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ORIGINAL ARTICLE

Evaluation of Total Polyphenolic Content in Various Grades of Black Tea Powder

Amrithendu Chandran[1], Archana Prabhat[2]*

1 Department of PG Studies in Food Science and Nutrition, Alva's College, Moodbidri, Karnataka, India
2 Head & Coordinator, Department of PG Studies in Food Science and Nutrition, Alva's College, Moodbidri, Karnataka

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* **Corresponding author**.
drarchanaprabhat@gmail.com

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Abstract

Tea is the most popular non-alcoholic beverage in the world. Among tea producing countries the principal producers are China, India, Sri Lanka, Kenya and Indonesia which account for 80% of global production. Tea leaf grading, which is used in the tea industry, is the process of assessing goods based on the character and state of the tea leaves themselves. The main target of the present study was the evaluation and comparison of total phenolic content among different tea grades. The tea grades with the highest and lowest polyphenolic contents were also analysed. Eight different grades of black tea were collected from the Devon tea estate, Chikkamagaluru. The prepared samples were assayed to evaluate their total phenolic content spectrophotometrically at 725nm. Folin-Ciocalteu method was used for estimation of total polyphenolic content. The results showed that, there were significant differences in the total polyphenol content from different grades of black tea samples. Tea grade SRD has the maximum amount of total polyphenols, or 172.68 g GAE (0.01726 g GAE per 100g), while grade BOP has the lowest amount, or 141.58 g GAE (0.014158 g GAE per 100g).

Keywords: Black tea; Tea grades; Phenolic content; Folin-Ciocalteu

1 Introduction

Among all beverages, tea has the second-highest consumption rate. Over 50 centuries ago, tea has been made from *Camellia sinensis* plant leaves. The tea plant originated in Southeast Asia and is now grown in more than 30 nations. Every year, three billion kilograms of tea are produced and consumed. Based on the production processes used, tea has been divided into three major categories. In terms of the total amount of tea produced, 78% of it is black tea, which is typically drunk in Western nations, 20% is green tea, which is frequently consumed in Asian nations, and 2% is oolong tea, which is mostly produced (by partial fermentation) in southern China. There are three different varieties of tea: fermented (black tea), semifermented (oolong tea) and unfermented (green tea).

The difference lies in the 'fermentation' which actually refers to oxidative and enzymatic changes in the tea leaves during processing [1].

Chinese tea is made from the leaves and leaf buds of the plant species *Camellia sinensis*. It belongs to the Theaceae family of flowering plants' genus Camellia. This species is used to harvest white tea, green tea, oolong tea, and black tea; however, each is processed differently to achieve a distinct level of oxidation. The *Camellia sinensis* plant is also used to make kukicha (twig tea), however twigs and stems are used instead of leaves. According to Namita et al. (2012) [2], common names for tea include shrub, tree, and plant.

Although the Chinese *Camellia sinensis* is now grown all over the world in tropical and subtropical areas, it is originally from mainland China, South and Southeast Asia. When grown for its leaves, it is an evergreen shrub or small tree that is often trimmed to a height of under two metres (six feet). Its taproot is robust. The flowers have 7 – 8 petals, are yellow-white, and measure 2.5 - 4 cm in diameter. Tea oil, a sweet-tasting flavouring and cooking oil that can be extracted from the seeds of *Camellia sinensis* and *Camellia oleifera*, should not be confused with tea tree oil, an essential oil that comes from a different plant and is used for medicinal and cosmetic purposes. The leaves are 2 – 5 cm wide and 4 – 15 cm long. For making tea, it is best to collect the young, light green leaves with short white hairs on the underside. Due to the differences in the chemical makeup of different leaf ages, different leaf ages result in diverse tea characteristics. Typically, the first two to three leaves and the tip (bud) are picked for processing. Every one to two weeks, this hand choosing is done again [2].

While green tea is more popular in Asia, black tea is more popular in North America, Europe, and North Africa. China and Taiwan are the main places where oolong tea is made. According to Yang et al. (2012) [3], three billion kilograms of tea are produced and consumed worldwide each year. The process of making black tea starts with picking, followed by withering, rolling, and drying. The leaves develop a shape that makes rolling easier throughout the withering process. The leaves' cell structure is broken down as a result of this process, and fermentation subsequently starts. About 75% of the catechins present in tea leaves go through an enzymatic transition during the manufacturing of black tea, consisting of partial polymerization and oxidation. Tea leaf polyphenol oxidase is the primary enzyme involved in these processes, thus it's critical to ensure that it has direct contact with air, oxygen and polyphenols. The technological procedure used in producing black tea determines the content of the finished product [4].

Tea leaf grading, which is used in the tea industry, is the process of assessing goods based on the character and state of the tea leaves themselves. The term "orange pekoe" is used for the best grades, and "fannings" or "dust" for the poorest.

Different varieties of pekoe tea are categorized according to how many adjacent young leaves (two, one, or none) were selected with the leaf buds. Only the leaf buds, which are selected with the balls of the fingertips, are used in premium pekoe grades. To prevent bruising, avoid using mechanical instruments or fingernails. The term "broken" refers to tea that has been crushed to create bagged teas, as in "broken orange pekoe" BOP. Fannings and dust, which are minute by-products of the sorting and crushing procedures, are included in these lower grades.

Orange pekoe is referred to as "OP". The grading system also includes classifications above OP, which are mostly based on leaf size and completeness. Orthodox teas that are broken, fannings, or dust have slightly varied grades. Another grading system exists for CTC teas, which are made of leaves that have been mechanically shaped into uniform fannings [5].

Worldwide, the consumer's preference is approximately 76-78 % of black tea followed by 20-22% for green tea and 2% for oolong tea. Black tea leaves are exposed to the highest levels of oxidization and fermentation compared to its other tea counterparts. When black tea is oxidized, the catechins are converted into theaflavins and the arubigins, which still act as antioxidants [6]. Due to their positive impact on human health, bioactive phytochemicals like phenolic compounds have been the subject of numerous epidemiological research in recent years. Free radical damage to biomolecules has been theorised as one of the primary causes of the majority of chronic diseases. Consuming foods high in phenolic compounds, which have the ability to scavenge reactive species, may therefore be a mechanism of protection, with these foods being recommended for optimum health advantages. These positive effects are mostly linked to the antioxidant abilities of tea polyphenols. Additionally, tea has been found to have anti-mutagenic, anti-carcinogenic, hypocholesterolemic, antimicrobial, and antiallergenic properties [7].

Polyphenols are organic molecules that are only produced by plants. Nearly all plants contain polyphenols, which are typically involved in the attraction of pollinators, the performance of structural tasks, protection from ultraviolet radiation, and defence against microbial invasion and herbivores. They have chemical characteristics with phenolic substances and have been shown to have biological effects on oxidative and inflammatory stress, digestion of macronutrients, and gut flora. These substances are widely found in foods including fruits, vegetables, nuts, seeds, flowers, and tree bark as well as popular drinks like wine, beer, and tea, making them an essential component of the human diet. Depending on the amount of polyphenolic compounds present, they play a role in the sensory and nutritional aspects of plant foods, such as astringency, colour, and odour.

At least one phenyl ring and one or more hydroxyl substituents are found in the chemical structure of polyphenols. Phenolics range from lightweight condensed tannins to simple tiny single aromatic ring structures. Polyphenols can be classified in a variety of ways. The split into flavonoids and non-flavonoids is the simplest, but they can also be categorised into a variety of substances depending on the amount of phenol units in their molecular structure, the type of connection between phenol units, substituent groups, and/or these factors[8].

Polyphenols are a well-known class of phenolic systems that are distinguished by having at least two phenyl rings and one or more hydroxyl substituents[9]. According to the amount of phenol units in their molecular structure, substitute groups, and/or the kind of connection between phenol units, polyphenols can be categorised simply into flavonoids and nonflavonoids or further separated into numerous subclasses[10]. Plants produce polyphenols, which are widely dispersed in plant tissues and primarily take the form of glycosides. Flavonoids can be found as glycosides or aglycones, even though their basic structure is an aglycone (the non-sugar portion of the corresponding glycoside)[11].

Researchers and the general public have been interested in tea polyphenols recently due to their possible health benefits, such as their anticancer, antioxidant, anti-cardiovascular, and antibacterial activity[12]. For southern Chinese women, regular tea consumption—whether it's green, black, or oolong tea—significantly lowers their chance of developing ovarian cancer[13]. According to a recent study[14], polysaccharides and polyphenols from oolong tea had synergistic effects on reducing tumour growth in a model of hepatocellular carcinoma and improving the antioxidant and immunological responses in mice.

Due to their ability to induce apoptosis, inhibit cell proliferation, and limit migration both *in vitro* and *in vivo*, theaflavins from black tea are typically thought to be useful for the prevention of carcinogenesis. When theaflavin was administered to breast cancer cells, p53-mediated Bax-transactivation, induction of mitochondrial outer membrane premobilization, and cancer cell death were significantly induced[15].

There are still certain restrictions on the use of tea polyphenols in therapeutic interventions, despite the fact that new evidence has demonstrated the health benefits of these compounds. Foods made from plants, such as flavonoids and ellagitanin, are rich in phenolic acids. However, after consuming 10–287–100 mg of a single phenolic substance, the plasma concentration of existing polyphenols is only 1 mM[16]. This is because dietary polyphenols have a relatively limited bioavailability.

The majority of polyphenols found in food matrixes or in their plant-based food sources are conjugated or bound, suggesting that most dietary polyphenols reach the colon and may then be metabolised by the gut microflora or excreted in bile and have a low bioavailability. This suggests that dietary polyphenols may play a part in influencing the microbiota of the gut in order to improve the host-microbial symbiosis in the lower intestine, which supports general health. Additionally, drinking tea will give the body modest amounts of polyphenols, which will support the body's natural ability to fight free radicals and restore oxidative equilibrium. However, due to the prooxidative activity of polyphenols, supplementing with pure tea polyphenols may increase the risk of oxidative stress[17]. Additionally, the body recognises tea polyphenols as xenobiotic chemicals that must be broken down and eliminated from the body. As a result, greater consumption of extracts high in tea polyphenols may place a metabolic burden on the body, which may compromise hepatic or gastrointestinal functions[18].

1.1 Objectives

1. To estimate the total polyphenol content in different grades of black tea of the same plantation crop.

2 Methodology

2.1 Sample collection

Black tea samples of 8 different grades were collected from the Devon tea estate, Chikkamagalur.

2.2 Sample preparation

1 gm. of each of the samples was weighed and 100 ml distilled water was added, boiled and filtered.

2.3 Preparation of reagents

Folin Ciocalteu reagent (FC reagent): 25ml of FC reagent was made up to 250ml with distilled water.

Sodium Carbonate solution (7.5% w/v): Weigh 18.75mg of sodium carbonate and make up to 250 ml with distilled water.

Standard preparation: A stock standard solution of gallic acid at a suitable concentration (usually around 1000 μg/mL) was prepared. From the stock solution, a series of working standard solutions with different concentrations were prepared.

2.4 Total polyphenol estimation

0.1ml of each sample was taken in different test tubes and 5ml of FC reagent and 4ml of sodium carbonate solution were added and the tubes were made upto 10ml using distilled water. The tubes were kept for incubation at room temperature for about 60 minutes. After incubation, absorbance was measured at 725 nm in a spectrophotometer using distilled water as blank. The concentration of polyphenols in the sample was derived from a standard curve of gallic acid.

2.5 Detection Procedure

0.1ml of each sample was taken in different test tubes and 5 ml of FC reagent, 4 ml of sodium carbonate solution were added and the tubes were made upto 10 ml using distilled water. The tubes were kept for incubation at room temperature for about 60 minutes. After incubation, absorbance was measured at 725nm in a spectrophotometer using distilled water as blank. The concentration of polyphenols in the sample was derived from a standard curve of gallic acid.

3 Results and Discussion

Tea has been made from Camellia sinensis plant leaves. More than 30 countries today grow the tea plant, which was first domesticated in Southeast Asia. Three billion kilos of tea are produced and consumed annually. The three main categories of tea have been determined by the production methods employed. There are three different types of tea: fermented (black tea), semi-fermented (oolong tea), and unfermented (green tea)[2]. In the tea industry, a technique known as ”tea leaf grading” is used to evaluate products based on the characteristics and condition of the tea leaves themselves. For the best grades, ”orange pekoe” is used, while ”fannings” or ”dust” is used for the poorest[5].

Tea has a significant amount of polyphenols, which are antioxidants obtained from plants that are thought to account for some of the health advantages of fruits, vegetables, chocolate, and red wine[19]. Tea contains a variety of polyphenols that belong to the flavonoid group. These include epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (EGCg), quercetin glycosides, theaflavins, and thearubigins. Together, these polyphenols are referred to as flavanol monomers. During the production of black tea, flavanol monomers and flavanol gallates are converted into theaflavins and thearubigins. According to Ruxton (2008)[20], it has been suggested that the overall polyphenol concentration of black and green tea is comparable, despite the fact that the types of flavonoids contained vary depending on how much they are oxidised during processing[21]. An average cup of tea contains around 200 mg of total flavonoids per cup[22].

Polyphenols are a well-known class of phenolic systems that are distinguished by having at least two phenyl rings and one or more hydroxyl substituents[9]. Polyphenols can be divided into flavonoids and nonflavonoids or many more subclasses depending on the number of phenol units in their molecular structure, replacement groups, and/or the type of linkage between phenol units[10]. Polyphenols, which are widely distributed in plant tissues and generally exist in the form of glycosides, are produced by plants. Although their basic structure is an aglycone (the non-sugar part of the corresponding glycoside), flavonoids can also be found as glycosides or aglycones[11].

Numerous research have looked into the possible health advantages of drinking tea. Black tea contains several bioactive chemicals that have anti-oxidant qualities. As a result, drinking tea increases the antioxidant status in vivo and reduces the risk of various illnesses, including stroke, coronary heart disease, cancer, and mutations[23]. Black tea's antioxidant properties are attributed to its polyphenol content. Gallic acid, theaflavins, and catechins all contribute to tea's antioxidant properties [Vinson and Dabbargh, 1998]. By chelating at the lower pH region of the stomach, theaflavins and their gallate esters, which are only found in black tea, can scavenge the metals[24]. Iron can be bound by tea polyphenols to create a redox inactive compound. Grinberg's tea polyphenol complex (Fe) —Tea polyphenol complex[25]. Black tea provides protection against reactive oxygen and nitrogen species. It was found to inhibit the production of compounds like NO and superoxide in murine peritoneal macrophages. Theaflavins were the most effective compounds in down regulating nitric oxide synthase and therefore black tea was found to be a better chemo-preventer than green tea[26].

Uneven fermentation may arise from the dhool's excessive moisture content, which can impede aeration and temperature regulation[27]. A leaf that has undergone excessive withering may have a concentration of catechins that inhibits the action of polyphenol oxidase (PPO) and lowers the expression of the cell contents on the surface[28]. Because of this, under conditions of limited oxygen, a sizable amount of the fermentation may take place inside the leaf particle. Moisture loss during withering does not appear to alter the optimum fermentation time[29].

In this study, various tea samples were collected from Devon tea estate Chikmangalur for the analysis of the total polyphenolic compounds. Total polyphenol estimation was done using the Folin Ciocalteu method. Eight samples viz, Super Red Dust (SRD), Pekoe Dust (PD), Broken Orange Pekoe (BOP), PEKOE, Red Dust (RD), Pekoe Fannings (PF), Super Fine Dust (SFD) and Broken Pekoe (BP) were collected. Each samples were boiled using distilled water and filtered.

Figure 1represents the gallic acid concentration plot obtained by plotting the absorbance against concentration of gallic acid (mg/ml) for each sample.Table 1 summarizes the concentration of total phenolic compounds in the samples determined as milligrams of gallic acid equivalent (GAE) using the equation obtained from the standard gallic acid graph.

Additionally, drinking tea will give the body modest amounts of polyphenols, which will support the body's natural ability to fight free radicals and restore oxidative equilibrium. However, due to the prooxidative activity of polyphenols, supplementing with pure tea polyphenols may increase the risk of oxidative stress[17].

Figure 2 is the graphical representation of the total polyphenolic compounds present in the samples. Polyphenols

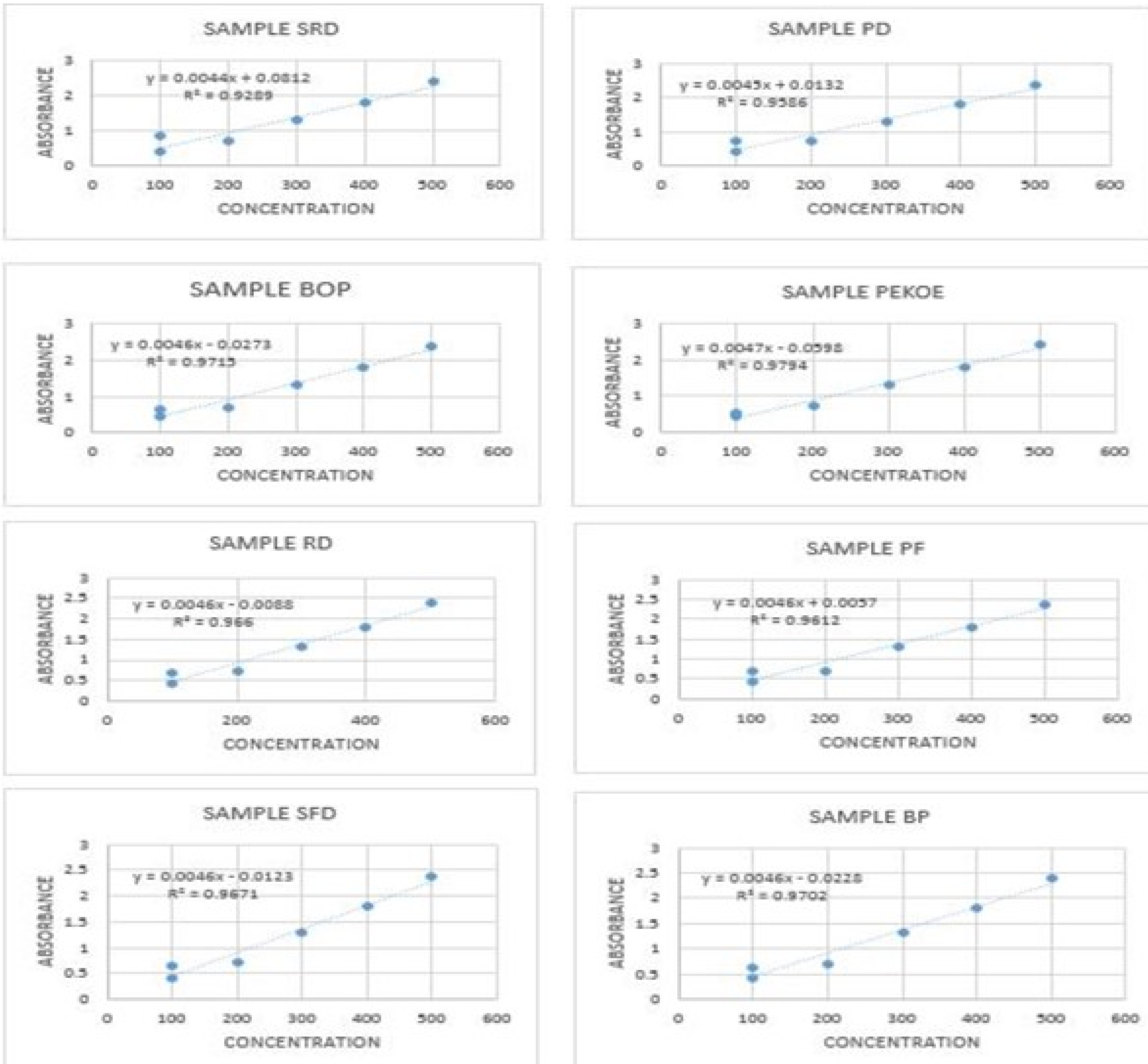

Fig 1. Polyphenolic concentration of various black tea powder samples

Table 1. Calculated value of phenolic compounds of various grades of Black tea powdersamples: Its optical density and absorbance

Samples	Sample OD	y+b or y-b	m	Results in μg equivalent of gallic acid	μg GAE in 100g sample	g GAE in 100g sample
SRD	0.841	0.7598	0.0044	172.68	17268	0.01726
PD	0.705	0.6918	0.0045	153.73	15373	0.015373
BOP	0.624	0.6513	0.0046	141.58	14158	0.014158
PEKOE	0.559	0.6188	0.0047	154.7	15470	0.01547
RD	0.661	0.6522	0.0046	141.78	14178	0.014178
PF	0.69	0.6843	0.0046	148.76	14876	0.014876
SFD	0.654	0.6663	0.0046	144.847	14484	0.014484
BP	0.633	0.6558	0.0046	142.565	14256	0.014256

are organic molecules that are only produced by plants. It has been demonstrated that they have biological impacts on oxidative and inflammatory stress, the digestion of macronutrients, and gut flora. They have chemical properties with phenolic compounds. These compounds are a crucial part of the human diet because they are extensively present in a variety of foods, including fruits, vegetables, nuts, seeds, flowers, and tree barks, as well as well-liked beverages like wine, beer, and tea [Antonio et al. 2002]. The body also recognizes tea polyphenols as xenobiotic substances that need to be degraded and expelled from the body. Because of this, consuming more extracts rich in tea polyphenols may stress the body's metabolism and impair hepatic or digestive functioning [(18)].

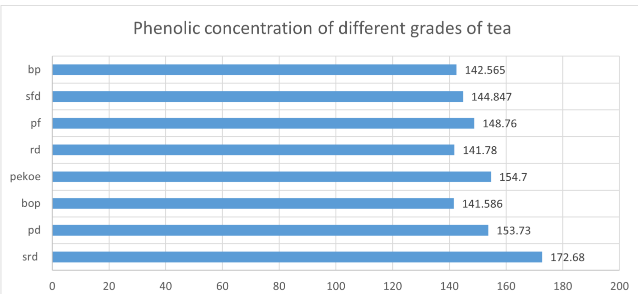

Fig 2. Phenolic concentration of different grades of black tea powder samples

For the grades SRD, PD, BOP, PEKOE, PF, SFD, and BP, the total polyphenolic content of the samples was determined to be 0.01726, 0.015373, 0.014158, 0.01547, 0.014178, 0.014876, 0.014484, and 0.014256 g GAE in 100 g of sample, respectively.

From the findings, the tea grade SRD possesses the highest concentration of total polyphenols i.e.172.68 μg GAE (0.01726 g GAE in 100g) and the grade BOP was found to have the lesser phenolic concentration i.e.141.58 μg GAE (0.014158 g GAE in 100g).

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