (HSCs) responsible for liver fibrosis [2], possesses anti-inflammatory effects, inhibits neural degeneration [14] and inhibits several diseases like leukaemia HL-60RG, prostate, colon cancer [24].

L-theanine (γ-glutamylethylamide) is a unique amino acid (Figure 1c) reported in only four plant species including tea [6, 7]. Theanine is responsible for the taste of non-aerated green teas. In tea comprises 1-2% (dry weight), contributes to 50% amino acids, and is the only free amino acid which does not occur in proteins [16]. It is synthesized from glutamic acid, alanine and ethylamine [6] and is converted to polyphenols [26]. Theanine improves memory, enhances recognition, reduces anxiety, helps in weight loss, has antioxidant properties and protects liver cells from injuries [21, 22, 41]. Additionally, it reduces mental and physical stress, fatigue, muscular pain, promotes neurotransmission and α wave production in the brain, neutralizes poisons and boosts the immune system [35].

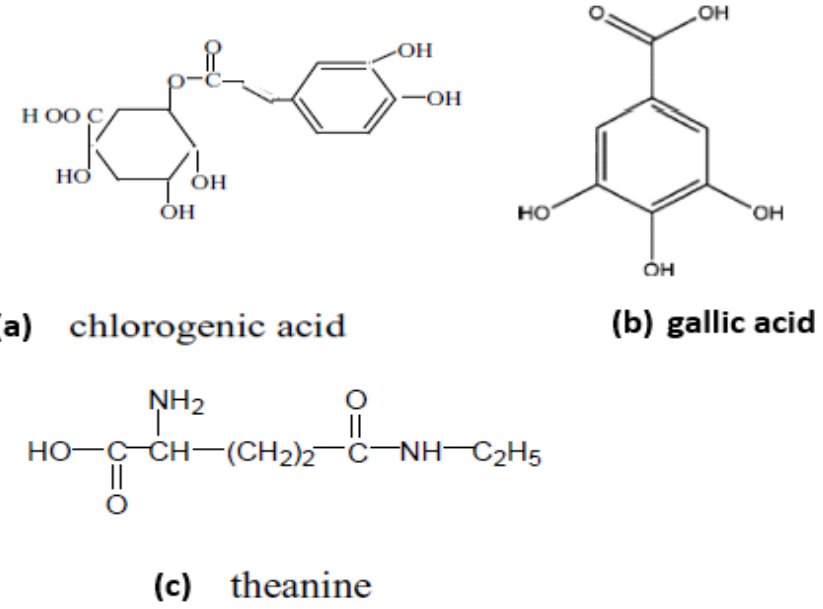

FIGURE 1: *Chemical structures of (a) Chlorogenic acid, (b) Gallic acid and (c) Theanine*

Processing affects the stability and configuration of tea biomolecules that impact on quality and health beneficts. This study focused on the effects of aeration and non-aeration processing on CGA, GA and theanine.

MATERIALS AND METHODS

Sample collection

Fresh green tea leaf samples were collected from Timbilil estate (0° 22'S, 35° 21'E, 2180 m asl), Kenya Agricultural and Livestock Research Organization- Tea Research Institute (KALRO-TRI). Six tea cultivars vis. TRFK 6/8, TRFK 11/4, TRFK 12/2, TRFK 31/8, TRFK 54/40 and TRFK 306 were used.

Sample preparation

Two leaves and a bud were harvested and processed as aerated (black), non-aerated (green) CTC and orthodox teas. Processing of aerated (black) tea involved withering (16-18 h), maceration (CTC / hand rolling), aeration and drying [27, 32, 36]. Non-aerated (Green) tea processing involved, steaming leaves at 100°C for 60s, withering for 3-4 h, maceration (CTC /hand rolling) and drying (Fluid bed dryer) to 4% Moisture Content [9, 10, 27, 28].

Physico-chemical analysis

Moisture content

Moisture was determined by the hot air oven drying at 103°C ± 2.0°C to a constant weight and results expressed in percentage [20].

Chlorogenic acid (CGA)

Chlorogenic acid was extracted with a mixture of methanol and water (5 g sample, 150 mL of boiling 50% methanol/water (v/v) (MeOH) into a 250 mL conical flask for 20 min). The extract was cooled to room temperature, filtered (membrane filters), diluted (1mL into 50mL volumetric flask with 50% methanol/water (v/v) (MeOH)) and transferred to vials for analysis (1mL). A Shimadzu LC 20 AT HPLC fitted with a SPD-20 UV-Visible detector and C_6 Column (250 x 4.6 mm) at 270nm was used. Isocratic elution was done (acetonitrile: 0.5% phosphoric acid, 15:85) at a flow rate of 1mL/min; column temp 35±0.5°C and 20µL injected. Chlorogenic acid (CGA) was calculated from CGA standard curve (25 mg CGA in 1mL of HPLC grade ethanol; Six concentrations-3.92, 7.84, 23.52, 39.2, 54.88 and 78.4 mg/L prepared) [20].

Gallic acid (GA)

Extraction of gallic acid was done according to the ISO procedures ISO14502-2-2005E (2005) for Catechin determination [15].

L-Theanine

Extraction was done with distilled water (1g in 100 mL of boiling double distilled water for 5 min) cooled to room temperature, filtered and topped to 100mL mark before transferring to vials for analysis (1mL). A Shimadzu LC 20 AT HPLC fitted with a SPD-20 UV-Visible detector and C_{18} column was used. Mobile phase A (double distilled water) and B (100% acetonitrile) at a flow rate of 1mL/min, column temp 35±0.5°C and 20µL injected. Binary gradient conditions: 100% solvent A for 10 min; wash time 8 min with 20% mobile phase A and 20% mobile phase B; 20min conditioning with 100% mobile phase A before the next injection. Theanine was quantified using a standard calibration curve (50 mg L-theanine dissolved into 50mL with distilled water and dilutions of 5, 10, 20, 50 and 100 mg/L made). The theanine content was computed and expressed as a percentage by mass on a dry matter basis using the relation in equation W_1= ((Dsample – D intercept) x Vsample x d x 100)/ (Sstd x M sample x 10000 x wDM, sample). Where: W_1 was weight of theanine; Dsample was the optical density for the sample; D intercept was the optical density at the point of the best-fit linear calibration line intercepts the y-axis; S std was the slope obtained from the best fit linear calibration; M sample was the mass (g) of the sample; Vsample was the sample extraction volume (10mL); d was the dilution factor and w DM sample was the dry matter content ((%) mass fraction) of the test sample [20, 32].

Data analysis

Data on Chlorogenic acid, Gallic acid and L-theanine was subjected to ANOVA and means separated ($p \leq 0.05$) using SAS statistical package [30].

RESULTS AND DISCUSSION

Chlorogenic acid (CGA)

A chromatograph of chlorogenic acid eluted in a sample tea assay is shown in Figure 2. The contents of chlorogenic acid in both CTC and orthodox teas from cultivars TRFK 306, TRFK 54/40, TRFK 11/4, TRFK 6/8, TRFK 31/8 and TRFK 12/2 are presented in Table 1.

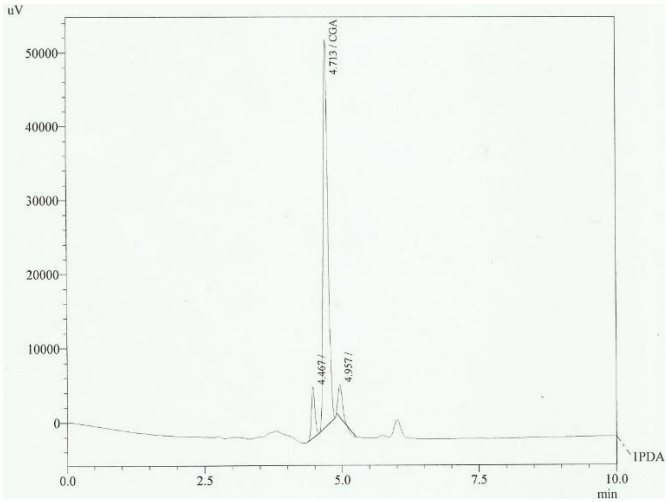

FIGURE 2: *Chlorogenic acid (CGA) peak eluted at 4.713 min A Shimadzu LC 20 AT HPLC fitted with a SPD-20 UV-Visible detector and C_6 column (150mm x 4.6mm) at 278nm.*

TABLE 1: *Chlorogenic acid of selected cultivars processed as aerated and non-aerated teas*

Tea Variety	Non Aerated CTC	Aerated CTC	Non Aerated Orthodox	Aerated Orthodox	MEAN	LSD	CV
TRFK 6/8	27.33±2.08	33.81±1.41	26.31±0.84	28.83±0.87	29.07	3.03	5.21
TRFK 31/8	27.62±0.71	29.44±4.36	22.31±1.14	33.44±1.32	28.20	3.73	6.63
TRFK 11/4	26.71±2.07	35.47±2.25	23.44±2.57	29.99±2.13	28.90	4.37	7.56
TRFK 54/40	29.53±3.16	36.22±1.68	20.72±1.31	26.46±2.04	28.23	4.52	8.02
TRFK 12/2	23.47±0.64	27.44±0.56	15.16±1.38	22.82±1.82	22.22	2.00	4.42
TRFK 306/1	31.75±2.64	36.94±0.29	15.16±0.00	39.14±1.15	30.75	2.80	4.55
Mean	27.73	33.22	20.52	30.11			
CV	7.61	5.18	5.48	5.57			
LSD	3.84	3.13	2.05	3.05			

Values are Means ± SD of three replicates. SD= Standard deviation, CV=Coefficient of Variation and LSD =Least significant difference. Columns show means for tea cultivars while rows show means for processing method.

Effects of processing methods on chlorogenic acid

Chlorogenic acid varied between aerated and non-aerated teas, increasing with aeration and maceration. Further, aerated teas showed higher CGA compared to non-aerated teas. Cut Tear and Curl teas registered significantly ($p \leq 0.05$) higher CGA compared to orthodox teas even for the same cultivar. TRFK 306 had the highest CGA in non-aerated CTC, aerated CTC and aerated orthodox teas, while TRFK 6/8 had the highest in non-aerated. It can be deduced that CGA formation is aided by aeration as well as the degree of cell disruption.

Research have shown that CGA in plants vary with type, physiological stage, storage and processing. TRFK 306/1, a purple tea cultivar, registered the highest CGA at 36.94% and 39.14% in aerated CTC and orthodox teas, respectively. Cultivar TRFK 12/2 recorded the least CGA at 27.44% and 22.82% for aerated CTC and orthodox teas, respectively. This means aeration method can be used to process teas with high CGA.

Effects of processing methods on gallic acid (GA)

Gallic acid (GA) was assayed and peaks eluted alongside the phenolics (Table 2; Figure 3). Aerated teas had significantly ($p \leq 0.05$) higher GA than non-aerated teas for orthodox teas across all cultivars. This could be attributed to oxidative de-gallation of phenolic esters [3].

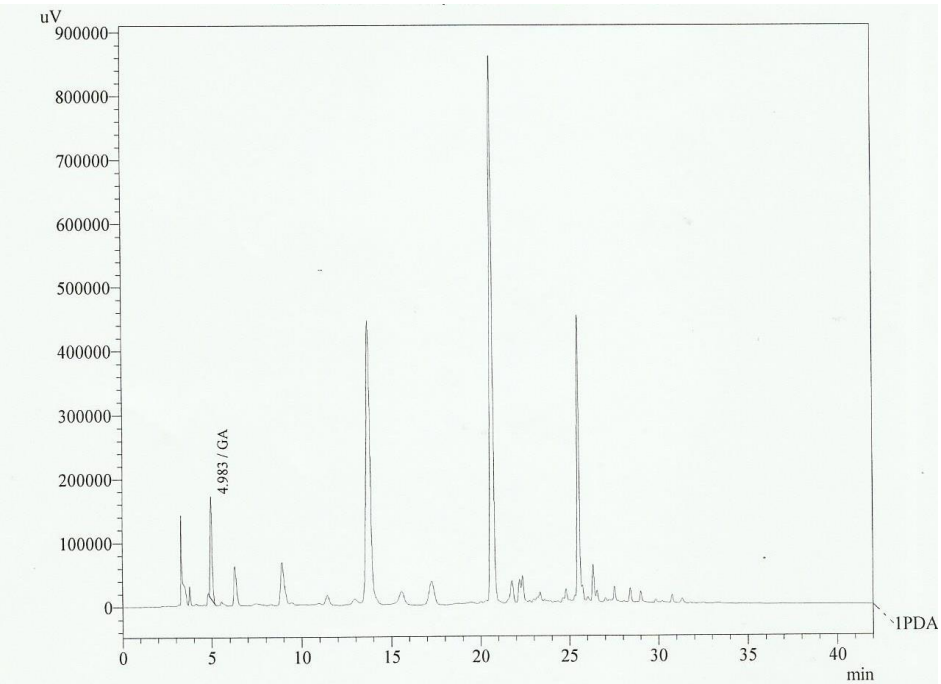

FIGURE 3*: Gallic acid (GA) peak eluted at 4.98 min. Shimadzu LC 20 fitted with SPD-20 UV-Vis detector, C_6 Column (250 x 4.6mm) at 278nm.*

TABLE 2: *Gallic acid of cultivars processed as aerated and non-aerated CTC and orthodox teas*

Tea Variety	Non Aerated CTC	Aerated CTC	Non Aerated Orthodox	Aerated Orthodox	Mean	LSD	CV
TRFK 6/8	0.28±0.01	0.28±0.07	0.26±0.02	0.35±0.11	0.29	0.12	21.14
TRFK 31/8	0.29±0.05	0.28±0.09	0.27±0.03	0.36±0.10	0.30	0.10	16.85
TRFK 11/4	0.36±0.04	0.28±0.09	0.34±0.07	0.38±0.11	0.34	0.17	25.10
TRFK 54/40	0.47±0.07	0.30±0.06	0.47±0.06	0.30±0.08	0.39	0.15	19.59
TRFK 12/2	0.26±0.01	0.33±0.01	0.21±0.00	0.32±0.02	0.28	0.02	3.45
TRFK 306/1	0.51±0.04	0.32±0.05	0.43±0.04	0.55±0.03	0.45	0.13	1.48
Mean	0.36	0.30	0.33	0.38			
CV	10.84	17.38	13.25	16.29			
LSD	0.07	0.09	0.08	0.11			

Values are Means ± SD of three replicates. SD= Standard deviation, CV=Coefficient of Variation and LSD =Least significant difference. Columns show means for tea cultivars while rows show means for processing method.

The lowest and highest GA for non-aerated CTC were 0.26 and 0.32%, while for orthodox it ranged from 0.21 to 0.43%, respectively. In aerated teas the range was 0.28 to 0.55%. The cultivar determines GA content in leaves. TRFK 12/2 had the least (0.26%), while TRFK 54/40 had the highest (0.36%). This indicates that aeration enhances GA in processed teas irrespective of the cultivars.

However, maceration does not appear to affect GA content for non-aerated teas, while GA content for aerated teas varied, albeit without any particular trend.

Effects of processing methods on L-Theanine

L-theanine chromatograph is shown in Figure 4 and the content for different cultivars presented in Table 3.

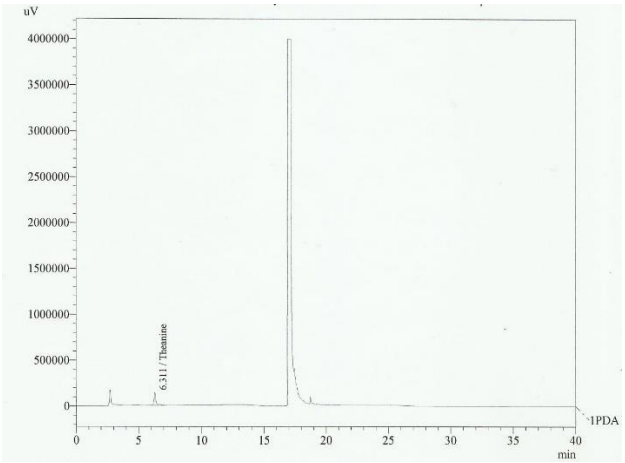

FIGURE 4*: Theanine chromatogram eluted at 17min using Shimadzu LC 20A series fitted with SPD-20 UV-Vis detector; C_{18}, aqua (250 x 4.6mm); at 210nm.*

TABLE 3: *Theanine in selected cultivars of aerated and non-aerated CTC and orthodox teas*

Tea Variety	Non Aerated CTC	Aerated CTC	Non Aerated Orthodox	Aerated Orthodox	MEAN	LSD	CV
TRFK 6/8	1.05±0.03	0.79±0.04	0.97±0.08	0.81±0.10	0.90	0.13	7.07
TRFK 31/8	1.30±0.17	0.76±0.08	1.54±0.20	1.20±0.40	1.20	0.51	21.23
TRFK 11/4	1.73±0.08	1.47±0.04	1.62±0.07	1.45±0.03	1.57	0.11	3.49
TRFK 54/40	1.40±0.08	1.08±0.04	1.40±0.09	1.19±0.09	1.27	0.15	5.77
TRFK 12/2	1.49±0.29	1.44±0.15	1.33±0.10	0.80±0.33	1.26	0.53	2.09
TRFK 306/1	1.19±0.05	1.19±0.27	1.82±0.01	0.89±0.04	1.27	0.25	9.75
Mean	1.36	1.12	1.44	1.06			
CV	10.09	11.01	8.00	21.20			
LSD	0.25	0.22	0.21	0.40			

Values are Means ± SD of three replicates. SD= Standard deviation, CV=Coefficient of Variation and LSD =Least significant difference. Columns show means for tea cultivars while rows show means for processing method.

Higher theanine was recorded in both non-aerated CTC and non-aerated orthodox teas with a mean of 1.36% (range: 1.05 - 1.73%) and 1.44% (range: 0.97 – 1.82%), respectively. compared to aerated CTC with a mean of 1.12% (range: 0.79 – 1.47%), while aerated orthodox mean was 1.06% (range: 0.80 – 1.45%). Cultivar TRFK 6/8 had the lowest theanine in aerated CTC, non-aerated CTC and non-aerated orthodox teas, while TRFK 11/4 had the highest theanine in non-aerated CTC and aerated orthodox teas. The studies showed aerated teas have low theanine, because of degradation during aeration [17, 29]. However, there was no significant difference ($p \leq 0.05$) between maceration types. The study shows that for the production of teas rich in theanine, non-aeration processing is most suitable compared to aeration. Processing methods that leads to higher L-theanine levels can be adopted for processing high value teas with health benefits.

CONCLUSION AND RECOMMENDATION

Tea contains phytochemicals that impart health benefits. Because of the growing lifestyle diseases due to oxidative reactions of reactive oxygen species (ROS) in our bodies, there is need to consume plant biomolecules for antioxidants and healthful biomolecules. The findings in this study show that processing methods alters the biochemical composition of teas, whereas cultivar determine biomolecules content. Non-aeration and aeration methods of processing should be used to process teas targeting specific biomolecules for health benefits. Aeration method should be used for CGA and GA while non-aeration method for

theanine rich teas. The style of maceration does not influence the composition of biomolecules during processing.

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