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**RESEARCH ARTICLE**

**ADVERSE EFFECTS OF TEA METABOLITES EXTRACTED DURING INDIAN HOUSEHOLD TEA PREPARATIONS ON DIGESTIVE ENZYMES AND IRON**

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**Abstract**

The study encompasses secondary metabolites and antioxidant potential estimation of green & black tea during infusions under Indian household tea making processes. Tea infusions for 5, 10 & 15 minutes in controlled (at 60, 80 & 100°C temperature) and uncontrolled environment (normal household preparation) were studied. Controlled infusions revealed optimised extraction conditions of each metabolite and served as the platform, based on which, normal household process was compared. Study suggests effective & maximal utilization of metabolites for minimizing nutrient loss by correlating inhibitory roles of total polyphenols and total tannins with the digestive enzymes and iron and calculated the volume of tea required for certain % of enzymatic & iron inhibitions. Green tea is more strong non-haem iron inhibitor (3.7- 4.6 times stronger than black tea) due to more total tannins and the volume of green tea required is almost half (48-55% lesser) than that of black variety to have same enzymatic inhibition % (due to more available polyphenols), thus making it almost twice (1.82-2.07 times than black) as effective as black tea to act as antidiabetic and antiobesitic agents by their inhibitory actions upon  $\alpha$ -amylase & lipase respectively. Drinking of green tea may not be suitable for the malnourished population, especially, in the Indian subcontinent due to higher available polyphenols & tannins possessing adverse effects on digestive enzymes & iron.

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**Introduction:-****The context**

Tea chemistry is complex. On the bush, tea leaves contain thousands of chemical compounds. When they are processed, these compounds break down, form complexes and form new compounds. Many tea chemicals have been categorized into broad groups and collectively we have some ideas of their fate what happens to these groups during processing and what flavours and aromas they are responsible for. Plant leaves are made up of mostly water, and when they are removed from the plant they begin to wilt and lose water. Black tea represents approximately 72% of total consumed tea in the world, whereas green tea accounts for approximately 26%. Recent interest in the health

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aspects associated with consumption of tea beverages has grown within the scientific community and has generated much excitement about tea polyphenols.

Brewing techniques vary widely according to cultural customs around the world (Wooward, 1980). Estimation of secondary metabolites intake from tea is subject to considerable variation (Stavric *et al.*, 1988). There is little published information on estimation of secondary metabolites under household conditions, but estimation of metabolites contents & antioxidant property of brewed tea with controlled time and temperature prompted the Working Group to compare the percentage change of metabolites contents & antioxidant property of Darjeeling Tea (Green & Black) during normal household tea making time and temperature (Temp<sub>uncon</sub>, Time<sub>var</sub>) with those of controlled conditions (Bunker *et al.*, 1979).

In recent years there has been more and more research on the effect of consumer preparation on composition and activity of tea infusions (Lakenbrink *et al.*, 2000, Su *et al.*, 2006, Kyle *et al.*, 2007, Molan *et al.*, 2009).

The extraction temperature, extraction time, water quality and water-to-tea ratio, tea particle size, extraction pH, and the number of extractions are all important factors which directly affected the quantity of the extracted antioxidants (Vuong *et al.*, 2011). A few evidences have been found from some of the few research work. Such as in the case of green tea it was reported that- the higher temperature of brewing, the higher the reducing power of the infusion (Molan *et al.*, 2009). Similarly, for black tea, the longer the time of brewing, the higher the antioxidant activity as well as the total phenolic and catechin contents (Kyle *et al.*, 2007).

A tea drinker typically consumes 180 to 240 mg of polyphenols from a strong cup of tea . Recent interest in the health aspects associated with consumption of tea beverages has grown within the scientific community and has generated much excitement about tea polyphenols.

#### **The genus Camellia:-**

The genus Camellia comprises evergreen shrubs which, in commercial cultivation, are maintained as a low bush in continuous vegetative growth. The genus contains a large number (Ca. 82) of species, but only the 'tea Camellia', Camellia *sinensis* is grown commercially. Other species, especially, Cam. *irrawadiensis* and Cam. *taliensis* are of importance as a source of genetic material (Varnam *et al.*, 1994).

Although the length of time for steeping and the amount of water in which the leaves are steeped can vary widely, these factors generally control the amount of solids extracted, and to a lesser extent influence the composition. A typical brew of one tea bag in one cup of water produces a solution of 0.35% wt/wt solids, and from this value the dose expected from consumption of one cup of tea can be calculated. This is typically how tea phytochemicals are consumed (Matthew *et al.*, 1997).

**Darjeeling Tea:-** Darjeeling, on the Southern slopes of the Himalayas in north east India. Darjeeling teas are cultivated at splendid altitudes of 800-2000 meters and it is the highest tea gardens. The region has just the right climatic conditions for cultivating fine tea bushes ([http://www.teagschwendner.com/US/en/Tea\\_Growing\\_Regions.TG](http://www.teagschwendner.com/US/en/Tea_Growing_Regions.TG)).

#### **Polyphenols & Flavonoids Present In Tea Leaves:-**

##### **Polyphenols:-**

Polyphenols refers to a categorization of compounds composed of many phenolic groups with potent antioxidant properties (believed to be greater than even vitamin C). They give tea its bitter flavour. Because green tea leaves are young and have not been oxidized, green tea has up to 40 percent polyphenols, while black tea contains only about 10 percent. These compounds are plant metabolites produced as a defence against insects and other animals. They are derived from amino acids via sunlight and therefore tea grown in the shade has a smaller concentration of polyphenols and a higher concentration of amino acids.

##### **Flavonoids:-**

Within the flavonoid group, flavanols (also known as flavan-3-ols) are the most prevalent. Flavanols are also referred to as tannins, and during oxidation are converted to theaflavins and thearubigins—the compounds responsible for the dark colour and robust flavours notably present in black teas. Tea flavanols are sometimes

collectively referred to as catechins. Besides flavanols, tea flavonoids also include flavonols, flavones, isoflavones, and anthocyanins; all of which contribute to the colour of a tea's infusion and its taste.

Black tea is produced by the full oxidation of tea leaves, which are then dried. Compounds from the flavonoids family called catechins make up 27% of the composition of unoxidised green tea; due to the oxidation process, this is reduced in black tea to around 4%. The oxidation products of catechins in black tea are polyphenols, which influence both its colour and flavour.

### **Proteins, Carbohydrates, Lipids and Iron Binding Action of Tea Metabolites:-**

#### **Action on Proteins, Carbohydrates, Lipids:-**

Tea Polyphenols have been observed to have a strong precipitating effect on proteins, denaturing and reducing enzymatic activity (Sekia *et al.*, 1984; Ozawa *et al.*, 1987).

Immobilized polyphenols bind proteins reversibly, with restoration of enzyme activity after elution (Oh *et al.*, 1980).

Dissolved ash metals such as hard water calcium may influence complexation, as in the case of tea scum formation (Spiro *et al.*, 1996a; Spiro *et al.*, 1996b). Green tea polyphenols have been shown to be more effective than traditional antioxidants such as BHA, BHT, ascorbic acid, and vitamin E (Tanizawa *et al.*, 1984; Namiki *et al.*, 1986; Zhao *et al.*, 1989).

Although it is commonly stated that there are no tannins (meaning hydrolyzable tannins such as pentagalloylglucose) in tea, this statement is not strictly true. In addition to the gallic acid esters of the catechins and their oxidation products (which can be hydrolyzed to produce gallic acid readily and precipitate proteins), there is also a small quantity of hydrolyzable tannin (Nonaka *et al.*, 1983; Yoshizawa *et al.*, 1987; Hatano *et al.*, 1989; Han *et al.*, 1997).

### **Experimental:-**

#### **Materials:-**

Folin-ciocalteu reagent (Spectrochem, 2(N), Batch No:2784208), Sodium Carbonate (Spectrochem, 99.5%, Batch No:327555), Methanol (Merck, 99.8%), Butylated hydroxyl toluene (Merck, 99%, Batch No: 61774805001730), Tannic Acid (Merck, 99%), Potassium Acetate (Merck, 99%, Batch No: 61792805001730), Aluminium chloride (Merck, 98%, Batch No: 380108302501730), Quercetin (SRL, 99%, Batch No: T836708), Diphenylpicrylhydrazyl (Sigma Aldrich), Gallic Acid (CHD, 99%, Batch No-050711).

**Instruments:** Spectrophotometer (Unicam 300 from Thermospectronics), Heating mantle, Conical flask, beaker, Micro pipette, Measuring cylinder.

#### **The Methodology:-**

##### **Sample preparation:-**

The tea leaves were from the gardens of Jungpana tea estate, Darjeeling.

The sample is stored in airtight container to prevent moisture gain.

For preparing samples, 0.5 gm tea leaves was added to 50 ml distilled water. Infusion was made at 60, 80 & 100°C for 5, 10 & 15min (for each temperature condition). This solution\* remained the sample for performing the experiments.

Another sample was prepared with same amount of tea leaves with respect to only the infusion time (water boiled at 100°C, removed from heat source and tea leaves were infused) i.e. household tea making process.

##### **Moisture content of tea leaves:-**

Tea leaves were analysed for the moisture (dried in hot-air oven at 103-105°C for two hours and constant weight was noted) content to get the dry weight of samples.

##### **Method of Determination of Total Phenolic Compounds (TPC):-**

0.1ml of sample solution\* was taken into a test tube and 1ml (10 fold diluted) Folinicalteu reagent, 0.8 ml 2% Na<sub>2</sub>CO<sub>3</sub> were added to it and the volume is made upto 10 ml (volumetric flask) with distilled water. Absorbance of the solution was measured at 740 nm wavelength ( $\lambda_{\max}$  of gallic acid) (Agbor *et al.*, 2014) against a standard

calibration curve. Gallic acid was used as a reference standard and the calibration curve is prepared in the range of 20 to 100  $\mu\text{g}$  using 20, 40, 60, 80, 100  $\mu\text{g}$  as standard concentrations.

#### **Method of Determination of Total Tannin Content (TTC):-**

0.1 ml sample\* was taken in a test tube and 7.5 ml of water, 0.5 ml of Folinicalteu reagent and 1 ml of 35%  $\text{Na}_2\text{CO}_3$  solution was added to it. Then the volume was made upto 10 ml (volumetric flask) by using distilled water and shaken well. The test tubes were incubated for 30 min at room temperature and absorbance was measured at 700 nm wavelength ( $\lambda_{\text{max}}$  of tannic acid) (Agbor *et al.*, 2014) against a standard calibration curve. Tannic acid was used as a standard and the calibration curve was prepared in the range of 20  $\mu\text{g}$  – 100  $\mu\text{g}$  using 20, 40, 60, 80, 100  $\mu\text{g}$  as the standard concentrations.

#### **Method of Determination of Total Flavonoid Content (TFC):**

1.9 ml of methanol was added to 0.1 ml of sample solution\* (taken in a test tube). 0.1 ml 10%  $\text{AlCl}_3$  solution and 0.1 ml of 1M  $\text{CH}_3\text{COOK}$  were added to it successively and volume was made upto 5 ml (volumetric flask) with distilled water. The solution was kept at room temperature for 30 min. Absorbance of the solution was measured at 415 nm wavelength ( $\lambda_{\text{max}}$  of quercetine) (Hassan *et al.*, 2013) against a standard calibration curve. Quercetine was used as standard reference material and the calibration curve was prepared in the range of 20  $\mu\text{g}$  – 100  $\mu\text{g}$  using 20, 40, 60, 80, 100  $\mu\text{g}$  as the standard concentrations.

#### **Method of Determination of DPPH Free Radical Scavenging Activity:-**

5 different concentrations (10, 25, 50, 100, 150 $\mu\text{g}$ ) of sample\* were taken in test tubes. The volume was made upto 1 ml by using 80% methanol. To each sample 3 ml of DPPH reagent was added. The solutions were incubated in dark for 30 min and absorbance was taken at 517 nm wavelength against a standard calibration curve. During this whole process light was avoided as much as possible.

#### **Preparation of DPPH solution:-**

0.004 gm of DPPH was dissolved in 100 ml of 80:20 methanol and the solution was kept in amber coloured bottle, avoiding light (Marsha *et al.*, 2012).

#### **The look out:-**

The authors thus tried to estimate the secondary metabolites and antioxidant property of tea during infusions under normal household tea making certain inhibition percentage. The values of controlled-temperature infusion served as the platform, based on which, the infusions of each process and to correlate the values of such metabolites with certain digestive enzymes and iron by suggesting the volume responsible for metabolite during normal household tea making process were compared.

#### **Results and discussion:-**

##### **The outcome of work:**

There are various ways by which consumers brew their tea. The main difference is in brewing time and temperature. Though there are some evidences that the antioxidant activity, polyphenols and tannin content increase with increase in brewing time and temperature, studying the influence of brewing time on polyphenols and antioxidants content of tea can contribute to the information on how to utilize the product effectively and maximally (Shonisani *et al.*, 2010).

Changes in normal household infusion methods on the basis of the research would optimise the extraction and utilisation of metabolites to deliver optimal health benefit. During household tea making processes (**Temp<sub>uncon</sub>**, **Time<sub>var</sub>**), tea was infused for 5, 10 & 15 minutes after water was boiled and removed from the heat source. Variations of metabolites and antioxidant properties have been observed, if we consider the values, as the infusion times have been increased. The 15min time was chosen as the highest duration of infusion because in household tea makings, infusions are not done beyond that generally (**Table1, Fig1**).

The study comprised of tea infusion for 5, 10 & 15 minutes in controlled and uncontrolled environment - viz., infusion at 60, 80 & 100° C controlled temperature and tea infusion in water after it is boiled and removed from heat source. Each infused extract was filtered and the metabolites were estimated.

The values of controlled-temperature infusion served as the platform, based on which, the infusions of each metabolite during normal household tea making process were compared.

**Table1:** Change of metabolites contents & antioxidant property of Darjeeling Tea (Black & Green) during normal household tea making time and temperature (**Temp<sub>uncon</sub>**, **Time<sub>var</sub>**)

Parameter	Time of infusion (min)	Infusion at Controlled Temperatures						Infusion at Uncontrolled Temperatures (Normal Household Infusion)	
		60°C		80°C		100°C		Water Boiled at 100°C, infused and periodically withdrawn	
		BLACK	GREEN	BLACK	GREEN	BLACK	GREEN	BLACK	GREEN
Polyphenols (mg/g)	5	150	357	195	518	300	609	100	182
	10	275	539	310	637	410	910	115	203
	15	155	448	280	602	395	686	125	259
Flavonoids (mg/g)	5	5.0	5.0	6.0	5.0	8.0	6.0	6.0	6.0
	10	5.5	5.5	8.0	6.0	11.0	7.0	7.0	7.0
	15	7.0	6.0	9.0	7.0	12.0	8.0	8.0	7.5
Tannins (mg/g)	5	125	357	205	532	315	665	85	392
	10	230	609	330	686	445	777	110	420
	15	200	455	280	637	425	728	120	448
Antioxidant Property (µgm/)	5	50.06	52.11	44.97	49.69	47.79	41.14	47.07	45.44
	10	49.16	47.99	43.03	36.68	40.81	39.29	37.23	42.45
	15	42.34	47.05	24.88	34.89	40.13	36.94	34.83	38.52

Experimental data depicted one interesting point that, the **optimisation** of each of polyphenols & tannins (w.r.t. the two varieties of Darjeeling tea under study), occurred at the same time-temperature combination, viz., at 10min-100°C infusion. Similarly, identical time-temperature combination of optimisation have been found out experimentally for the antioxidant property; viz., at 15min-80°C infusion. On the other hand, w.r.t. flavonoids, the increasing trend continued and no optimisation could be recorded during the experimental period (15 minutes) of the two varieties of Darjeeling tea, pointing to the fact that higher temperature and higher time facilitated more and more extraction. For each variety, the lowest values for each of the metabolites were recorded experimentally during the lowest temperature-lowest time period of infusion combination.

During household tea making process, for each variety, the highest values for each of the metabolites was recorded experimentally during the highest temperature-highest time period of infusion combination. The household tea making, however, could not reveal the optimised value of each metabolite, pointing to the conclusion that, such uncontrolled temperature of infusion was not sufficient to maximise the extraction. The highest experimental values observed for each metabolite (during household extraction process) were lesser than the lowest values of corresponding metabolites (during controlled infusions) (**Table1**).

#### Polyphenols:-

The amount of polyphenols in green variety was much higher than that in the black; the result was in agreement with the chemistry of oxidation of tea leaves. In case of uncontrolled temperature, as the optimization of polyphenol release was not reached till 15 min., so the upward trend continued. However, the rate of extraction varied in black & green teas; At lower time period of infusion (5-10min), the black tea resulted in more polyphenolic extraction rate (15.05%) than green tea (11%); whereas, when the infusion time period was increased, the trend reversed (Black 8.7%; Green 27%) (**Table1**). The rate variation of extractions in black & green tea may be explained thus: the unoxidised polyphenols require more time & temperature to be available in the medium.

**Flavonoids:-**

In normal household tea making process, the flavonoid content remained almost constant for both black & green varieties of Darjeeling Tea (**Table1**) suggesting that there exist a lesser effect of time-temp variation upon this substance.

**Tannins:-**

The tannins content in black tea showed sharp upward trend (29.41%) while moving from 5 to 10 min, but decreased drastically (9.09%) in the case of 10 to 15 min. duration. However, in case of green tea, the rate of extraction was almost same during the entire time period of experiment, i.e. while moving from 5 to 10 (7.14%) and from 10 to 15 min. (6.67%). It may be concluded that at controlled heat, tannins may have been optimally released from the food mesh, with its optimum at around 10 min. This result shows that lesser infusion time (5-10min) at uncontrolled temperature facilitated the maximum extraction of tannins (**Table1**). But, due to sustained heat beyond 10 min., tannins undergo polymerization, like the polyphenols leading to precipitation (**Table1, Fig1**).

**Antioxidant property:-**

Although the AO activity continued to increase during the experimentation period, the rate of change varied with time of infusion. While in case of black variety, a sharp higher increment has been observed while moving from 5-10min., the AO activity of the green variety followed a gradual upward trend as the infusion time was increased from 5 to 15 minutes.

In case of controlled infusions, the highest polyphenols and tannins content due to tea infusions were observed after 10min. infusion at 100°C (410 & 445mg/gm resp.) and the lowest (150 & 125mg/gm resp.) was found in 5min. infusion at 60°C. While, the lowest flavonoids content was observed in both 5min. infused sample at 60°C (5mg/gm), the highest has been depicted by the 15min infused tea at 100°C (12.0mg/gm). On the other hand, the highest AO activity (24.88µgm/µl) due to tea infusions was observed after 15min. infusion at 80°C and the lowest (50.06µgm/µl) in 5min. infusion at 60°C.

TP plays an important role in protein precipitation and enzyme inhibition, through forming various complexes (Cartriona *et al.*, 1988; Shi *et al.*, 1994). It is known that most polyphenols, such as tannic acid, gallotannin, catechin and proanthocyanidin, can react with proteins. TP exhibits strong complexing abilities with enzymes. This should inevitably result in the change of enzyme molecular configuration and lead to the loss of catalytic activity. Many enzymes, such as tyrosinase, peroxidase, trypsin (Huang *et al.*, 2004), decarboxylase (Bertoldi *et al.*, 2001) squalene epoxidase (Abe *et al.*, 2000) and ribonuclease (Ghosh *et al.*, 2004) were found to be denatured by tea polyphenols. So it could be speculated that TP should bind and precipitate digestive enzymes and thereby reduce food digestibility. The EGCG, ECG and GCG account for more than 82% (w/w) of the TP sample. It is therefore speculated that TP will take part in complexing reactions with protein and, to some extent, affect the activities of digestive enzymes.

Tea polyphenols showed the strongest inhibition of  $\alpha$ -amylase which has the highest molecular weight. In the presence of 0.05 mg/ml of TP,  $\alpha$ -amylase had the highest loss of enzyme activity and the residual activity is only 39% of the original. However, in comparison with pepsin, trypsin has a smaller molecular weight but a higher activity loss, suggesting that the inhibitory effects of tea polyphenols on enzymes do not completely depend on the molecular weight of the enzyme. The inhibitory effect of TP on lipase activity was also established (Nakai *et al.*, 2005), where the effect of oolong tea polyphenols on pancreatic lipase was investigated.

The main mechanism of TP-protein (TP-enzyme) bonding is considered to be non-covalent interactions (Siebert *et al.*, 1996; Dreosti *et al.*, 2000). Tea polyphenols contain hydroxyl groups and galloyl groups in their molecular structure. The phenolic groups can form hydrogen bonds with the polar groups (amide, guanidine, peptide, amino and carboxyl groups) of protein. In other words, the composition and quantity of the polar groups in the enzyme protein will affect the formation and stability of hydrogen bonds between TP and the enzyme. Moreover, the galloyl groups in TP exhibit certain hydrophobicity (He, Shi, & Yao, 2006). With the recognition that there are many hydrophobic amino acids present in enzyme protein, such as proline, phenylalanine and tyrosine, it could be considered that tea polyphenols should strongly bind enzymes through hydrophobic association.

So, it could be concluded that tea polyphenols might act as an antinutritional factor, in terms of their inhibitory effects on digestive enzymes, which may be due to the cooperative effects of hydrophobic association and hydrogen bond formation between TP and the enzymes.

According to the studies by Siebert, Troukhanova, & Lynn, 1996 & Dreosti, it is clear that the TP-gelatin interaction occurs easily even in dilute solution. Further, with the increase of gelatin and TP concentrations, the bonding ability of TP with gelatin was strengthened.

Hence, based on the above studies of Siebert, Troukhanova, & Lynn, 1996 & Dreosti, 2000, there could be a reduction of the digestibilities of carbohydrates, proteins and lipids, whose hydrolyzation reactions in the gut are enzyme mediated. Hence, TP might act as an antinutritional factor, in terms of its potential to inhibit the activities of digestive enzymes.

The authors, therefore, tried to correlate the total polyphenols extracted during Indian household tea making processes with the percentage inhibition of Digestive Enzymes and calculated the volume of tea required to attain the prescribed dose of 0.05mg/ml (Siebert, Troukhanova, & Lynn, 1996; Dreosti, 2000) for the inhibition. The authors further, depicted the number of cups of tea required as per International Cup volumes.

From **Table 2**, it can be noticed that the volume of green tea required is almost half (48-55% lesser) than that of black variety, for attaining the same percentage inhibition of digestive enzymes, due to the availability of more polyphenols. Thus, it can be said that green tea is almost twice (1.82-2.07 times) as effective as black tea to act as antidiabetic and antiobesitic agents by their inhibitory action upon  $\alpha$ -amylase & lipase respectively. But, for the malnourished population, especially, in the Indian subcontinent, drinking of green tea may not be recommended.

#### **Action on Iron:-**

Iron is one of the most important essential trace elements for human life. Although quite abundant in the environment, iron deficiency still remains one of the most common deficiency syndromes in India, since the availability of iron for biological systems is limited. Therefore iron is spared within the human body, but there is no possibility of depleting surplus iron. Severe iron overload results in significant tissue damage, and even small amounts of excess iron may be harmful, leading to increased risk of cardiac infarction (Salonen *et al.*, 1992).

Black tea in particular, a commonly used beverage, has been shown by Disler (1975) and others (Brune *et al.*, 1989; de Alarcon *et al.*, 1979; Morck *et al.*, 1983) to reduce the uptake of iron from various sources of inorganic iron by about two thirds as compared with water intake (Tuntawiroon *et al.*, 1991). Polyphenols are assumed to act by binding heavy metals in the gastrointestinal lumen. **Table 1** shows that total tannin (TT) content in household-tea preparation varied with infusion time and the respective values in green tea are much more than that in the black variety. Further, from this data it can be suggested that that to obtain 66.8% Chelating Effect of Ferrous Ions by TT (0.015 mg/ml) (Gulcin *et al.*, 2010), how much volume of tea is required (**Table 3**), as it is known that tannates have the ability to inhibit iron absorption (Gulcin *et al.*, 2010). **Table 3** depicts that the green tea happens to be a strong iron-chelating agent, ranging from 3.7- 4.6 times as potent iron binder as black tea due to the presence of higher amount of total tannins. Thus, the action of tea polyphenols (tannins) may be ascribed in two contradictory ways – firstly, they may act as a beneficial agent to reduce iron overload in human systems by binding the non-haem iron (as has already been suggested by de Alarcon *et al.*); secondly, they may act as an inhibitory agent for the people suffering from anaemia (Zijp *et al.*, 2000; Samman *et al.*, 2001).

**Table2:** % Inhibition of Digestive Enzymes by Extracted Total Polyphenols (TP) due to Household Tea Preparations (**Temp<sub>uncon</sub>**, **Time<sub>var</sub>**)

Total Polyphenols (TP)			% Inhibition of enzymes by TP (0.05 mg/ml)				No. of Cups of tea required			
Time of infusion (min)	Variety	Conc. of TP extracted (ppm)	Enzymes				Vol. of prepared tea req to obtain the conc. of 0.05mg/ml* (ml)	Metric Cup (250ml)	Imperial Cup (284ml)	US Customary Cup (236.59ml)
			$\alpha$ -amylase	Pepsin	Trypsin	Lipase				
			% inhibition							
5	Black	100	61	32	38	54	500.00	2.0	1.8	2.1
	Green	182					274.73	1.1	0.9	1.2
10	Black	115	61	32	38	54	434.78	1.7	1.5	1.8
	Green	203					246.31	0.9	0.9	1.1
15	Black	125	61	32	38	54	400.00	1.6	1.4	1.7
	Green	259					193.05	0.8	0.7	0.8

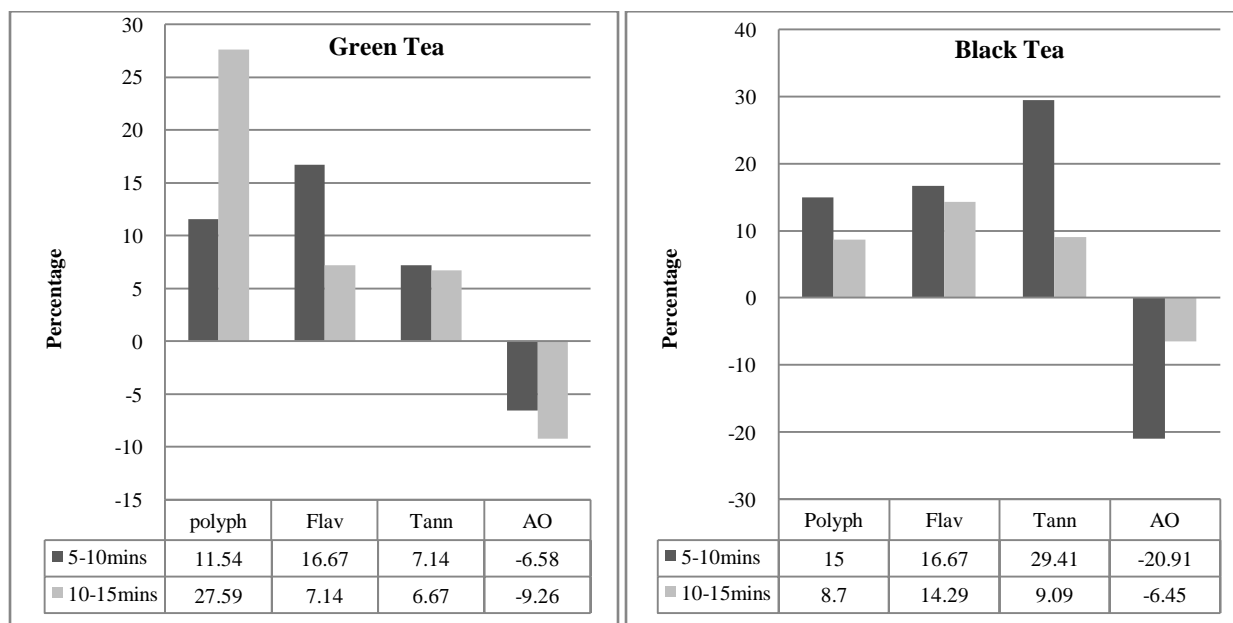
Ref: \*(Siebert, Troukhanova, & Lynn, 1996; Dreosti, 2000)

**Table 3:-** Chelating Effect of Ferrous Ions ( $Fe^{2+}$ ) by the Tannins (**Temp<sub>uncon</sub>**, **Time<sub>var</sub>**)

Total Tannins (TT)			To Obtain 66.8%* Chelating Effect of Ferrous Ions by TT (0.015 mg/ml)	No. of Cups of tea required		
Time of infusion	Variety	Conc. of TT extracted (ppm)	Vol. of prepared tea req to obtain the conc. of 0.015mg/ml* (ml)	Metric Cup (250ml)	Imperial Cup (284ml)	US Customary Cup (236.59ml)
			5			
5	Green	392	38.27	0.153	0.135	0.162
10	Black	110	136.36	0.545	0.480	0.576
	Green	420	35.71	0.143	0.126	0.151
15	Black	120	125.00	0.500	0.440	0.528
	Green	448	33.48	0.134	0.118	0.142

\*ref: Ilhami Gulcin, Zubeyr Huyut, Mahfuz Elmastas, Hassan Y and Aboul-Enein;

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**Fig 1:-** Percentage Variations of metabolites and antioxidant property in household tea making processes.



**Conclusions:-**

From the data presented it is evident that regular tea drinking with meals may exert a negative effect with respect to normal digestion processes as the tea metabolites possess some inhibitory properties against various digestive enzymes besides iron. Thus, tea – green tea in particular, may act as strong inhibitory agent against digestive enzymes and iron which might result in severe malnutrition and anaemia, especially in countries like India, where a good number of populations is still thriving below the poverty line. On the contrary, these negative effects of tea drinking with meals can be recommended as an additional therapeutic tool for the maintenance of normal body functions of persons suffering from diabetes, obesity and iron overload; as the inhibitory effects of tea metabolites might lessen the calorie intake along with iron. Therefore, drinking of tea, (green/black) may be said to be either beneficial or deleterious, when the physical conditions and nutritional status of the concerned individual is known. The study leaves a scope for further work using animal models to establish the proper enzymatic and iron inhibitions.

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