IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 11, Issue 10 Ver. II (October. 2017), PP 39-43
www.iosrjournals.org

Analysis Of The Diversity Of Some Arabica And Robusta Coffee From Kenya And Uganda By Sensory And Biochemical Components And Their Correlation To Taste.

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***Abstract*:** *Arabica coffee (Coffea arabica L.) is known for the production of high quality beverage while Robusta coffee (Coffea. canephora Pierre) has been characterized as a neutral, weak flavored and occasionally with strong acid and pronounced bitterness.Coffea arabica(Arabica) and Coffea canephora (Robusta) are the two main coffee species cultivatedcommercially in the world.However, bulk of the coffee in producing countries is sold as raw green coffee with very limited value addition. This study sought to establish correlations between some chemical components (caffeine, trigonelline, chlorogenic, citric, malic and phosphoric Acid) in the green coffee and the final beverage qualityof 10 coffees, 4 of them Arabica from Kenya and 6 Robusta from Uganda.HPLC analyses were used to determine the contents of caffeine, trigonelline, malic, citric and chlorogenic acids while the concentration of phosphoric acid was determined using a spectrophotometer. The sensory characteristics fragrance/aroma, flavor, aftertaste, acidity, body, balance,uniformity, clean cup, sweetness and overall perception were assessed by a panel of five judges. The results indicated significant ($p<0.05$) variations among the coffees for all the sensory attributes and biochemical components except trigonelline.There were positive significant correlations(at $P< 0.01$) among all the sensory characteristics with each other. Caffeine had negative correlation with all the sensory variables at $P<0.01$ level of significance.Citric acid showed significant ($P<0.05$) correlations with flavour and acidity.*

***Key words*:** *Coffee, Arabica, Robusta, Sensory variables, biochemical components, correlations*

Date of Submission: 29-09-2017 Date of acceptance: 10-10-2017

I. Introduction

Coffee beans are the seeds of a perennial evergreen tropical plant, which belongs to the family *Rubiceae* and genus *Coff*ea. Two species namely arabica (*Coffea arabica* Linnaeus) and Robusta (*Coffea canephora* Pierre) are cultivated commercially[1, 2] and to a limited extent liberica *(Coffea* liberica) and excelsa (*Coffea excelsa*) [3]. The distinct taste and aroma of coffee could be the main reason why it is widely and almost universally accepted as a refreshing beverage [4]. The green coffee contains all of the necessary precursors to generate the coffee flavour. However, the levels and biochemical status of these precursors may vary in relation to factors such as species, variety of bean, geographic origin, soil conditions, and storage of the beans, duration and temperature of the roasting procedure, genetic traits, environmental factors, maturation level, postharvest treatment, and storage[5]. Green coffee biochemical composition of has been used to discriminate between Arabica and Robusta [6, 7]. The biochemical composition and beverage quality has also been used to compare Arabica hybrids grown at various elevations in Central America[8]. Caffeine, chlorogenic acids, sucrose and trigonelline have been used for characterization of coffee species as well as varieties within a species[9].

Different levels of biochemical components in coffee contribute variously to the final quality of the cup[10]. Trigonelline is a pyridine derivative known to contribute indirectly to the formation of appreciated flavour products including furans, pyrazine, alkyl-pyridines and pyrroles during coffee roasting [11]. Chlorogenic acids (CGA) play an important role in the formation of roasted coffee flavour and have a marked influence in determining coffee cup quality[12]. They are known to be responsible for coffee pigmentation, aroma formation, bitterness and astringency [13].

Acidity has been recognized as an important attribute of the sensory quality in coffee. The International Standard ISO-5492[14] defines acidity as a basic taste produced by dilute aqueous solutions of most acid substances. Acidity rises from the presence of hydrogen ions from the ionization of constituent acids (both inorganic and weak organic) in aqueous solution. Among the coffee tasters, sourness has a particular

connotation, generally unfavorable, whereas, acidity is a favorable characteristic Washed Arabicas (or milds) usually have fine acidity whereas dry processed Robustas are neutral with varying degrees of harshness This study soughtto establish correlations between some chemical components (caffeine, trigonelline, chlorogenic, citric, malic and phosphoric Acid) in the green coffee and the final beverage quality

II. Materials And Methods

2.1Roasting and sensory evaluation

All procedures were performed according to the protocol described by the Specialty Coffee Association of America - SCAA [15].Roasting of the green coffee was done to attain a medium roast level using a Probat laboratory roaster within 24 hour of evaluation and allowed to rest for at least eight hours. The roasted coffee bean samples were weighed out as whole beans to a predetermined ratio of 8.25g per 150 ml of waterand ground immediately prior to sensory evaluation, (no more than 15 minutes before infusion with water) and ground individually into the cup (five cups per sample).Clean and odor free water was used for coffee beverage preparation and was brought to approximately 200º F (93ºC) at the time of pouring onto the ground coffee. The hot water was poured directly onto the grounds in the cup to the rim of the cup, making sure to wet all of the grounds. The grinds were allowed to steep undisturbed for 3-4 minutes before evaluation. The sensory characteristics fragrance/aroma, flavor, aftertaste, acidity, body, balance,uniformity, clean cup, sweetness and overall were assessed by a panel of three trained judges. The sensory attributes were scored on a ten point scale.The total score, which is a reflection of the broad coffee quality performance, was calculated by adding all the parameters including uniformity, clean cup and sweetness.

2.2 Sample preparation prior to biochemical components extraction

Green coffee beans from Robusta and Arabica coffees were lyophilized using liquid Nitrogen and then ground to a fine powder using an IKA Wilmington,NC28408 USA Blade grinder and larger particles were removed by passage through a 0.425 MM screen. After grinding, the samples were kept in a freezer at -4°C until analysis.

2.3 Extraction and quantification of caffeine, trigonelline and total chlorogenic acids (CGA)

Caffeine, trigonelline and CGA were extracted from the green coffee powder by refluxing in distilled water. Caffeine, trigonelline and CGA were analysed using a HPLC system (KNEUR) equipped with a Supel Co. discovery diode array detector at three wavelengths, 278nm for caffeine, and 266nm for trigonelline and 324nm for CGA. Identification of caffeine, and trigonelline CGA was done by comparing the retention times of standards and their concentrations calculated from peak areas using calibration equations.

2.4 Extraction, analysis and quantification of organic acids

Five(5) grams of the green coffee powder wasweighed into a 250 mL conical flasks and 150 ml of deionized water (18.2 MQ) at 70°C added.The flask with the contents was agitated in a ultrasonic bath for 5 minutes and then placed into a water bath set at 70°C for 30 min. The flask content was filled to 250 mL mark with deionized water (18.2 MQ) and then filtered. Three milliliters of the resulting extract was filtered again in a C18 cartridge (SEP PAK) that had been previously conditionedwith methanol and 5 mL of water. The filtrate was acidified using 1M sulphuric acid to pH 2 and partitioned using ethyl acetate. Ethyl acetate was evaporated to dryness using Rotorvapor at low temperatures followed by quantitative determination of organic acids.

2.5 Quantitative determination of organicacids

The concentration of organic acids was measured in two replicates using a high performance liquid chromatography (HPLC) (Knauer, Germany), refractive index (Model Smartline S 2300) detector, with a pump Knauer (Model Smartline S1000) set at 0.6Ml/Min, Oven (Model Smartline S 4050) set at 40^0 and Column Eurokat H 10µm, Mobile phase 0.01N H_2SO_4. Standard solutions of malic acid, and citric acid were used for peak identification in the chromatograms and for the calculation of the sample concentration. The organic acid levels of the samples were quantified in percentage of dry matter basis (% dmb).

2.6 Extraction, analysis and quantification of phosphoric acids

Phosphoric acid was determined according to [16]. One gram of the coffee powder was weighed into a dry test tube and 3 mls of nitric acid and Molybdivanadate reagent mixed in the ratio of 1:1and heated to boiling and gently simmered for 30 minutes in the digestion rack. The solution was treated 5 mg of activated charcoal and diluted to 20 ml. and vigorously shaken. After filtration 5 mls were transferred to a clean test tube and 2 ml of the molybdivanadate reagent added using an automatic pipette, shaken and diluteto 10 ml, and left undisturbed for 10 minutes. The color of the sample was compared to the color of standards similarly prepared. Absorbance

was recorded using a spectrophotometer the concentration of phosphoric acid in the samples calculated using a calibration equation.

2.7 Statistical analysis

The sensory and biochemical data was subjected to analysis of variance (ANOVA) using the software SPSS 19 and effects declared significant at 5% level. Student-Newman-Keuls (SNK5%) test was used to separate the means at 5 % level of significance. The computer programme IBM SPSS Statistic 19 was used to perform statistical correlation analysis using Pearson Correlation Coefficients.

III. Results

Analysis of variance revealed the coffees varied significantly ($P<0.05$) in all sensory characteristics except the variable overall as shown in TABLE 1. Aftertaste,body, balance and acidity was significantly different between Arabic and Robusta coffee samples. The flavour of Robusta-1480and Robusta-1460 was not significantly different($P<0.05$) from that of the the Arabicas coffees assessed. Similarly, the fragrance of Robusta-1480 was not significantly different($P<0.05$) from that ofarabica coffees Batian and Ruiru 11.

Table 1: Mean sensory characteristics of four Kenyan Arabica and six Ugandan Robusta coffees

Source	Sample description	Fragrance	Flavour	Aftertaste	Acidity	Body	Balance	Overall	Total
Kenya	Arabica-SL 28	7.88a	7.88a	8.00a	7.88a	7.88a	7.88a	8.13a	85.50a
Kenya	Arabica-R11	7.88a	7.75a	8.00a	7.88a	7.75a	7.88a	7.88a	85.00a
Kenya	Arabica- Batian	7.75ab	7.63a	7.63a	7.75a	7.63a	7.63a	7.63a	83.63a
Kenya	Arabica- K7	7.63ab	7.50a	7.75a	7.63a	7.63a	7.63a	7.75a	83.50a
Uganda	Robusta-1500	6.88c	6.75b	6.63b	6.63bc	6.63b	6.63b	6.38a	76.50b
Uganda	Robusta-1480	7.13bc	6.63ab	6.50b	6.63bc	6.13bc	6.13c	6.88a	75.00b
Uganda	Robusta-1560	6.88c	6.13b	5.88b	6.00d	6.13bc	6.13c	6.88a	71.00c
Uganda	Robusta-1460	5.75d	6.88ab	6.50b	6.75b	6.38b	6.13c	5.75a	65.13d
Uganda	Robusta-1240	6.50c	6.13b	6.13b	6.13d	5.50cd	6.00c	6.50a	52.88e
Uganda	Robusta-1520	5.88d	5.75b	5.63b	5.25e	5.25d	5.13d	6.00a	48.88f

Means along a column not sharing a letter are significantly different ($P<0.05$) using Student-Newman-Keuls test.

Analysis of variance showed that the coffees portrayed significant differences ($P<0.05$) in all the biochemical components assessed except trigonelline. Caffeine levels were higher in the Robusta coffee than Arabica coffees (TABLE 2).

Table 2: Mean trigonelline, caffeine, citric, malic, phosphoric and total chlorogenic acids (CGA) % dry weight basis (DWB) for fourKenyan andsix Ugandan Robusta coffees

Source	Sample description	Trigonelline	Caffeine	Citric acid	Malic acid	Phosphoric acid	CGA
Kenya	Arabica K7	0.92a	1.05e	1.35a	0.22ab	0.31cd	7.11d
Kenya	Arabica-Batian	1.25a	1.08e	0.98ab	0.27a	0.48abcd	7.45cd
Kenya	Arabica-Ruiru11	1.18a	1.34de	1.20ab	0.19abc	0.36bcd	7.94b
Kenya	Arabica-SL28	1.20a	1.23de	1.00ab	0.16bc	0.74a	7.15d
Uganda	Robusta- 1460	0.96a	2.48ab	0.80b	0.14bc	0.26cd	7.34cd
Uganda	Robusta-1240	1.10a	2.41ab	0.89b	0.13bc	0.35 bcd	7.40cd
Uganda	Robusta-1480	1.22a	1.96c	1.05ab	0.18abc	0.59 bcd	7.41cd
Uganda	Robusta-1500	1.00a	2.30ab	1.18ab	0.22ab	0.68 ab	7.65bc
Uganda	Robusta-1520	1.20a	2.73a	1.04ab	0.15bc	0.36 bcd	7.75bc
Uganda	Robusta-1560	1.21a	2.77a	0.91b	0.11c	0.16d	8.23a

Means along a column not sharing a letter are significantly different ($P<0.05$) using Student-Newman-Keuls test.

3.1 Correlation among biochemical and sensory variables

There were positive significant correlations among all the sensory characteristics (at $P< 0.01$) with each other

(TABLE 3). Flavour and acidity showed significant (P<0.05) correlations with citric acid. Caffeine had negative correlation with all the sensory variables at P<0.01 level of significance. The caffeine content of green beans showed negative and statistically significant correlations with all sensory quality attributes.

Table 3: Correlation coefficients of sensory and biochemical variables

Variables	Fragrance	Flavour	Aftertaste	Acidity	Body	Balance	Overall	Citric	Malic	Phosphoric	Chlorogenic	Trigonelline
Flavour	0.812**											
Aftertaste	0.856**	0.986**										
Acidity	0.839**	0.991**	0.981**									
Body	0.858**	0.979**	0.972**	0.970**								
Balance	0.902**	0.965**	0.978**	0.972**	0.982**							
Overall	0.968**	0.818**	0.871**	0.833**	0.860**	0.901**						
Citric	0.185	0.613*	0.529	0.617*	0.569	0.514	0.219					
Malic	-0.141	0.259	0.212	0.198	0.233	0.131	-0.031	0.690*				
Phosphoric	0.443	0.451	0.444	0.41	0.399	0.400	0.362	-0.218	-0.393			
Chlorogenic	-0.256	-0.517	-0.514	-0.499	-0.423	-0.420	-0.315	-0.253	-0.013	-0.532		
Trigonelline	0.323	0.053	0.067	0.036	0.063	0.084	0.347	-0.241	-0.055	0.270	0.117	
Caffeine	-0.874**	-0.926**	-0.956**	-0.931**	-0.913**	-0.926**	-0.887**	-0.424	-0.165	-0.446	0.585	-0.141

*. Correlation is significant at the 0.05 level (2-tailed).
**. Correlation is significant at the 0.01 level (2-tailed).

IV. Discussion

The two species of *Coffea* that have acquired worldwide economic importance are Arabica andRobusta. Coffee beverage quality is a complex characteristic which depends on a series of factors. Robusta coffee has been characterized as a neutral, weak flavored and occasionally with strong acid and pronounced bitterness[17]. However,a study by[18] found Ugandan Robusta coffee having good cup with some qualities comparable with Arabica. Similar results were observed in this study the some of the Robusta coffee having flavour which was not significantly different from the Arabica.

The levels of trigonelline, caffeine, citric, malic, and phosphoric and chlorogenic acids evaluated in this study were within the ranges reported in other studies. Caffeine levels ranged from 1.05% to 1.34% (dwb) for Arabica and 1.96% to 2.77% (dwb) for Robusta levels showing there were within those reported in literature [5, 19, 17]. The average level of trigonelline varied from 0.92% to 1.25%. [20] reported carboxylic acid profile of Arabica coffee at 0.5%dm citric acid and 0.5% malic acid. In another study, [20] gave an average of 5.6 g/kg for malic acid and 12.3 g/kg for citric acid in arabica coffees, while values for Robusta coffees averaged 3.0 g/kg for malic acid and 8.6 g/kg for citric acid. Report by [21] indicated arabica coffees containing less phosphoric acid (average 1.3 g/kg) than Robusta varieties (average1.7 g/kg).

Results of this study showed some and significant correlations among some of the sensory attributes.The caffeine content of green beans showed negative and statistically significant correlations with all cup quality attributes.[22] analysed green beans for caffeine and found, the highest and lowest caffeine levels to be the highest and lowest quality samples, respectively.However results like that would only be possible when analyzing coffee from same species. This study did not show any discernable trend of caffeine levels and beverage quality. Chemically, caffeine remains stable during coffee roasting except for minute amounts that sublime [22]. Beside its stimulatory effect mainly attributed to caffeine, coffee is appreciated and/or consumed for its pleasing aroma and taste.

The acidity of coffee brews has always been recognized as an important attribute of their sensory quality. Some of the acids contributing to this sensation are formed during the development of the coffee bean while some are generated during roasting [23]. Carboxylic acids such as citric acid, malic acid, and the chlorogenic acids are important sources of hydrogen ions in coffee. No significant correlations were observed between cup quality and total chlorogenic acids.Citric acid formed during the development of the coffee bean was found to have significant (P<0.05) correlations to flavour and acidity. However, [24] reported a correlation between the coffee astringency to chlorogenic acids while [25] associated individual contents of chlorogenic acid with bad coffee. In their study, [12] found 3, 4-dicaffeoylquinic acid levels in green coffee correlating strongly with high quality. The fact that coffees with high total chlorogenic acids had equally good flavour underscores the importance of analyzing specific chlorogenic acid fractions in coffee.

V. Conclusion

The study demonstrated high variation in sensory and biochemical composition in the samples analysed. Significant correlations observed between citric acid and cup quality traits indicate that biochemical components in green coffee plays an important role in determining the sensory quality of coffee. It further indicates that chemical analysis of green beans may be used as an additional tool for coffee quality evaluation.

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Yash Khapre. "Analysis Of The Diversity Of Some Arabica And Robusta Coffee From Kenya And Uganda By Sensory And Biochemical Components And Their Correlation To Taste." IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT), vol. 11, no. 10, 2017, pp. 39–43.