Flow Injection Spectrophotometric Determination of Total Polyphenols in Black Tea Processed and Marketed in Ethiopian

Terefe Tafese Bezuneh
Department of Chemistry, College of Natural Science, Arbaminch University, Arbaminch, Ethiopia
teretafe@gmail.com/teretafe2007@gmail.com

Abstract
The present study proposes an alternative flow injection spectrophotometric method for the quantification of total polyphenols in tea samples. The method was based on the reaction between polyphenols and 4-aminoantipyrine (4-AAP) in the presence of hexacyanoferrate in alkaline medium. A reddish-brown coloured product is formed as a result of a reaction between polyphenols and 4-AAP in the presence of oxidizing reagent, hexacyanoferrate, in alkaline medium which was detected at a wavelength of 520 nm. Standard gallic acid solutions prepared in the range of 10- 500 mg/L was used to plot a calibration graph. A good linear calibration graph (r= 0.997, n= 5) was obtained by plotting A (520nm) values versus gallic acid standards solutions (10-500mg/L). The effect of several experimental conditions such as the effect of pH, reaction coil length, flowrate, the concentration of 4-AAP and concentration of hexacyanoferrate (HCF) were optimized and applied to flow injection analytical system. The proposed method was applied for the quantification of total polyphenol in selected tea samples and the result obtained are given as gallic acid equivalent (GAE) mg/L with RSD < 5.
Keywords: Flow Injection, Amino-Antipyrine, Total-Polyphenol

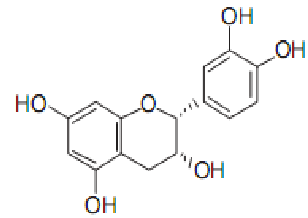
1. Introduction

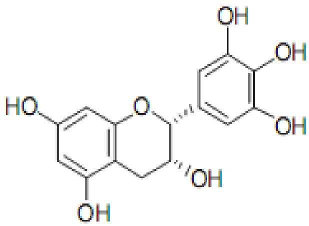
Tea is liquor prepared from the dried leaves of the tea plant *Camellia sinesis* (Lasheen *et al.*, 2008; Sharma *et al.,* 2007). Tea remains the most widely consumed non alcoholic beverage because of its special aroma and taste as well as its various health related benefits (Susanna *et al.*, 2013).

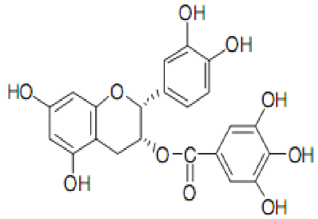
Commercially available tea can be processed from hybrids of two distinct ecotype of tea plant the Assam-type (var. assamica) and China type (var. sinensis) (Janendra *et al*., 2007). Tea can be processed as; black tea, green tea, white tea and oolong tea. Black tea is fully oxidized while green tea is un-oxidized Ruxton, 2008; Claudia *et al*., 2008). Each type of tea can be characterized by special aroma and test (Susanna et al. 2013). Among various types of tea produced in different countries, more than 70 % is produced as a black tea. This makes black tea the most widely consumed type of tea (Lasheen *et al.*, 2008; Gardner *et al.*, 2007).

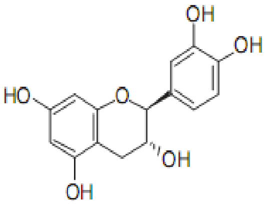
Tea most commonly cites as good sources of chemical compounds known as polyphenols (Theo *et al.*, 2005). Polyphenols are characterized by the presence of thehydroxyl functional group attached to an aromatic ring. They are classified into several groups based on their chemical structure; flavonoids, phenolic acids, phenolic alcohols, stilbenes, lignans, cinnamic acids, benzoic acids, coumarins, and lignins (Ilja and Peter, 2005).

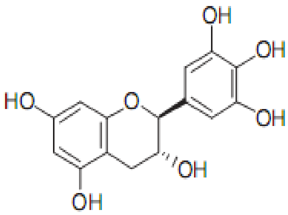
Flavonoids are secondary plant metabolites with complex phenolic structure (Anton and Sheila, 2003; Leamsomrong *et al.*, 2009). Flavonoids can be classified as flavonols, flavones, flavanones, isoflavones, anthocyanidins flavanols, catechins and proanthocyanidins ((Ilja and Peter 2005; Rong, 2010).The major flavonoids found in tea includes; epigallocatechin (EGC), epicatechin (EC), epigallo-catechingallate (EGCG), epicatechingallate (ECG) (collectively known as cathechins), quercetin glycosides, theaflavins and thearubigins (Leonida *et al.*, 2013). Catechins comprises of one third of polyphenols found in plant (Kashmir *et al*., 2008). Catechins are mainly found in green tea and are attributed for the astringency and bitterness characteristic of tea (Massimo et al., 2007; Hasan and Nihal 2000). Black tea mainly contains theaflavin and thearubigin polyphenols Fig. 1 (Hasan and Nihal 2000).

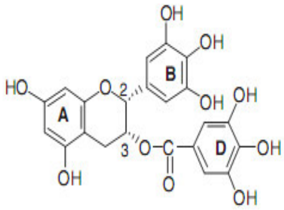

(–) epicatechin (EC)

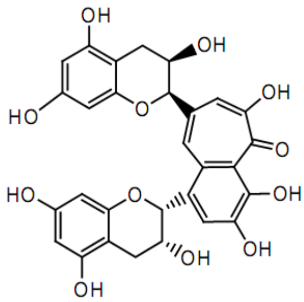

(–) epigallocatechin (EGC)

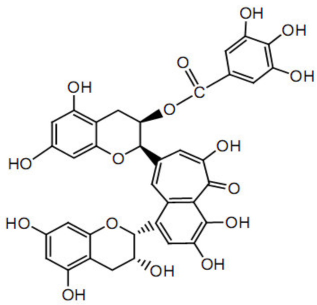

(–) epicatechingallate (ECG)

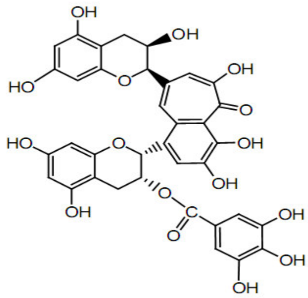

(+) catechin


(+)gallocatechin (GC)


(–)epigallocatechingallate (EGCG)


Free theaflavins(TF-f)


Theaflavin-3-gallate (TF-3-G)


Theaflavin-3'-gallate(TF-3’-G)

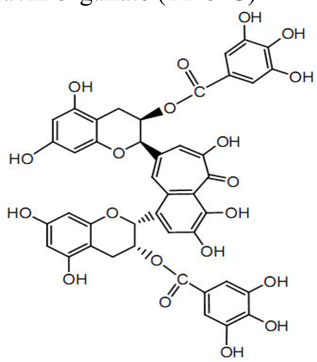

Theaflavin-3-3'-digallate(TF-3-3’-DG)

Figure 1. *Chemical structures of the major polyphenols present in green and black tea (Nihal et al., 2007; Sinija and Mishra 2008; Nurulain, 2006).*

Tea polyphenols are known to be a protective agent and many in vitro and in vivo effects of polyphenols have been reported (Claudine *et al*. 2005). Metal chelating and antioxidant characteristics of polyphenols, attributed tea for its various health related benefits (Mangenya *et al*., 2014). Antioxidant characteristics of tea have been related to various health benefits as; lower the risk of coronary heart disease (CHD) and cancer, protection of DNA, lipid, proteins, tissue, cell and its components from damage, by free reactive radical (peroxide, hydroperoxide or lipid peroxyl) (Shizuo, 2011; Sandip *et al*., 2012).

Different methods have been developed for the determination of polyphenols in tea sample. Determination of individual polyphenol is difficult as more than 8000 varieties of polyphenols have been identified so far. Due to this reason it is convenient to determine polyphenols collectively as a total polyphenols (Sergio *et al*., 2011; Siripat and Daranee, 2008).

There are different analytical procedures developed for the quantification of total polyphenols in tea samples. The larger number of method developed are based on reaction of polyphenols with colorimetric reagents. A coloured product formed by such reaction is used as a base for the measurement of total polyphenol in the visible region (Theppakorn and Ploysri, 2014).

FC method is one of the colorimetric methods used for the determination of total polyphenols in tea samples. This method is based on the reaction of polyphenols with FC reagent which is a mixture of phosphomolybdate and phosphotungstate. The blue coloured product formed by this reaction has an absorption maximum around 765 nm which can be related to the total polyphenolic content found in a sample (Singleton *et al*., 1999; Andressa *et al*., 2013). FC method is most widely utilized and it is also used as an ISO standard for the quality control of green and black tea (ISO14502-1, 2005).

The Oxitester method is based on measurement of reduction in absorption,which is as a result of reduction-oxidation reaction between polyphenols with that of coloured free radicals reagents. Total polyphenol in a sample can be correlated to that of reduction in absorption, which can be measured at 505 nm wavelength and expressed as gallic acid equivalent (GAE) (Barbara *et al.*, 2012).

The reduction of Fe(III) to Fe(II) by polyphenols can also be used to quantify total polyphenols. This method is based on reduction of Fe(III) to Fe(II) by polyphenols in which the reduced Fe(II) forms a coloured complex with 1,10-phenanthroline which has a maximum absorbance at 510 nm. The concentration of total polyphenol is related indirectly to the amount of $Fe(phen)_3^{2+}$ complexes formed (Mônica *et al*., 2013; Conselo *et al*., 1993).

Even though, FC method is most commonly utilized method for the quantification of total polyphenol (TP), it might be difficult to use this method in case when the reagent is not commercially available and also the reagent is quite expensive especially for developing countries. Due to this reason, the great concern of the present study is to develop an alternative method which uses low cost and commercially available reagents.

In this study a simple and rapid FI-Spectrophotometric method for the determination of total polyphenol in tea was developed. Experimental parameters such as effect of concentration of 4-AAP, concentration of $K_2[Fe(CN)_6]$, flow rate of carrier stream, and pH, were studied.

2.0 Materials and Methods

2.1 FIA Setup

A schematic diagram of FIA system is presented in Fig.2. A peristaltic pump was used to deliver the carrier solution and reagents with a fixed carrier flow rate. Sample injection valve, IV, was used for introducing standard solutions, as well as sample solutions, into a carrier stream. Flow lines including reaction coil were made of PTFE tubing (i.d= 0.89 mm). Single beam UV/VIS (LK 2151, Germany) spectrophotometer was used to measure the absorbance.

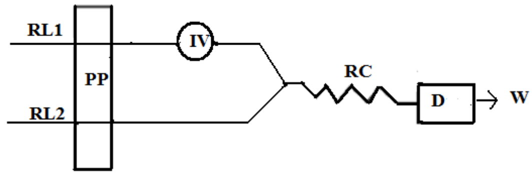


Figure 2.*Schematic diagram of a FIA system and its operations. (RL1)(RL2) Reagent line 1 and 2, PP, peristaltic pump, IV, Injection Valve, RC, Reaction coil, D, Detector, W, waste.*

2.2. Reagents

The 4-aminoantipyrine, potassium ferricyanide (Merck, Germany), sodium hydroxide (BDH, England), boric acid (Hopkins and Williams, England), gallic acid (Riedel-de Haen), sodium hydroxide (BDH, England), tartaric acid (Mallinckrodt, New York), sucrose (SiscoResearch Laboratory, India), fructose (SiscoResearch Laboratory, India), are reagents that were used. All reagents used were of analytical grade.

2.3. Procedure

2.3.1. Tea Infusion Preparation

Different types of black tea products produced in Ethiopia were assessed for their total polyphenols. Tea extract was made by soaking 1g of tea powder samples in hot distilled water for about 15 minute, after which the extract was cooled, filtered and then adjusted to a 100mL volumetric flask.

2.3.2. Determination of Total Polyphenols

Determination of total polyphenols by the proposed method is performed as follows: Tea sample is injected into a carrier stream of buffered hexacyanoferrate (III) solution and then carried to the second stream which comprises of buffered 4-AAP solution. The polyphenols found in the sample starts reacting with 4-AAP and produces a coloured product which is measured at λ= 520 nm.

3. Results and Discussion

3.1. Optimization of the FIA Methods

The pH from 8 to 12 was selected to evaluate the effect of pH on the reaction of polyphenols and 4-

aminoantipyrine. This pH range was selected because the reaction between polyphenols and 4-amminoantipyrine is favored under alkaline solution. The maximum absorbance was recorded at pH 10 and pH 10 was selected as the optimum pH.

The effect of the flow rate of carrier stream, which was 4-AAP and HCF, was studied in the range of 0.3 to 1.7 mL/min. The maximum response was recorded at a flow rate of 1.3 mL/min. The flow rate of 1.3 mL/min was chosen for further experiments. The flow rate of the 4-AAP and HCF reagent were equal.

The effect of the concentration of HCF solution was examined in the range of 0.5 to 5.00 g/L. The maximum response was observed at the concentration of 2 g/L. Therefore 2 g/L of HCF is recorded as an optimum concentration of HCF and used for the rest of the procedure.

The effect of the concentration of 4-aminoantipyrine was also studied in the range of 0.25 to 2 mM. Maximum absorbance was recorded at a concentration of 0.5 mM. Therefore 0.5mM was used as an optimum concentration for the rest of the procedure.

Reaction coil is an important section of the FI setup where mixing and reaction between the reagent, 4-AAP and polyphenols takes place. The length of reaction coil is optimized in the range of 28.75 cm to 34.00 cm. The maximum absorbance was recorded at coil length of 31.75 cm. The coil length of 31.75 cm is used as an optimum for the rest of the procedure.

3.2. Optimum Conditions

The optimum condition obtained for the determination of TP in tea samples can be listed as follows: The concentration of carrier stream reagents, 4-AAP and HCF 0.5mM and 2 g/L, respectively, flow rate of 1.3 mL/min, reaction coil lengths 31.75 cm, wavelength for detection of polyphenols 520 nm and optimum pH of 10 were obtained. The result obtained from optimization procedure was used to determine the total polyphenolic content of tea samples.

3.3. Application of the Proposed Method to Tea Samples

The total polyphenols determination of selected tea samples is performed by applying the optimized conditions to the tea extract.

Table 1. Total polyphenol content of selected tea samples.

Tea samples	Total polyphenol concentration(expressed GA, mg/L) (n=3)	%RSD
Black lion tea	578±7.20	1.25
Good morning tea	511±13.20	2.59
Abyssinia tea	434±14.00	3.24
Wushwush tea	596±7.20	1.21
Ahadu tea	639±0.50	0.08

Five tea samples that are processed and marketed in Ethiopia were selected for the present study. The results obtained were presented in Table 1. The level of total polyphenols in the selected black teas varied between 434± 3.24 % mg/L GAE in Abyssinia tea and 639±0.08 % mg/L GAE in Ahadu tea.

The highest concentration of total polyphenol was found in the Ahadu tea sample, 639±0.08% mg/L GAE. The lowest concentration of total polyphenol was found in the Abyssinia tea sample, 434±3.24% mg/L GAE.

3.4. Interference Study

Evaluation of interferences is important as there might be non phenolic compounds from a sample matrix which can abscure the absorption of total polyphenols at the probing wavelength. Interference from tartaric acid, sucrose, ascorbic acid and fructose is mostly reported in the determination of total polyphenols by UV-visible spectrophotometrey.

Different techniques have been used to minimize the effect of interferent, according to Monica G S et al (2013), it is possible to minimize the effect of interferent by diluting the sample before analysis. According to their investigation, dilution from 5 to 25- folds is required to hinder the effect of the interfering compounds (Mônica *et al*., 2013).

In the present work the influence of: tartaric acid, sucrose, ascorbic acid and fructose have been investigated. This have been done by recording the absorbance both in the presence and absence of the interfering compounds with respect to gallic acid equivalent. Solutions containing a fixed concentration of gallic acid (280 mg/L) and known concentration of the possible interfering species were analyzed. The results revealed no interaction from those compounds.

4.0. Conclusion

As it is known FC method is the most widely accepted method applied for the quantification of total polyphenols in tea samples. In this work, a simple and rapid method is proposed for the determination of the total phenolic compounds in tea samples. This method can be used as an alternative method for the quantification of total polyphenols in different samples.

References

Andressa B, Gisely C L and João Carlos P M (2013). Application and Analysis of the FolinCiocalteu Method for the Determination of the Total Phenolic Content from LimoniumBrasiliense L.Molecules, 18, 6852-6865.

Anton R and Sheila W (2003). Antioxidant Effects of Tea: Evidence from Human Clinical Trials. The Journal of Nutrition, 133, 3285S-3292S.

Barbara G, Joana C, Marlene C et al. (2012). A simplemethod for the determination of bioactive antioxidants in virgin olive oils. Journal of Science Food and Agriculture, 93, 1727–1732.

Claudia A, Graciela E F, and Rosana F (2008). Total Polyphenol Content and Antioxidant Capacity of Commercially Available Tea (Camellia sinensis) in Argentina. Journal of Agricultural And Food Chemistry, 56, 9225–9229.

Claudine M, Gary W, Christine M, et al. (2005). Bioavailability and bioefficacy of polyphenols in humans. American Journal of Clinical Nutrition, 81, 230S–42S.

Conselo T, Mercedes C, Andreu C, et al. (1993). A new flow-injection spectrophotometric method for the determination of tannins in tea and beer using iron (III) and 1,10-phenantroline. Food chemistry, 47, 201-204.

Gardner E J, Ruxton C H S and Leeds A R (2007). Black tea – helpful or harmful? A review of the evidence. European Journal of Clinical Nutrition, 61, 3–18.

Hasan M and Nihal A (2000). Tea polyphenols: prevention of cancer and optimizing health. American Journal of Clinical Nutrition, 71, 1698S-1702S.

Ilja C W A and Peter C H H (2005). Polyphenols and disease risk in epidemiologic studies. American Journal of Clinical Nutrition, 81, 317S–25S.

ISO, 2005. ISO14502-1: 2005 Determination of substances characteristic of green and black tea. Part 1. Content of total polyphenols in tea-colorimetric method using Folin–Ciocalteu reagent. International Organization for Standardization, Geneva, Switzerland.

Janendra W A, Costa M D, Mohotti A J, et al. (2007). Ecophysiology of tea. Brazilian Journal of Plant Physiology, 19(4), 299-332.

Kashmir S, Arti R, Sanjay K, et al. (2008). An early gene of the flavonoid pathway, flavanone 3-hydroxylase, exhibits a positive relationship with the concentration of catechins in tea (Camellia sinensis). Tree Physiology, 28, 1349–1356.

LasheenYF, Awwad N S, El-khalafawyA,et al. (2008). Annual effective dose and concentration levels of heavy metals in different types of tea in Egypt. International Journal of Physical Sciences, 3(5), 112-119.

Leamsomrong K, Suttajit M and Chantiratikul P (2009). Flow Injection Analysis system for the Determination of Total Phenolic compounds by Using Folin-Ciocalteu Assay. Asian Journal of Applied Science, 2(2), 184-190.

Leonida C, Samson M K, Amos A, et al. (2013). Variation in Catechin Composition of Popularly Cultivated Tea Clones in East Africa (Kenya). American Journal of Plant Sciences, 4, 628-640.

Mangenya T M, Kelvin O M, John K W, et al. (2014). Selected Inorganic Nutrients in Black Tea from Three Tea. American Journal of Plant Sciences, 5, 473-479.

Massimo D, Carmela F, Roberta D, et al. (2007). Polyphenols, dietary sources and bioavailability. Ann InstitutoSuperiore di Sanita, 43(4), 348-361.

Mônica G S, Cecilia V N, Horacio D M (2013). A new method for quantification of total polyphenol content in medicinal plants based on the reduction of Fe(III)/1,10-phenanthroline complexes. Advances in Biological Chemistry, 3, 525-535.

Nihal T, Sedat V Y, Ferda S, et al. (2007). Effect of Extraction Conditions on Measured Total Polyphenol Contents and Antioxidant and Antibacterial Activities of Black Tea. Molecules 12, 484-496.

Nurulain T Z, (2006). Green tea and its polyphenoliccatechins: Medicinal uses in cancer and noncancer applications. Life Sciences, 78, 2073-2080.

Rong T (2010). Chemistry and Biochemistry of Dietary Polyphenols. Nutrients, 2, 1231-1246.

Ruxton C H S (2008). Blaack tea and health. British Nutrition Foundation Nutrition Bulletin, 33, 91-101.

Sandip P, Debjani G, Chabita S et al. (2012). Total polyphenol content, antioxidant activity and lipid peroxidation inhibition efficacy of branded tea (Camellia sinensis) available in India. International journal of Tea Science, 14-20.

Sergio P, Flurin W, Alexia N G, et al. (2011). Flow Injection Analysis for the Direct Quantification of the Total Polyphenol Content in Coffee Brews. Journal of Flow Injection Analysis, 28(1), 23–28.

Singleton V L, Orthofer R, and Lamuela-Ravento´s, R.M (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of FolinCiocalteu reagent. Method Enzymology, 299, 152–178.

Sinija V R , and Mishra H N (2008). Green tea: Health benefits. Journal of Nutritional & Environmental Medicine, 17(4), 232–242.

Siripat S and Daranee P (2008). Flow Injection Analysis-Spectrophotometry for Rapid Determination of Total Polyphenols in Tea Extracts. Journal of Flow Injection Analysis, 25(1), 61–64.

Sharma V K, Bhattacharya A, Kumar A, et al. (2007). Health Benefits of Tea Consumption. Tropical Journal of Pharmaceutical Research, 6 (3), 785-792.

Shizuo T (2011). Polyphenol Content and Antioxidant Effects in Herb Teas. Chinese Medicine, 2, 29-31.

Susanna B, Antonella C, Paola M, et al. (2013).The Joint Use of Electronic Nose and Electronic Tongue for the Evaluation of the Sensorial Properties of Green and Black Tea Infusions as Related to Their Chemical Composition. Food and Nutrition Sciences, 4, 605-615.

Theo P M, Anton G R, and Johan M A (2005). Consumption of both black tea and green tea results in an increase in the excretion of hippuric acid into urine. American Journal of Clinical Nutrition, 81, 256S–60S.

Theppakorn T and Ploysri K (2014). Development of a visual test kit for estimation of total polyphenols in tea. International Food Research Journal, 21(2): 501-506.