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# Determination of Caffeine Content and Antioxidant Activity of Coffee

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## To cite this article:

Belete Tewabe Gebeyehu, Solomon Libsu Bikila. Determination of Caffeine Content and Antioxidant Activity of Coffee. *American Journal of Applied Chemistry*. Vol. 3, No. 2, 2015, pp. 69-76. doi: 10.11648/j.ajac.20150302.16

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**Abstract:** Attempt has been made to look into caffeine contents and antioxidant activity of coffee grown at *Wembera*, *Goncha*, *Zegie*, and *Burie* localities of North-West Ethiopia. The caffeine content of the extracts in % w/w has been found to be  $1.53 \pm 0.003$  for *Wembera* coffee,  $1.41 \pm 0.040$  for *Goncha* coffee,  $1.29 \pm 0.033$  for *Zegie* coffee and  $0.97 \pm 0.049$  for *Burie* coffee. The antioxidant activities of the coffee extracts were measured by using ferric reducing power assay and Rancimat assay. Ferric reducing power assay was used to measure the total antioxidant power of water soluble components of coffee and is expressed as ascorbic acid equivalent antioxidant capacity in milligram per gram of the dried coffee samples. The ferric reducing power values of the extracts were  $9.532 \pm 0.201$ ,  $9.159 \pm 0.441$ ,  $8.955 \pm 0.180$ ,  $6.751 \pm 0.284$  for *Wembera*, *Burie*, *Goncha* and *Zegie* coffees, respectively. The Rancimat assay was also used to measure antioxidant activity of lipid soluble portions of coffee extracts using sunflower oil as the oxidizable substrate. It was found that all the coffee extracts improved the oxidative stability of sunflower oil. The protection factors were  $1.36 \pm 0.027$ ,  $1.31 \pm 0.027$ ,  $1.26 \pm 0.069$  and  $1.17 \pm 0.015$  for *Wembera*, *Burie*, *Goncha* and *Zegie* coffees respectively. Based on these results, it is suggested that *Wembera* coffee has the higher caffeine content and antioxidant activities than *Burie*, *Goncha* and *Zegie* coffee varieties.

**Keywords:** Coffee, Caffeine, Antioxidant Activity, Ferric Reducing Power, Rancimat Assay

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## 1. Introduction

The name coffee is derived from the name of the province *Keffa* where shepherds from Abyssinia/Ethiopia discovered the coffee beans in the 6<sup>th</sup> century [1]. Since then, coffee has become one of the most widely consumed beverages throughout the world [2] due to its pleasant taste [3], aroma [3], [4] stimulant effect [5] and health benefits [6].

Coffee beans are the seeds of a shrub belonging to the botanic family *Rubiaceae* and the genus *Coffea* [7]. The generic name covers over sixty different species only three of which, *Coffea Arabica*, *Coffea Robusta*, and *Coffea Liberica* have commercial values [6]. The two most important commercial species are *Coffea Arabica* and *Coffea Canephora*, usually known as *Arabica* and *Robusta* varieties, respectively [6], [8]. *Arabica* is considered to be a higher quality bean, prized for its complex aroma and flavors [9], [10] and is usually the most expensive one in the world market [8]. Most of the coffee in the world market is produced by developing countries including Ethiopia [9],

[11]. While most of the coffee plants cultivated in Ethiopia are *Coffea Arabica*, there are, however, wide ranges of variability among coffee cultivars in the country [1], [12], [13]. This variability in coffee beans has been attributed to variation in the soil, altitude and climate of the coffee growing areas [9], [12]-[16]. These factors are believed to influence coffees characteristics (chemical content, flavor or aroma) [14]-[16]. For example, Brazilian researchers after screening 300 Ethiopian coffee trees discovered three naturally decaffeinated varieties, which they named AC1, AC2 and AC3 [17]. Analysis of these varieties showed they contain less than 0.07% caffeine compared to the caffeine found in natural coffee beans [20].

Caffeine has been the subject of extensive research for its occurrence in nature and its long history of use [21]. It is a naturally occurring alkaloid [22] which is found in the leaves, seeds or fruits of over 63 plants species worldwide [1], [21]. The most common sources of caffeine are coffee [12], [16], [21], [23], cocoa beans, cola nuts and tea leaves and the worldwide consumption of products derived from these

natural materials means that caffeine is one of the most popular and commonly consumed drugs in the world [21].

Caffeine's popularity stems mainly from the fact that it is a pharmacologically active substance [24]-[26] and a mild central nervous system stimulant [20]. Coffee has been consumed for a long time mainly for its stimulant effects on the central nervous system due to presence caffeine [1], [24], [25]. However, recent literature reports associate coffee consumption with improvement of health among humans [23], [27]. This has been attributed to the presence of bioactive compounds [28]-[31] in coffee which possess antioxidant behavior [32]-[33].

Antioxidants [34] are substances that when present at low concentrations, compared with those of the oxidizable substrate significantly delay or inhibit oxidation of that substrate [30], [32], [43]. In living system, antioxidants have played an important role in scavenging excessive free radicals [35] such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) [36]-[39] which are formed during normal cellular metabolism in high concentration [33], [40]. Researchers have shown that the intake of cereals, fruits, vegetables, tea, and coffee [41], [42] are important to lower risk of diseases that are formed as consequence of free radicals [43]. Among those dietary species, coffee is a major source of antioxidants and is estimated to provide half of total antioxidant intake in several populations [20], [41], [42]. Thus, coffee extracts have received a lot of attention in recent years and numerous studies have proven that the biologically active components of the extracts such as chlorogenic acids (caffeoylquinic acids [29], dicaffeoylquinic acids [29], feruloylquinic acids [29], [30], [31]), [34] and *p*-coumaroylquinic acids [29]), melanoidns [37], [39], [44] and lipid soluble heterocyclic compounds (alkaloids) [45], [46] play significant role for its antioxidant behavior in both *in vitro* and *in vivo* [32].

The amount of chlorogenic acids, melanoids and heterocyclic compounds (caffeine) in coffee may be influenced by its species, origin, brewing procedures and roasting conditions which in turn affect the quality of its beverages [1], [12]-[16], [23]. It is thus justifiable to attempt to determine the caffeine content and antioxidant activity of coffee. Among the various methods that have been employed for the determination of caffeine in coffee, Uv-visible spectroscopy technique is fast, simple, cheap, and available in most laboratories [13].

Antioxidant activity of coffee can be evaluated by ferric-reducing antioxidant power (FRAP) assay [47], [48]. This is a simple, speedy and inexpensive, assay based on the ability of phenolics to reduce  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$  [49], [50]. The FRAP assay is valid to quantify samples with hydrophilic antioxidants [23]. The antioxidant activity of coffee can also be studied by the Rancimat assay which is an automatic determination of the oxidative stability of oils and fats without the need for expensive and environmentally hazardous chemicals [49], [51], [52]. The relative activity of the antioxidants is expressed by the protection factor, oxidative stability or antioxidant index [53].

Geographic location is known to affect the caffeine level and antioxidant activity [1], [12], [18], [54] of coffee belonging to the same species [1], [12]. To the best of our knowledge, very little research has been done on the chemistry of coffee varieties in Ethiopia. Therefore, the aim of the present study is to evaluate the caffeine level and the antioxidant activity of indigenous coffee varieties produced in four different localities (*Wembera*, *Burie*, *Zegie* and *Goncha*) of North-West Ethiopia.

## 2. Materials and Methods

### A. Instruments and apparatus

Traditional coffee roasting machine and electronic blending device model-NM 8300 (Nima, Japan) were used for roasting, grinding and homogenizing of coffee samples. Electronic balance (Ohaus, Switzerland) was utilized to measure mass of standards, reagents and samples. SP 65 UV/Visible spectrophotometer (SANYO, UK) was used to measure absorbance of the samples. Filter paper (Whatman number 542, 90 mm Dia, England), quartz cuvette, centrifuge (West Sussex, UK), Incubator (Tuttlingen, Germany), pH-meter model 3310 (Hanna, Italy) and Rancimat Metrohm model 743 (Herisan, Switzerland) were also used for different purpose during the experiment.

### B. Chemicals, reagents and samples

Dichloromethane, trichloroacetic acid, potassium hexacyanoferrate, ferric chloride, ascorbic acid, sodium phosphate dibasic, and sodium phosphate monobasic dihydrate all obtained from Blulux India, caffeine standard (St.Louis, Germany), and sunflower (Bahir Dar, local oil factory) were used in determination of caffeine content and antioxidant activity of coffee samples. Distilled water was used throughout the experiment for sample preparation and rinsing of materials. Four different coffee samples which were grown in *Wembera*, *Zegie*, *Burie*, and *Goncha* localities of North-West Ethiopia were collected from their production sites.

### C. Experimental procedure for determination of caffeine by Uv-visible spectrophotometer method

Exactly 20 g of coffee from each sample was roasted by using conventional coffee roasting machine. Each of the roasted coffee samples was ground and screened through 250 $\mu\text{m}$  sieve to get a uniform texture. An accurately weighed amount of sieved coffee (50 mg) was dissolved in 100 mL of distilled water in a temperature range of 80-90 °C. The solution was stirred for 30 min using magnetic stirrer and heated gently to remove caffeine easily from the solution. In addition the solution was filtered by using filter paper to get rid of particle from the solution. Series of working standard solutions of caffeine in the range of 0.016 mM to 0.102 mM concentration were prepared in distilled water.

The extraction of caffeine from coffee has been done according to a procedure reported by Belay [13]. In a typical experiment, the coffee sample solution was mixed with dichloromethane by volume ratio (25:25 mL) and the resulting mixture was stirred for 10 min using magnetic

stirrer. From the mixture, the organic phase containing most of the caffeine (solubility of caffeine in dichloromethane is 140 mg/mL) [55] is separated from the aqueous phase with a separating funnel. Dichloromethane is the most efficient (98-99%) solvent for extraction of caffeine from coffee [56]. The aqueous phase is extracted four times with 25 mL dichloromethane and the fractions from the organic phase were mixed together. The absorbance of this solution was measured in the range of 243–320 nm against the corresponding reagent blank (dichloromethane). The measurement of the absorbance was repeated three times for each sample.

#### D. Experimental procedure for determination of antioxidant activity by FRAP assay

The reducing power was determined by the method of Oyaizu [57] with little modification. 1 mL of different concentration of each coffee sample extracts (1.25, 2.5, 5.0 and 10% v/v) was mixed with 2.5 mL sodium phosphate buffer (0.2 M, pH 6.6) followed by addition of 2.5 mL of 1% potassium hexacyanoferrate to the mixture. The mixture was incubated at 50°C for 20 minutes then 2.5 mL of 10% trichloroacetic acid was added to the mixture to stop the reaction; the mixture was centrifuged 3000 rpm for 10 min. The supernatant (2.5 mL) was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride solution and allowed to stand for 10 min. The absorbance was measured at 700 nm against the blank (distilled water) to determine the amount of ferric ferrocyanide (Prussian blue) formed.

Series of ascorbic acid solutions in the range of 0.025 mg/g to 0.2 mg/g was used as standard to determine the reducing power of coffee extracts. The antioxidant results were expressed as mg of ascorbic acid equivalent antioxidant capacity per g of coffee (dry matter).

#### E. Experimental procedure for determination of antioxidant activity of coffee by Rancimat assay

5 g of each type of sieved coffee samples (*Wembera*, *Zegie*, *Burie*, and *Goncha*) was macerated in 25 g of sunflower for 10 hours at room temperature before analysis. Samples were centrifuged for 45 minutes at 5000rpm and the filtrate was made ready for analysis. 3 g of each filtrate samples was placed in four different reaction vessels and then placed in the heating block of the wet section. In parallel to this 3 g of sunflower oil was run as a reference in one reaction vessel. 60 mL of distilled water was also measured in five reaction vessels containing the electrodes and placed in the heating block of the wet section. The reaction vessels were connected with plastic tubes with the instrument as described by Rancimat 743 operating procedures. A temperature at 120 oC and air flow of 20 L/h was adjusted on the computer with Rancimat software.

#### F. Statistical data analysis

For the determination of caffeine content and antioxidant activity of coffees collected from four different geographical locations of north –west Ethiopia, all measurements and analyses were carried out in triplicates. The results were expressed as means  $\pm$  standard error of three parallel

replicates. Analysis of variance was performed by using one-way ANOVA. The results with  $P < 0.05$  were regarded to be statistically significant. Data were statistically analyzed using origin 7.0 programs.

## 3. Results and Discussion

### A. Determination of caffeine content by Uv-visible spectrophotometer method

The absorbance of four working standard solutions of pure caffeine in the range of 0.016 mM and 0.102 mM was measured at 273 nm using UV-Vis spectrophotometer. And then the absorbance versus concentration graph (Fig.1) was constructed to validate the UV-Vis absorption of caffeine in terms of linearity, sensitivity, precision and for calibration purpose to determine the caffeine content of various coffee samples. From the calibration curve (Fig.1), the calibration equation was:  $y = 10.63305x + 0.01486$ ,  $R = 0.9996$ ,  $SD = 0.0133$ , where  $y$  is absorbance,  $x$  is concentration of caffeine and  $R$  is the linear regression coefficient. This equation indicated that the current studies were carried out according to the Beer's law ranges in terms of linearity, sensitivity and precision of the method. Thus, the proposed method allowed for determination of caffeine in coffee samples.

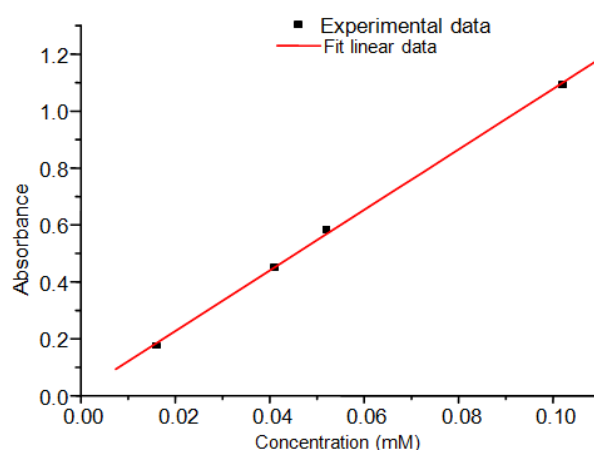


Fig. 1. Calibration curve of caffeine standard for Uv-visible spectrophotometer analysis

A UV-Vis spectrophotometer method cannot be used directly for determination of caffeine in coffee beans owing to the matrix effect of UV-Vis spectrophotometer absorbing substances in the sample matrix [13]. In order to overcome this difficulty the coffee samples were first dissolved in water and then the caffeine was extracted from the solution using dichloromethane. After extraction, the absorbance of the solution was measured using Uv-Vis spectrophotometer (Fig.2). As it can be seen from Fig.2, the maximum absorbance of caffeine extracted was obtained at 276 nm. The result (Fig.2) showed the highest amount of caffeine was detected at *Wembera* coffee sample followed by *Goncha*, *Zegie* and *Burie* coffees respectively.

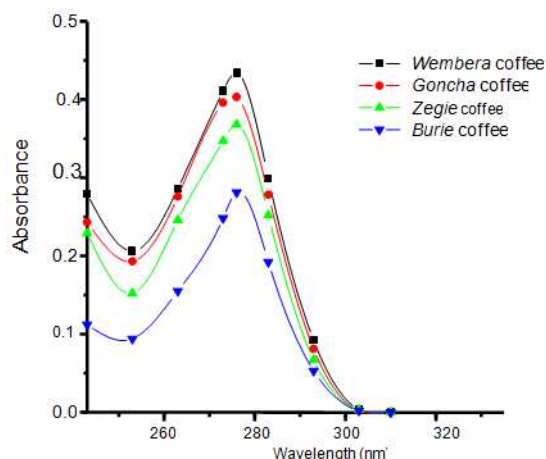


Fig. 2. Absorbance versus wavelength of caffeine from coffee samples

The caffeine levels of the samples were calculated from the regression equation of the best line fit (Fig. 1) of the standards ( $y = 10.63305x + 0.01486$ ,  $y$  is absorbance of caffeine in coffee at 276 nm and  $x$  is concentration of caffeine calculated). By using these concentrations the mass of caffeine in coffee was determined from the relation  $m = MCV$  ( $m$  is mass of caffeine in mg,  $M$  is molar mass of caffeine in g/mol,  $C$  is concentration of caffeine in mM calculated at 276 nm,  $V$  is volume of solution in mL containing caffeine) [58]. Moreover, the percentage (w/w) of caffeine was calculated by taking the mass of caffeine obtained from Beer's law and the mass of the coffee sample on dried weight (50 mg), Table 1.

Table 1. Mass and percentage (% w/w) of caffeine in coffee sample

Coffee samples	Mass of caffeine (mg)	Caffeine (% m/m)
Wembera	$0.761 \pm 0.004$	$1.53 \pm 0.003$
Goncha	$0.709 \pm 0.020$	$1.41 \pm 0.040$
Zegie	$0.646 \pm 0.016$	$1.29 \pm 0.033$
Burie	$0.487 \pm 0.024$	$0.97 \pm 0.049$

At 0.05 levels, the means of all the coffees are significantly different ( $P < 0.05$ ).

As Table 1 shows the caffeine contents(w/w) are  $1.53 \pm 0.003\%$ ,  $1.41 \pm 0.04\%$ ,  $1.29 \pm 0.033\%$ , and  $0.97 \pm 0.049\%$ , in terms of mass of caffeine to mass of coffee sample for *Wembera*, *Goncha*, *Zegie* and *Burie* coffees respectively. There was a significant difference ( $P < 0.05$ ) in caffeine contents among all the coffee samples. This indicated that the caffeine content of coffee beans growing in different geographical locations was different.

In support to this study different value of caffeine contents in coffee beans have been reported by the previous researchers [13], [9], [59] - [61]. For example, an average value of 1.10% by HPLC methods for 42 Ethiopian coffee samples [9], and in the range  $0.96 \pm 0.01\%$  -  $1.23 \pm 0.06\%$  for *Arabic* green coffee beans [59] were reported for their caffeine contents. There are more reports that describe the average value of caffeine to be less than 1.5% for *Arabic*

coffees [60]. Using derivative spectrophotometer it was also reported that the percentage of caffeine in coffee beans was  $1.36 \pm 0.03\%$  [61]. Moreover, the caffeine content in Ethiopian *Arabica* coffee grown in *Bench Maji*, *Gediyo Yirgachefe*, *Tepi*, and *Godere* has been determined by UV-Vis spectroscopy to be  $1.1 \pm 0.01\%$ ,  $1.01 \pm 0.04\%$ ,  $1.07 \pm 0.02\%$ , and  $1.19 \pm 0.02\%$ , respectively [13]. Therefore, these values are in reasonable degree of agreement with the findings of the present work.

In summary, the caffeine level of coffee beans produced in the study areas decreased in the order of *Wembera*, *Goncha*, *Zegie* to *Burie* (Table 1). All the coffee samples have the caffeine level in the range of the caffeine contents of different coffee *Arabica* reported previously [9], [13], [60]-[61]. Even though all the samples were roasted between 8-12 minutes until the medium level roasting; the literatures [59] also describe roasting does not significantly affect the content of caffeine other than causing a slight relative increase due to the loss of other component. Hence the variation in caffeine level of coffee samples may be due to geographical origins which might have different altitude, soil type, rain fall and other agricultural as well as environmental conditions. The variations in caffeine content of coffees are well documented in literatures [20], [23].

#### A. Ferric reducing antioxidant power assay

A calibration curve was obtained using four different concentrations of ascorbic acid in the range of 0.025- 0.2 mg/mL for the determination of the antioxidant reducing power of coffee extracts. The equation of the calibration curve was obtained from the resulting absorbance versus concentration curve and the equation used was:  $y = 3.528x + 0.087$ ;  $R = 0.978$ . Where:  $y$  is the absorbance at 700nm,  $x$  is concentration of ascorbic acid in mg/mL and  $R$  is linear regression coefficient.

As the confirmatory test for the reducing ability of coffee extracts an aqueous solution of ferric chloride was mixed with the reaction mixture after incubation at 50 °C for 20 min. It was observed that the color of the solution changed from yellowish to Prussian blue. This is an indication for the reduction of  $Fe^{+3}$  to  $Fe^{+2}$  that might be occurred due to the presence of water soluble antioxidants components of coffee [20]. The formation of  $Fe^{+2}$  can be monitored by measuring the absorbance of Prussian blue at 700 nm [49].

As it was observed during the experiments, there was a variation in the intensity of the color among the different concentrations of coffee extracts. The color of the complex was varied in the range of lighter Prussian blue to deep blue when the concentrations of coffee extracts were changed from 1.25%, 2.5%, 5% to 10% (v/v) and also there was a difference among the coffee extract types of the same concentration. As shown in Fig.2, the absorbance of the Prussian blue complex was changed with the difference in concentrations and coffee varieties. In all of the coffee extracts, the values of the absorbance were increased with the increase in concentration of the samples (Fig.3). Moreover, the absorbance of the extracts decreased in the order of *Wembera*, *Burie*, *Goncha* to *Zegie* coffees (Fig.3). Since an



increase in absorbance is indicative for the higher reducing power of coffee extracts, *Wembara* coffee would have the highest ability in reduction of ferric to ferrous form while *Zegie* coffee extract has the lowest one (Table 2).

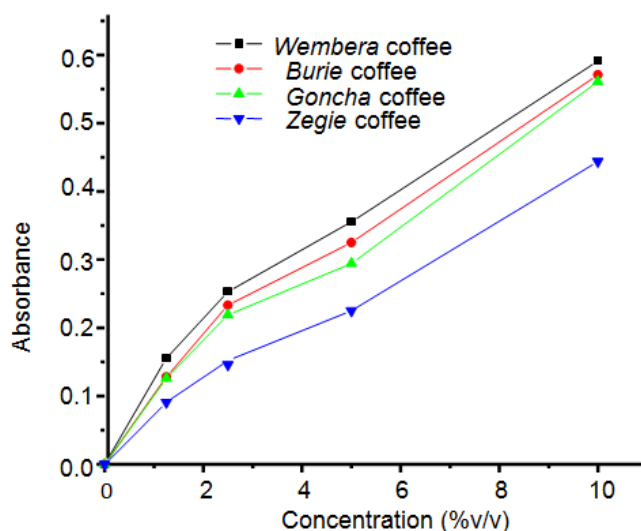


Fig. 3. Absorbance versus concentration of samples

The ferric reducing antioxidant power of coffee extracts was expressed in terms of ascorbic acid equivalent antioxidant capacity [62]. Briefly, the results were expressed as the maximum concentration of coffee extract having ferric reducing ability equivalent to that of 0.2 mg/mL of ascorbic acid, particularly expressed as milligram of ascorbic acid equivalent per gram of coffee sample in the dried weight. The antioxidant activity of coffee extracts was also described as the relative percentage reducing power of extracts in comparison with ascorbic acid [63] the results are summarized in Table 2.

Table 2. FRAP values and percentage reducing power (%RP) of coffee samples

Types of coffee	FRAP values in AEAC (mg/g)	Reducing power (% RP)
<i>Wembara</i>	9.532 ± 0.201 <sup>a</sup>	77.907 ± 1.57 <sup>a</sup>
<i>Burie</i>	9.159 ± 0.441 <sup>a</sup>	75.253 ± 0.51 <sup>a</sup>
<i>Goncha</i>	8.955 ± 0.180 <sup>a</sup>	73.947 ± 3.42 <sup>a</sup>
<i>Zegie</i>	6.751 ± 0.284 <sup>b</sup>	58.463 ± 0.52 <sup>b</sup>

Values are mean ± standard deviation of triplicate measurements (n = 3). Values of the column followed by the same letter indicates no significant difference (p > 0.05) and different letters are significantly different (P < 0.05).

Table 2 shows that the reducing power of coffee extracts varied with the variation in the coffee samples. Particularly, *Wembara* coffee exhibited relatively strong reducing power (better able to donate an electron) in comparison with other extracts, which may be due to its high contents of water soluble antioxidants. Literatures reveal coffee beans contain efficient water soluble antioxidants, such as chlorogenic acids, caffeic acids, ferulic acids, *p*-coumaric acids, melanoids and alkaloids; [20], [38], [39], [46] and their

content depends mainly on the coffee species, origin and degree of roasting [13], [23], [44], [54], [64]. Hence the coffee samples in this study were roasted in same way, the variation in their reducing power might be due the difference in their geographical locations (origin) and their species. In agreement with the literatures; the results of the study (Table 2 and Fig.3) also revealed that coffee samples from various geographical locations of North- West Ethiopia have possessed different antioxidant activities.

#### B. Determination of antioxidant activity by Rancimat assay

The oxidative stability of sunflower oil in the presence and absence of different coffee samples was determined by measuring its induction time using Rancimat assay [52]. When coffee extracts were exposed to a stream of airflow 20 L/h at temperature 120 °C, the volatile oxidation products were transferred to a measuring vessel by the air stream and absorbed in the measuring solution (distilled water). As the conductivity of this measuring solution was recorded continuously its induction time was recorded automatically and the mean values are shown in Table 3.

It was observed that in rapid production of volatile acids at the end of the induction time, expressed in hours, induces an increase of the water conductivity. The induction time of the substrates with coffee extracts were: 2.670 ± 0.010, 2.573 ± 0.100, 2.480 ± 0.185 and 2.290 ± 0.080 h for *Wembara*, *Burie*, *Goncha* and *Zegie* coffees respectively (Table 3).

As shown in Table 3, the time required for the formation of a sufficient concentration of initiating radicals was greater when the coffee samples were added to the substrate, delaying the onset of the propagation phase of the radical chain reaction [65]. This might be happened due to the presence of different concentration of lipophilic antioxidants such as polyphenolic acids, melanoids and heterocyclic compounds [38], [39] in coffee extracts that slow down the oxidation process of the substrate through donation of hydrogen atom.

According to statistical analysis of variance (Table 2), there was a significant difference (P < 0.05) between *Zegi* with *Wembara* and *Burie* coffees to delay the oxidation of substrate. This shows that *Zegie* coffee might have different antioxidant activity from *Wembara* and *Burie* coffees. The values of protection factors were increased from *Zegie*, *Goncha*, *Burie* to *Wembara* coffees as shown in Table 3. Hence, PF > 1 and its values are different, coffee samples added might delay the formation of hydroperoxides within a different rate by offering various protection for the substrate [65]. Particularly, *Wembara* coffee was better in providing protection for the substrate from being oxidized. This might be due to the difference in concentrations of lipid soluble antioxidant components of coffee.

Similar to the results of present findings, researcher using Rancimat assay indicates that coffee beverages from different geographical locations have offered different protection to the oxidation stability of the substrates (oil and butter) [64]. The variations in protection factors have been explained due to the difference in concentrations of lipid soluble constituents of coffee extracts like polyphenolic acids,

melanoids and volatile heterocyclic compounds [39].

**Table 3.** Induction time (IT (h)) and protection factor (PF) values of coffees with sunflower oil.

Samples and sunflower oil	IT (h)	PF
Wembara coffee	2.670 ± 0.010	1.36 ± 0.027
Burie coffee	2.573 ± 0.100	1.31 ± 0.027
Goncha coffee	2.480 ± 0.185	1.26 ± 0.069
Zegie coffee	2.290 ± 0.080	1.17 ± 0.015
Sunflower oil	1.963 ± 0.041	—

Values are mean ± standard deviation of triplicate measurements ( $n = 3$ ).

In spite of the experimental differences, the Rancimat assay and the FRAP assay were found to show similar trends when comparing extracts of different coffee samples in their ranking order of antioxidant activities. In both cases the antioxidant activities of coffee samples were in the decreasing order of: *Wembara*, *Burie*, *Goncha* to *Zegie* coffees. But there was a slight difference in their significant level. This might be the difference in concentration of water and lipid soluble antioxidant components of coffees.

On the other hand, as it was revealed in this study the caffeine level of coffee samples were in a decreasing order of: *Wembara*, *Goncha*, *Zegie* to *Burie* coffees. This indicates that *Burie* coffee extract possesses the least value of caffeine content but the second higher value in its antioxidant activity. And also *Zegie* coffee has higher caffeine content than *Burie* coffee but the lower in its antioxidant activity. This shows that caffeine might not be the major antioxidant components of coffee and its role is almost negligible and does not have significant contribution to the overall antioxidant activity of coffee.

In light of this study results all coffee samples have higher antioxidant activities in both assays. The high antioxidant efficiency of coffee might be attributed to the high content of water soluble and lipid soluble compounds such as chlorogenic acids, caffeic acids, melanoids and heterocyclic compounds present in coffee. The presences of these compounds and their antioxidant activity have already been reported by the previous researchers [20], [32], [31], [36], [45].

## 4. Conclusion

The objective of this study was to determine the caffeine content and antioxidant activities of coffee varieties obtained from four different localities of North-West Ethiopia. The caffeine contents (%w/w) of coffee samples grown in *Wembara*, *Goncha*, *Zegie*, and *Burie* have been found to be  $1.53 \pm 0.003\%$ ,  $1.41 \pm 0.04\%$ ,  $1.29 \pm 0.033\%$  and  $0.97 \pm 0.049\%$ , respectively, suggesting dependence of caffeine content on the location where the coffee plants are grown. FRAP and Rancimat assays were employed for the determination of antioxidant activities of the coffee samples obtained from the four localities of North-West Ethiopia. The results show that the trends in antioxidant activities of the

samples of coffee in both assays were comparable. The ferric reducing power values of water soluble portions of coffee extracts in terms of ascorbic acid equivalent antioxidant capacity were  $9.532 \pm 0.201$ ,  $9.159 \pm 0.441$ ,  $8.955 \pm 0.180$ ,  $6.751 \pm 0.284$  mg/g dried weight for *Wembara*, *Burie*, *Goncha* and *Zegie* coffee, respectively. In a similar note, lipid soluble portions of coffee extracts using Rancimat assay improved the oxidative stability of the substrate (sunflower oil) from being oxidized by delaying the onset of propagation through donation of hydrogen. The protection factors (oxidative stability indexes) were  $1.36 \pm 0.027$ ,  $1.31 \pm 0.027$ ,  $1.26 \pm 0.069$  and  $1.17 \pm 0.015$  for *Wembara*, *Burie*, *Goncha* and *Zegie* coffees respectively.

In both cases *Wembara* coffee has shown the highest value of caffeine content and antioxidant activity among the other coffee varieties. However, the trend in caffeine content and antioxidant activities of *Burie*, *Goncha* and *Zegie* coffee extracts are not proportional. This study shows that the consumption of coffee with higher caffeine content might not be directly related with the benefits of higher antioxidant activities. Thus, the antioxidant behavior of coffee is mainly attributed by other compounds like chlorogenic acids, ferulic acids, caffeic acids and melanoids.

In light of the results of the present study, it may be suggested that normal dose coffee intake may exert several health beneficial effects by virtue of its antioxidant constituents which protect the body against diseases caused by oxidative stress. These findings warrant the need to further carry out similar studies so as to promote cultivation of those coffee plants most promising for health advancement. As caffeine contents and antioxidant activities of the coffee samples varied on the basis of geographical locations; there should be further study on agricultural and environmental factors that resulted in these differences. Since antioxidant activities of coffee extracts were tested only with the FRAP and Rancimat assays, there should be a need to test with other assays.

## References

- [1] T. Ranheim, B. Halvorsen, "Coffee consumption and human health-beneficial or detrimental? Mechanisms for effects of coffee consumption on different risk factors for cardiovascular disease and type 2 Diabetes mellitus," *Molecular Nutrition and Food Research*, 49, 274-284, 2005.
- [2] M. R. Camargo, C. F. Toledo, G. Farah. "Caffeine daily intake from dietary sources in Brazil," *Food Additives Contaminants*, 16, 79-87, 1999.
- [3] S. Schenker, C. Heinemann, M. Huber, R. Pompizzi, R. Perren and F. Escher, "Impact of roasting conditions on the formation of aroma compounds in coffee beans," *Journal of Food Science*, 67, 60-66, 2002.
- [4] A. M. Costa, C. Parreira, and L. Vilas-Boas, "The use of an electronic aroma sensing device to assess coffee differentiation comparison with SPME gas chromatography mass spectrometry aroma patterns," *Journal of Food Composition and Analysis*, 14, 513-522, 2001.

- [5] A. Aresta, F. Palmisano, C. G. Zambonin, "Simultaneous determination of caffeine, theobromine, theophylline, paraxanthine and nicotine in human milk by liquid chromatography with diode array UV detection," *Food Chemistry*, 93, 177-181, 2005.
- [6] V. J. Higdon, B. Frei, "Coffee and health: a review of recent human research," *Crit. Rev. Food Sci. Nut.*, 46, 101-123, 2006.
- [7] M. N. Clifford, C. L. Gibson, J. J. R. Rakotomalala, E. Crost, A. Charrier, "Caffeine from green beans of Mascarocoffea," *Phytochemistry*, 30, 4039-4040, 1991.
- [8] M. J. Martin, F. Pablos, A. G. Gonzalez, "Characterization of green coffee varieties according to their metal content," *Analytica Chimica Acta.*, 358, 177-183, 1998.
- [9] D. Yigzaw, M.T. Labuschagne, G. Osthoff, L. Herselman, "Variation for green bean caffeine, chlorogenic acids, sucrose and trigonelline contents among Ethiopian Arabica coffee accessions," *SINET: Ethiopian Journal of Science*, 30, 77-82, 2007.
- [10] J. Gray, "Caffeine, coffee and health," *Nutrition and Food Science*, 6, 314-319, 1998.
- [11] K. F. Wiersum, T.W. Gole. "Certification of wild coffee in Ethiopia: Experiences and challenges," 2008.
- [12] J. J. Barone, H. R. Roberts "Caffeine consumption," *Food Chem. Toxicol.*, 34, 119-129, 1996.
- [13] A. Belay, K. Ture, M. Redi, A. Asfaw, "Measurement of caffeine in coffee beans by UV- Vis spectrometer," *Food chem.*, 108, 310-315, 2008.
- [14] R. P. Heaney, "Effects of caffeine on bone and the calcium economy," *Food and Chemical Toxicology*, 40, 1263-1270, 2002.
- [15] D. Perrone, C. M. Donangelo, A. Farah, "Fast simultaneous analysis of caffeine, trigonelline, nicotinic acid and sucrose in coffee by liquid chromatography-mass spectrometry," *Food Chem.*, 110, 1030-1035, 2008.
- [16] H. Ashihara, H. Sano, A. Crozier, "Caffeine and related purine alkaloids: Catabolism, function and genetic engineering," *Phytochemistry*, 69, 841-856, 2008.
- [17] M. B. Silvarolla, P. Mazzafera, L.C. Fazouli, "A naturally decaffeinated arabica coffee," *Nature*, 429, 826, 2004.
- [18] I. Hecimovic, A. Belscak-Cvitanovic, D. Horzic, D. Komes, "Comparative study of polyphenols and caffeine in different coffee varieties affected by the degree of roasting," *Food Chem.*, 129, 991-1000, 2011.
- [19] K. Singh, A. Sahu, "Spectrophotometer determination of caffeine and theophylline in pure alkaloids and its application in Pharmaceutical formulations," *Anal. Bio. Chem.*, 349, 176-180, 2006.
- [20] A. Svilaas, A. K. Sakhi, L. F. Andersen, T. Svilaas, E. C. Strom, J. D. R. Jacobs, L. Ose, R. Blomhoff, "Intake of antioxidants in coffee; wine and vegetables are correlated with plasma carotenoids in human," *J. Am. Nutr. Sci.*, 134: 562-567, 2004.
- [21] S. Kolayli, M. Ocak, M. Kucuk, R. Abbasoglu, "Does caffeine bind to metal ions?" *Food Chem.*, 84, 383-388, 2004.
- [22] P. M. Dewick, "Medicinal natural products: a biosynthetic approach" England: John Wiley and Sons, 392-396, 2001.
- [23] C. W. Huck, W. Guggenbichler, G.K. Bonn, "Analysis of caffeine, theobromine and theophylline in coffee by near infrared spectroscopy (NIRS) compared to HPLC coupled to mass spectrometry," *Analytica Chimica Acta.*, 538, 195-203, 2005.
- [24] A. A. P. Almeida, A. Farah, D.A.M. Silva, E.A. Nunan, M. B. A. Glória, "Antibacterial activity of coffee extracts and selected coffee chemical compounds against enterobacteria," *J. Agric. Food Chem.*, 54, 8738-8743, 2006.
- [25] C. A. Brown, C. Bolton-Smith, M. Woodward, M. H. Tunstall-Pedoe, "Coffee and tea consumption and the prevalence of CHD in men and women: results from the Scottish Heart Health Study," *Journal of Epidemiological Community Health*, 47, 171-175, 1993.
- [26] Z. Textor, M. Beer, M. Anetseder, H. Ko-Stler, E. Kagerbauer, W. Kenn, D. Hahn, N. Roewer, "Caffeine impairs intramuscular energy balance in Patients," *Muscle Nerve*, 28, 353- 36, 2003.
- [27] R. L. Prior, X. Wu, K. Schaich, "Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements," *J. Agric. Food Chem.*, 53, 4290-4302, 2005.
- [28] J. M. Tunnicliffe, J. Shearer, "Coffee, glucose homeostasis, and insulin resistance: physiological mechanisms and mediators," *Appl. Physiol. Nutr. Metab.*, 33, 1290-1300, 2008.
- [29] G. S. Duarte, A.A. Pereira, A. Farah, "Chlorogenic acids and other relevant compounds in Brazilian coffees processed by semi-dry and wet post-harvesting methods," *Food Chem.*, 118, 851-855, 2010.
- [30] M. N. Clifford, "Chlorogenic acid and other cinnamates-nature, occurrence, dietary burden, absorption and metabolism," *J. Sci. Food Agric.*, 80, 1033-1043, 2000.
- [31] V. D. Truong, R. F. Mcfeeters, R. T. Thompson, L. L. Dean, B. Shofran, "Phenolic acid content and composition in leaves and roots of common commercial Sweet potato (*Ipomea batatas* L.) Cultivar in the United States," *J. Food. Sci.*, 72, 343-349, 2007.
- [32] A. Gomez-Ruiz, M. Ames, S. Leake, "Antioxidant activity and protective effects of green and dark coffee components against human low density lipoprotein oxidation," *Eur Food Res. Technol.*, 22, 1017-1024, 2008.
- [33] R. H. Stadler, R. J. Turesky, O. Mller, J. Markovic, P. M. Leong-Morgenthaler, "The inhibitory effects of coffee on radical-mediated oxidation and mutagenicity," *Mutat. Res.*, 308, 177-190, 1994.
- [34] A. Stalmach, W. Mullen, C. Nagai, A. Crozier, "On-line HPLC analysis of the antioxidant activity of phenolic compounds in brewed, paper-filtered coffee," *Brazilian Journal of Plant Physiology*, 18, 253-262, 2006.
- [35] M. Namiki, "Antioxidants/antimutagens in food," *Crit. Rev. Food Sci. Nutr.*, 29, 273-300, 1990.
- [36] M. D. del Castillo, J. M. Ames, M. H. Gordon, "Effect of roasting on the antioxidant activity of coffee brews," *J. Agric. Food Chem.*, 50, 3698-3703, 2002.
- [37] K.W. Kang, S. J. Oh, S. Y. Ryu, G.Y. Song, B. H. Kim, J. S. Kang, S. K. Kim, "Evaluation of the total oxy-radical scavenging capacity of catechins isolated from green tea," *Food Chem.*, 121, 1089-1094, 2010.

- [38] A. Farah, T. de Paulis, D. P. Moreira, L. C. Trugo, P. R. Martin, "Chlorogenic acids and lactones in regular and water decaffeinated arabica coffees" *J. Agric. Food Chem.*, 54, 374–381, 2006.
- [39] S. Shizuuchi, F. Hayase, "Antioxidative activity of the blue pigment formed in a D-xylose-glycine reaction system," *Biosci. Biotechnol. Biochem.*, 67, 54–59, 2003.
- [40] J. M. McCord, "The evolution of free radicals and oxidative stress," *Am. J. Med.*, 108, 652–659, 2000.
- [41] J. G. Dorea, T. H. M. da Costa, "Is coffee a functional food?" *British Journal of Nutrition*, 93, 773–782, 2005.
- [42] R. Pulido, F. Saura-Calixt, "Contribution of beverages to the intake of lipophilic and hydrophilic antioxidants in the Spanish diet," *European Journal of Clinical Nutrition*, 57, 1275–1282, 2003.
- [43] A. Podsedek, "Natural antioxidants and antioxidant activity of *Brassica* vegetables:" *A review LWT-Food Sci. Technol.*, 40, 1–11, 2007.
- [44] C. Delgado-Andrade, A. Josea, R. Henares, J. Morales, "Assessing the Antioxidant Activity of Melanoidins from Coffee Brews by Different Antioxidant Methods," *J. Agric. Food Chem.*, 53, 7832–7836, 2005.
- [45] K. Yanagimoto, H. Ochi, K. G. Lee, T. Shibamoto, "Antioxidative activities of fractions obtained from brewed coffee," *J. Agric. Food Chem.*, 52, 592–596, 2004.
- [46] S. Azam, N. Hadi, N. U. Khan, S. M. Hadi, "Antioxidant and prooxidant properties of caffeine, theobromine and xanthine," *Med. Sci. Monit.*, 9, 325–330, 2003.
- [47] K. Fujioka, T. Shibamoto, "Chlorogenic acid and caffeine contents: In various commercial brewed coffees," *Food Chem.*, 2008, 106, 217–221.
- [48] N. Hunda-Faujan, A. Noriham, A. S. Norrakiah, and A. S. Babji, "Antioxidant activity of plants methanolic extracts containing phenolic compounds," *Afr. J. Biotechnol.*, 8, 484–489, 2009.
- [49] I. F. F. Benzie and J. J. Strain, "The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay," *Analytical Biochemistry*, 1996, 239, 70–76.
- [50] K. I. Berker, K. Güçlü, I. Tor, R. Apak, "Comparative Evaluation of Fe(III) Reducing Power-Based Antioxidant Capacity Assays in the Presence of Phenanthroline, Bathophenanthroline, Tripyridyltriazine (FRAP), and Ferricyanide Reagents," *Talanta.*, 72, 1157–1165, 2007.
- [51] M. H. Gordon, E. Mursi, "A comparison of oil stability based on Metrohm Rancimat with storage at 20 °C," *J. AOCS.*, 71, 649–651, 1994.
- [52] J. O. Ragnarsson, T. P. Labuza, "Accelerated shelf life test for antioxidant rancidity," *Food Chem.*, 2, 291–308, 1977.
- [53] A. M. Vera, M. A. Martinez-Tome, M. Jimenez, A. Jimenez, M. Honrubia, "Antioxidant activity of edible fungi (truffles and mushroom): losses during industrial process" *Journal of food protection*, 65, 1614–1622, 2002.
- [54] R. Amarowicz, "Antioxidant activity of Maillard reaction products," *Eur. J. Lipid Sci. Technol.*, 111, 109–111, 2009.
- [55] B. Stavric, "Methylxanthines: toxicity to humans," *Food Chem Toxicol.*, 26, 645–662, 1988.
- [56] M. Haenen, H. V. den Berg, A. Bast, "Applicability of an improved Trolox equivalent antioxidant capacity (TEAC) assay for evaluation of capacity measurements of mixtures," *Food Chem.*, 66, 511–517, 1999.
- [57] M. Oyaizu, "Studies on products of browning reactions: Antioxidant activities of products of browning reaction prepared from glucosamine," *Jpn. J. Nutr.*, 1986, 44, 307–315.
- [58] D. Harvey, *Modern Analytical Chemistry*. Mc Graw Hill, 1<sup>st</sup> edition, 15–18, 2000.
- [59] A. Farah, M. C. M. Monteiro, V. Calado, A. S. Franca, L. C. Trugo, "Correlation between cup quality and chemical attributes of Brazilian coffee," *J. Food Chem.*, 98, 373–380, 2006.
- [60] E. Illy, "The complexity of coffee," *Scientific America*, 86–91, 2002.
- [61] G. Alpdogan, K. Karbina, S. Sungur, "Derivative spectrophotometer determination of caffeine in some beverages," *Turkish Journal of Chemistry*, 26, 295–302, 2002.
- [62] S. Coban, "Development of Biosensor for determination of the total antioxidant capacity," *Thesis*, Izmir Institute of Technology, 132–135, 2008.
- [63] A. Rohman, S. Riyanto, N. Yuniarti, W. R. Saputra, R. Utami, and W. Mulatsih, "Antioxidant activity, total phenolic and total flavonoid of extracts and fractions of red fruit (*Pandanus conoideus* Lam)," *International Food Research Journal*, 17, 97–106, 2010.
- [64] N. V. Yanishlieva, and E. M. Marinova, "Antioxidant effectiveness of some natural antioxidants in sunflower oil," *Fur Lebensmittel-Untersuchung und Forschung*, 203, 220–223, 1996.
- [65] M. Martinez-Tome, A. M. Jimenez, S. Ruggieri, N. Frega, R. Strabbioli, M. A. Murcia, "Antioxidant properties of Mediterranean spices compared with common food additives," *Journal of Food Protection*, 64, 1412–1419, 2001.