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Effect of Different Fermentation Methods on Physicochemical Composition and Sensory Quality of Coffee (*Coffea arabica*)

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Abstract: *Fermentation of coffee beans is primarily done to remove mucilage and can be done using two methods; dry and wet fermentation methods. This research aimed at determining the effect of different fermentation methods on physicochemical composition and the sensory quality of coffee. Coffee cherries were pulped and subjected to natural fermentation methods in different fermentation containers; plastic bucket, sack and cement tank. After fermentation, the parchment were washed and dried. The green coffee beans were evaluated for physicochemical composition and sensory attributes. The results showed that different fermentation methods did not have significant variations in most of the physico-chemical parameters analysed. However, significant variations were observed in the levels of pH with the wet fermented coffee samples showing lower levels as compared to dry fermented samples. Sensory evaluation results showed that wet fermented coffee samples had better colour of green beans, least silver skin discoloration and overall quality compared to dry fermented coffee samples. There were no significant differences in the body, acidity, colour, flavour and overall class among the coffee samples fermented using different containers. Hence different containers used during fermentation do not affect coffee quality and processors can adopt materials that are cheaper to reduce expenses during coffee processing.*

Key Words: *Arabica coffee, fermentation methods, physicochemical composition, cup quality*

I. Introduction

In Kenya, coffee is largely processed by wet method. The stages included during wet processing of coffee include; harvesting, sorting, removal of pulp, fermentation followed by washing, grading drying and finally storage[1]. Coffee is fermented to ease the removal of a layer of mucilage surrounding the bean, after which it is washed and dried. During fermentation the mucilage is degraded by natural enzymes in coffee fruits and the growth of microorganisms[2]. Chemical changes that occur during fermentation are pectin degradation by pectinase enzymes and breakdown of sugars in the mucilage[3]. Fermentation is concluded when the mucilage is easily detached by washing. Apart from mucilage removal fermentation also has impact on chemical composition of coffee bean and resulting cup quality. Microbial fermentation yields different flavor materials by breaking down the carbohydrates in the coffee mucilage and thus the superior aroma characteristics of wet-processed coffees[4].

Wet fermentation, involves water being added to submerge the coffee parchment in fermentation tank. For dry fermentation, the coffee parchment fermented in a tank without addition of the water [5]. In Kenya, coffee parchment fermentation is commonly done under dry or wet fermentation methods. Wet fermentation method is commonly used when conditions are too hot and fermentation conditions need to be controlled. Similarly coffee processors may utilize different equipment during the fermentation of coffee parchment [6]. This variations in the method and equipment used may cause variations in several parameters which could influence the final coffee quality. However, there is limited information on the effect of different fermentation methods and materials used on the chemical composition and the final cup quality. This research aimed at determining the effects of different fermentation methods and materials used on physicochemical composition and cup quality of coffee.

II. Methods And Methods

2.1Sample Collection and Treatment

Coffee cherries of Ruiru 11 cultivar was obtained from Dedan Kimathi University of Technology coffee farm, located in Nyeri County, Kenya. The ripe cherries were harvested from coffee plants grown in the same block by selective hand picking method to obtain uniform ripe cherries. The cherries were pulped and then subjected to four different treatments which included dry fermentation of coffee parchment in a sack, dry fermentation in a plastic bucket, dry fermentation in a cement tank and wet fermentation in a plastic bucket. For dry fermentation in a plastic bucket, perforations at the bottom of the container allowed the excess water to drain

while for wet fermentation the parchment was submerged in water in a plastic bucket. A control sample was also prepared by fermentation of coffee parchment in a cement tank by the dry method. The end of fermentation was assessed by rubbing a few beans of the fermenting coffee in the hand to check whether they felt gritty and can easily be washed. After fermentation the coffee parchment was thoroughly washed to remove the degraded mucilage and then dried to reduce moisture content to a level of 9.5 to 10%.

2.2 Physicochemical Composition

2.2.1 Preparation of Green Beans for Chemical Analysis

The parchment were hulled and ground to obtain fine powder of particle size 0.6mm for analysis of physicochemical components including; crude protein, titratable acidity, pH, sucrose, caffeine, chlorogenic acids and trigonelline.

2.2.2 Determination ofCrude Protein

Crude protein was determined using Kjedhal method as described in [7]. The Protein % was calculated by multiplying nitrogen value obtained by protein factor (6.25).

2.2.3 Determination Titratable Acidity

Total acidity in the coffee samples was determined by adding 10 g of the coffee sample in a conical flask with 75 ml 80% alcohol. Agitation was done using a shaker for 16 hours followed by filtration. Ten ml of the filtrate was diluted to 100 ml followed by titration with 0.1N Sodium hydroxide using phenolphthalein as an indicator [8].

2.2.4 Determination of pH

The pH of coffee samples was determined using the method as described by[9].Three grams of ground coffee were added to 50ml hot water. The extract was cooled to room temperature and pH measured using a pH meter.

2.2.5 Determination of Sucrose Content

Sucrose was determined using High Performance Liquid Chromatography (HPLC) analysis as described by[10]. In sample preparation, 0.5g of the sample was added to a flask containing 50ml 96 % ethanol. The mixture was refluxed for one hour, then ultrasonicated for six minutes. This was followed by cooling and filtration using a Whatman filter paper no.42.The filtrate obtained was evaporated to dryness using rotor vapors. It was then reconstituted with10 ml mobile phase containing acetonitrile: water 80:20 and filtered using micro filter (0.45 µm). A sample of 20µm was injected into HPLC fitted with a refractive index.

2.2.6 Determination ofCaffeine Content

Extraction and purification of caffeine was done as described by [11]. Five (5) grams of the ground coffee powder was weighed in a 250 ml Erlenmeyer flask before adding 3.5 grams of magnesium oxide and 200 ml of double distilled water. The mixture was refluxed for 25 minutes and then left to cool. It was then filtered under vacuum on celite and the filtrate recovered in a 250 ml flask topping up the volume with distilled water. Twenty (20) ml of this preparation was then pipetted into 100 ml flask adjusting to volume with mobile phase consisting of 35% v/v methanol, 65% v/v distilled water and 0.1% v/v glacial acetic acid. The extracts were then filtered using 0.45 µm micro filters. A sample volume of 20 µl was injected into HPLC with pulsed diode array (PDA) detector set at 278 nm. Peak areas were used for calculating the concentration of caffeine.

2.2.6 Determination of Chlorogenic Acid Content

The coffee samples were prepared for HPLC analysis according to the method described by [12]. Two grams of each ground coffee sample were weighed accurately in 250 ml beakers and 100 ml distilled water was added to each sample. The mixture was boiled for 5 minutes while stirring, and then cooled. Subsequently, the solution was filtered using 0.45 µm filter paper. The clear filtrate with some dilution was used for the HPLC analysis with detector, pulsed diode array set at 324 nm. A 100µl amount of the filtrate was diluted with 900 µl of demonized water and pipetted into clean 1000 µl volumetric flasks. Peak areas were used for calculating the concentration of chlorogenic acids.

2.2.7 Determination of Trigonelline Content

Trigonelline content was determined using HPLC analysis according to method described by[13].Samples preparation was done by weighing 0.5g of ground coffee sample and adding it 30 ml of an acetonitrile: water solution (5:95 v/v) at 80 °C for 10 min. Extracts were filtered and diluted in the mobile phase before injection. Peak area were used for calculating the concentration of trigonelline.

2.3 Sensory Evaluation

The quality of the coffee samples was analyzed using the method of [14]. Three hundred grams of green bean were used for raw bean analysis and 100g during roasting for each sample. A sensory evaluation

report form was used to record quality attributes for each coffee on raw and liquor quality. During raw bean quality analysis, color and quality attributes were assessed. Liquor was assessed using acidity, body and flavor attributes. Color of raw bean evaluated as greyish blue, greyish green with blue tinge, greyish green, greyish green with brown tinge, brownish grey green, greenish and brownish. Quality and flavor evaluated as fine, good to fine, good, fair to good FAQ fully, FAQ, poor to fair, poor and very poor. Acidity and body evaluated as pointed/ full, medium, light medium, light and lacking. The overall quality of the coffee was then evaluated as fine, good to fine, good, fair to good FAQ fully, FAQ, poor to fair, poor and very poor. The overall quality given infers to the class of the coffee sample.

III. DataAnalysis and Presentation

Data was analyzed using Statistical Packages for Social Scientist (SPSS) software version 20. Measures of variability of means among the samples was done using ANOVA. The means were subjected to Duncan Multiple Range test. The significance level was established atp≤0.05. Sensory data was subjected to analysis of variance and multivariate analysis. Mean seperation was done using Student-Newman-Keuls ($SNK_{5\%}$) test by Costat. Discriminant Function analysis was done using XL-STAT 2017.

IV. Results And Discussion

4.1 Physicochemical Composition of Coffee Processed Using Different Fermentation Methods

The levels of crude protein, pH, and titratable acidity and biochemical compounds in the coffee samples are presented in Table 1. In this study, the protein content of the green beans ranged between 10.62 ± 0.20 and 10.82± 0.15 for all the treatments. The results were within the range reported in literature. Green coffee beans contain about 9 to 16 % of protein in dry weight basis [15]. The results showed no significant differences between the treatments used in regard to the protein content ($p \leq 0.05$). Therefore, the wet fermentation method which allows the mucilage to degrade slowly did not cause any changes in the protein content of the beans and did not vary with the level in the dry fermented samples. However, [16] reported that free amino acids could be released in the bean following the degradation of proteins during the fermentation process in coffee.

Table 1: physicochemical composition of coffee processed using different fermentation methods (%)

Parameter	Treatment			
	Dry fermentation in cement tank	Dry fermentation in a sack	Dry fermentation in a plastic bucket	Wet fermentation in a plastic bucket
Crude Protein	10.67 ± 0.81 [a]	10.82± 0.15[a]	10.79 ± 0.54[a]	10.62 ± 0.20[a]
Titratable acidity	1.79± 0.2 [a]	1.66 ± 0.17 [a]	1.96 ± 0.58 [a]	1.62 ± 0.12 [a]
pH	6.06 ± 0.01 [a]	6.06 ± 0.01 [a]	6.05 ± 0.06[a]	6.00 ± 0.20 [b]
Sucrose	7.76 ± 1.15 [a]	7.92 ± 1.27 [a]	7.76 ± 1.11 [a]	8.03± 1.51 [a]
Caffeine	1.12± 0.44 [a]	1.13 ± 0.13 [a]	1.19± 0.41 [a]	1.17 ± 0.56 [a]
Chlorogenic acids	5.48± 1.03 [a]	6.34 ± 1.20 [a]	5.94±1.24 [a]	6.09± 0.74 [a]
Trigonelline	0.82 ± 0.14 [a]	0.87 ± 0.6 [a]	0.91± 0.43 [a]	1.01± 0.24 [a]

Values are means (± SD) of triplicate determinations. Means with the same letter are not significantly different at $p \leq 0.05$

It could be expected that the wet fermentation method causes more degradation of coffee than the dry fermentation due to the soaking by water and the long period of fermentation involved but this was not noted in the study. Losses of components such as protein in green beans could be minimal since protein and peptides are reported to complex with polyphenols and hence remain intact in the cell structure[16]. A study by [17]noted that the total concentration of free amino acids and protein content does not change significantly with the chemical reaction occurring during the harvest season and the postharvest processing steps, such as fermentation, drying and storage. Protein, peptides, and free amino acids are vital for coffee flavor since they are needed for the Maillard reaction [18]. They serve as flavor precursors as they play major roles during roasting of coffee to produced aroma compounds such as furans, pyrroles, pyrazines [15].

The mean titratable acidity of the green coffee beans that were fermented in a cement tank and in a sack was 1.79 ± 0.2and 1.66 ± 0.17 respectively. Titratable acidity of green beans for dry and wet fermented samples in a plastic bucket was 1.96 ± 0.58and 1.62 ± 0.12, respectively (Table 1). Similar results were obtained by[19]who reported titratable acidity of 1.32 to 1.60 mL $NaOHg^{-1}$[20]noted that dry processed coffee has higher titratable acidity as compared to wet processed coffee due to high levels of fermentation. However there were no significant differences between the fermentation methods used in terms of titratable acidity. Regarding the pH level, the wet fermentation method showed a significantly lower pH level. This could be attributed to the longer fermentation time taken during wet fermentation and the view that formation of aliphatic acids is increased by underwater fermentation[21]. Wet fermentation method takes longer because the water lowers the temperature and reduce the microbial growth hence degradation of mucilage from the beans proceeds at a slower phase. Hence acids generated by the microorganisms during fermentation accumulates in the beans. A report by

[22]indicate that microbial metabolites such as acetic acids produced during post-harvest processing accumulate in the coffee beans. The presence of these acids could lower pH levels.

Sucrose is an important biochemical component in coffee beans since their high levels in coffee beans correlate with better cup quality. Sucrose levels in the coffee samples as analyzed in this study, were found within the range of 7.76% and 8.03% for all the treatments (Table 1). These findings concur with [10] who reported that sucrose level of Arabica coffee ranges between 5- 8.5%. There were no significant differences between the treatments used in relation to the sucrose level. Although sucrose is soluble in water, these results indicate no loss of sucrose to the fermenting water as in the case of wet fermentation where coffee parchment is immersed in water and possible leaching of sucrose could occur. Studies on the effect of coffee processing on content of sugars showed that only reducing sugars such as glucose and fructose may be lost to the processing water [23]. Therefore sucrose as a disaccharide is not significantly affected by coffee fermentation and remains intact as a storage compound [24].The level of caffeine in all the coffee samples for all the treatments used ranged from 1.12% to 1.19% (Table 1). The level of caffeine in the samples were therefore within the range reported in literature. Arabica coffee contains about 1.2% of caffeine with a range of 0.6 to 1.9%[20]. From the study, there were no significant differences in the levels of caffeine in the coffee samples processed by the different fermentation methods. The levels of caffeine mainly vary between coffee species and genotypes [25]. There was no indication of loss of caffeine to the water used during fermentation as in the case of wet fermentation. This could be because caffeine is less soluble or it's strongly bound to the alkaloid and other compounds in the coffee bean [26].Caffeine plays an important role in coffee quality as it contributes to the perceived strength and to a small extent the bitterness of the brew[27]. Chlorogenic acids are among some of the aroma precursors in green coffee that are considered to contribute to acidity and formation of aroma compounds during roasting[28]. From the results, the chlorogenic acid content values of the samples varied from 5.48% to 6.34% (Table 1). The values of chlorogenic acid content obtained were in agreement with what is reported in the literature. The levels of chlorogenic acids range from 4% to 8.4 %[27]. Similarly, in the study, there were no significant differences ($p \leq 0.05$) between the different treatments used in relation to the level of chlorogenic acids content. Hence different fermentation methods does not affect the level of chlorogenic acids in green coffee beans. The wet fermentation method did not show any loss of this compound to the processing water.

4.2.1Sensory Quality of Coffee Processed By Different Fermentation Methods

Sensory quality evaluation was done to assess the influence of various fermentation methods on the quality of coffee. The method of Devonshire, [14]is commonly used for the assessment of the raw bean color, roast quality and liquor quality of coffee which aid in categorizing coffee into various classes. The attributes are given scores that range from 1 to 7 with the lowest score indicating the best level of the attribute. From the results, the color of the green coffee beans that were fermented in a cement tank and in a sack showed a mean score of $4.00 \pm 0.87$and 4.33 ± 0.50,respectively. The color of green beans that were obtained by dry and wet fermentation in a plastic bucket showed a mean score of $4.33 \pm 0.50$and 2.67 ± 0.0respectively (Table 2). There were significant differences ($p \leq 0.05$) between the treatments in terms of color. The wet fermented samples in a plastic bucket showed a significantly lower score than the other treatments in terms of color. This was described as a good greyish green color compared to the greyish green with brown tinge color shown by the other coffee samples obtained by the dry fermentation method. The dry fermented samples also showed more silver skin discoloration as compared to wet fermented coffee (Table 3). Considering overall quality, the wet fermented coffees samples was categorize as class 4+ (fully FAQ), followed by dry fermented coffee in a cement tank; class 4- , (About FAQ). The samples that were dry fermented in a sack and a plastic buckets both scored class 5- , (Poor to fair quality). From the study, it was noted that there were significant differences ($p \leq 0.05$) among the treatments in terms of color and overall quality (Table 2). Wet fermentation method have shown better overall quality and produces better color and with less silver skin discoloration. This improved overall quality may be attributed to the controlled longer fermentation time which allows increased complexity of acids and the possible leaching of some soluble substances bitter compounds from the beans to the water [29]. Moreover, it allows controlled fermentation to occur for longer which results in coffee with desirable attributes in terms of raw bean color and overall quality[2].

The acidity and body was described as medium for all the coffee samples for the different treatments. Hence no variations in the level of the attributes for all the treatments used (Table 3). This indicates that the different fermentation methods did not affect the perceived acidity and body of the brew. Although the pH level of the green beans was found to be significantly different between the treatments, the titratable acidity on the other hand was not significantly different ($p \leq 0.05$) between the treatments. Hence the level of acids in the brew for all the cofffee samples in the brew after roasting could be similar.The acids present in green beans include citric, malic, chlorogenic acids and quinic acids. The level of acids such as citric, malic and chlorogenic acid are shown to decrease during roasting while quinic acids increase due to the breakdown of chlorogenic acids[30].

Table 2: Mean sensory variables of raw bean color and coffee brew of coffee subjected to different fermentation treatments

Treatment	Color of green bean	Flavor	Overall class
Dry fermentation in cement tank	4.00 ± 0.87 [b]	5.00± 0.00 [a]	4- [b]
Dry fermentation in plastic bucket	4.33± 0.50 [b]	5.33± 0.50 [a]	5+ [b]
Dry fermentation in sack	4.33± 0.50 [b]	5.22± 0.44 [a]	5+ [b]
Wet fermentation in plastic bucket	2.67± 0.0a	5.00± 0.00 [a]	4+ [a]

Values are means (± SD) of triplicate determinations. Means with the same letter are not significantly different at $p \leq 0.05$.

Table 3: Description of Raw and Overall quality of coffee processed using dry and wet fermentation methods

Treatment	Raw quality	Body, Acidity	Overall quality
Dry fermentation in cement tank(Control)	Greyish green with brown tinge, Rather coated	Medium	About FAQ
Dry fermentation in sack	Greyish green with brown tinge, Rather coated	Medium	Poor to fair
Dry fermentation in plastic bucket	Greyish green with brown tinge, Rather coated	Medium	Poor to fair
Wet fermentation in plastic bucket	Greyish green	Medium	Fully FAQ

Key: FAQ- Fair Average Quality

4.2.2 Discriminant Function Analysis of Sensory Quality Variables of Coffees Samples In Different Containers

The quality data was subjected to discriminant function analysis (DFA). Results of the discriminant function analysis showed that the first discriminant factor explained 92.56 % total variation (Fig. 1). Using sensory variables, the coffees were not distinctly separated since overlapping of points were observed for the samples obtained from different containers used. (Fig. 1). This shows that the different containers used during the fermentation process did not have effect on the color, flavor and overall quality of coffee. This indicate that the different containers did not affect or created different conditions for the fermentation of parchment. Hence it can be deduce that, the use of different containers during fermentation process does not affect the color of green bean, flavor and over quality of coffee brew.

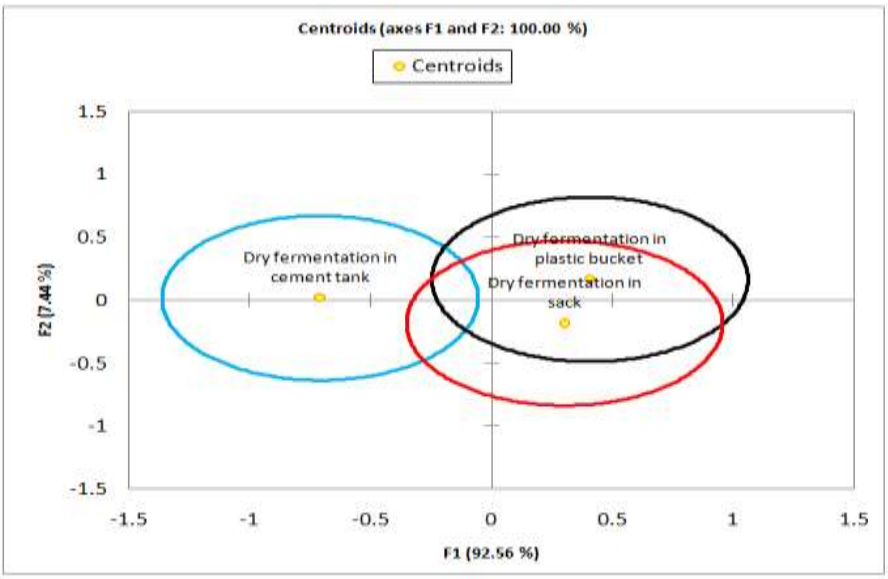

Figure 1: Discriminant factor analysis (DFA) based on quality data of dry fermented coffee in different containers.

V. Conclusion

From the study, the different fermentation methods used did not affect the level of crude protein, titratable acidity, sucrose, caffeine, and chlorogenic acids content of green coffee beans. However, pH was significantly different between the treatments. The wet fermentation method of coffee processing showed better quality than the other methods used. The use of different containers during fermentation showed non-significant differences on quality parameters such as color, flavor and overall class. Hence using different containers for fermentation under good practice does not affect the quality of coffee.

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