IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

Effect of brewing conditions on infusible fluoride levels in *Camellia sinensis*

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Abstract: This study investigated the fluoride content in infusions of various tea types' i.e. black, green, oolong, and white tea, commonly consumed in India. Fluoride concentrations were determined using a fluoride ion-selective electrode (FISE) method. The objectives were to understand the effect of brewing conditions on fluoride release and to assess the associated health risks. For a 2-minute brewing period, fluoride content ranged from 0.16 to 1.41 mg/l. This increased significantly after 5 minutes of brewing, ranging from 0.43 to 2.98 mg/l. A further, albeit smaller, increase was observed at 10 minutes of brewing. The repeated infusions from the same tea leaves showed the initial 2 - minute infusion yielded a certain fluoride concentration, the subsequent 5 - minute re-infusion showed a significant increase in fluoride. However, fluoride levels then decreased in the third (10 - minute) and fourth (15 - minute) re-infusions. The influence of tea leaf size on fluoride release showed that crushed tea leaves consistently yielded significantly higher fluoride concentrations in their infusions compared to uncrushed leaves, particularly for black and green teas. It must be emphasized that the amount of fluoride in the analyzed infusions of teas was not high enough to cause a risk of fluorosis, even if left to brew up to 10 min.

Index Terms **- Brewing, fluoride, infusion, *Camellia sinensis*, Chronic Daily Intake, Hazard Quotient**

I. IINTRODUCTION

Black tea is consumed primarily in western countries and in south Asian countries such as India and Sri Lanka, whereas green and oolong teas are consumed mainly in East Asian countries such as China, Japan, and Taiwan. Teas of *C. sinensis* undergo different manufacturing processes; green tea is produced by steaming (Japan) or panning (China) to prevent catechin oxidation by polyphenol oxidase (Graham, 1999). With no fermentation, green tea leaves retain their green colour and almost all of their original polyphenol content. The different processes of manufacturing give the various teas their characteristic colors and flavors. Oolong tea has an excellent characteristic combining the freshness of green tea and the fragrance of black tea. White tea is non-oxidized tea produced from young shoots of *Camellia sinensis* (Alcazar et al., 2007). Globally tea is growing in over 35 countries and India holds second position in the tea production (Das and Zirmire, 2018).

Fluoride is an essential micronutrient, and an appropriate amount of intake of this mineral, daily, can prevent the development of dental caries. However, numerous studies have indicated that excessive fluoride ingestion over a long period can lead to potentially severe dental and skeleton fluorosis, hypertension, damage to the neurological system, and a lower intelligence quotient (Lung et al., 2008; Valdez-Jiménez et al., 2011; Mohammadi et al., 2017; Razdan et al., 2017; Yuan et al., 2020). Tea (*Camellia sinensis*) is a plant exhibiting high tendency for accumulation of fluorides. Up to 98 % of fluorides are accumulated in the leaves used for the preparation of widely consumed tea infusions. Fluorides from tea leaves are released into infusions, which make the second after drinking waters important source of fluorine for humans. A narrow margin between acceptable fluoride concentration and health risk requires a reliable examination of a large variety of tea products for fluoride contents. The present work is an attempt to determine fluoride content in infusions of different types of tea leaves. The objectives were to understand the effect of brewing conditions on fluoride release.

II. RESEARCH METHODOLOGY

2.1 Chemicals and Glassware

All reagents used were of Merck Company and glassware was acid washed with 10% (w/v) nitric acid and triple rinsed with de-ionized water. De-ionized water was prepared by Lab-Q Water Purification System. Chemicals used were Sodium nitrate ($NaNO_3$), Sodium fluoride (NaF), Sodium acetate (CH_3COONa), Sodium hydroxide (NaOH), Acetic acid (CH_3COOH). NaF was dried at 110°C for two hours and after cooling was used as standard solutions needed for construction of calibration curve. Instruments used in the investigation are Industrial Oven, Heating Plate, Weighing Balance, PH meter and Fluoride ion selective electrode.

2.2 Sample Collection and preparation

All samples of the tea were purchased at supermarkets and local marketplaces. Approximately 200 g of each tea was collected and individually stored in airtight plastic bags (referred to as uncrushed tea samples). Approximately 100 g of each type of tea was crushed using an electrical grinder and individually packed into plastic bags. The concentrations of fluoride ions in 15 different samples of black, green, white and oolong tea were analyzed. Tea infusions were prepared on a customary way of tea preparation. For all measurement de-ionized water was used so that we can be sure that measured fluorides are from tea. From each tea were randomly selected five samples and dried at 80°C for five hours and analyzed on fluoride content. The infusion was prepared using 2.0 g of tea leaves boil with 200 ml de-ionized water for 2 min.

2.3 Stock and standard solution preparation

0.221gm NaF was dissolved in a liter of de-ionized water and stored in polyethylene bottle (for 100 ppm). Before weighing, NaF was dried for an hour at 110°C. Serial concentrations of sodium fluoride solution were prepared, and 25 ml of each concentration was added to equal volume of TISAB solution (Colina et al, 1990).

2.4 Analysis of Fluoride

The amount of fluoride in tea infusion was analyzed by potentiometric methods using previously described model. Samples were prepared for analysis by 25 ml of TISAB solution to 25 ml of the unknown solution to keep ionic strength and pH constant. During the measurement solutions were constantly mixing, and temperature was kept at 25°C. All measurement was made for five samples of each tea and the results presented here were average. Fluoride ion selective electrode was immersed in the solutions and readings on the ion analyzer were recorded. The fluoride concentration in tea infusions was analyzed based on the different brewing time, after repeated infusion and different leaf size

III. RESULTS AND DISCUSSION

The present result showed the general rank with respect to the fluoride concentration in infusions: Black tea > Green tea > Oolong tea > White tea. The effect of brewing time, repeated infusion and tea leaf size is described below.

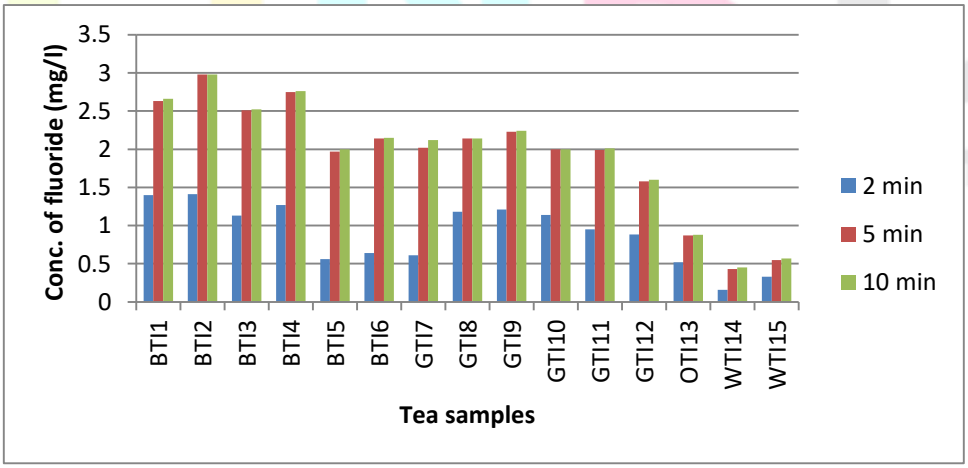

Fig.1: Concentration of fluoride in tea infusions (mg/l) after different brewing times (min)

3.1 Effect of different brewing times (min) on fluoride concentration of infusions

Since 1% tea to water ratio was the ratio used for makings of commercial tea drinks and used in other reports (Duckworth and Duckworth, 1978; Fung et al., 1999), tea infusions were prepared in 1% tea to boiling water ratio for this study. Moreover, the longer the brewing time, the higher the concentration is observed in the infusion, particularly of black tea, rising from 0.559 mg/l (lowest) – 2.98 mg/l (highest). It is observed (Fig.1) that most of fluoride is released within the first 5 minutes of infusion, passing from 2 to 5 minutes, and at 10 minutes there is a further increase. The longer brewing time of tea led to increase in concentration of fluoride, although it remained very low, in comparison to the 2 and 5 min of brewing. From the result it can be seen that there is a positive correlation between infusion times and F^- release. The value of fluoride infused from 15 brands of tea leaves ranged from 0.16 mg/l– 2.98 mg/l, indicating about differences in infusion efficiencies among different tea types. In general, white tea infusion had the lowest fluoride and black tea had the highest.

Present results obtained for black tea infusions after 5 min brewing are comparatively lower than the data presented by other authors 0.6–5.5 (Waugh et al., 2016), 1.15–6.01 (Cao et al., 2006), 1.47–5.45 (Das et al., 2017) and 1.057–6.680 mg/l (Satou et al., 2021). The fluoride content at the level of 3 mg/l or lower was also often reported: from 0.56 to 3.06 mg/l (Koblar et al., 2012) 0.57–3.72 mg/l (Emekli-Alturfan et al., 2009), 0.32–2.76 (Malinowska et al., 2008), 1.38 (Esfehani et al., 2018), 0.53–2.60 (Mahvi et al., 2006). On the other hand, the studies conducted earlier in Poland by Kłódka et al., (Kłódka et al., 2008) showed significantly lower fluoride concentrations in black tea from 0.121 to 0.652 mg/l that is far lower than the present data.

3.2 Effect of repeated infusion times (2, 5, 10, 15 min) on fluoride concentration of infusions

The fluoride content in tea leaves after repeated infusion of 2 to 15 min of repeated brewing showed highest increase during 2 to 5 min of brewing and afterwards the concentration decreases at 2nd and 3rd infusion for 10 min & 15 min. The almost same pattern of fluoride concentration in repeated infusion was observed for four types of tea leaves.

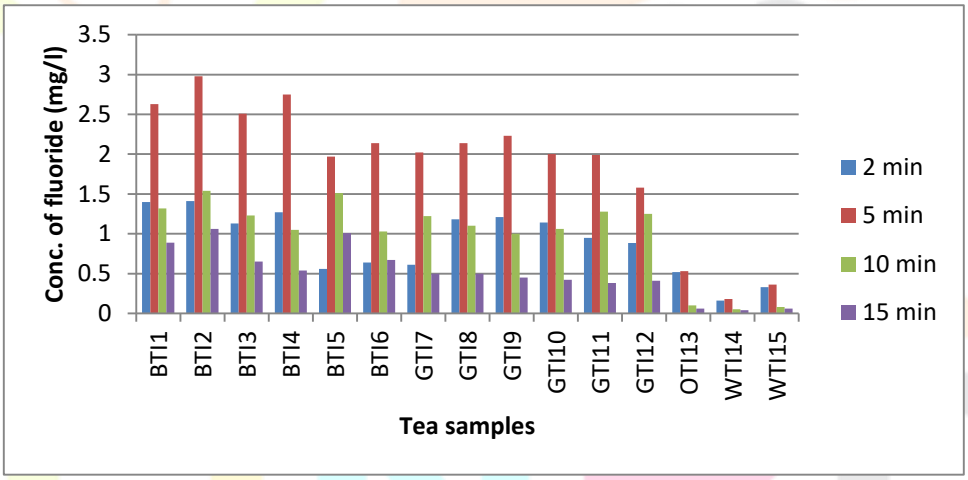

Fig. 2: Concentration of fluoride in tea infusions (mg/l) after repeated infusion of same tea leaves

The observed decrease in fluoride concentration during subsequent re-infusions (beyond the initial peak release) aligns well with existing literature. Studies by Malinowska et al. (2008) directly corroborate this, reporting significantly lower fluoride levels in second and third infusions in various tea types. This phenomenon is further supported by reviews (e.g., Ozsvath, 2009) indicating that most soluble fluoride is extracted in initial minutes, leading to depletion in subsequent brews. Similarly, research by Waugh et al. (2016) and others consistently shows that re-steeping tea leaves multiple times results in diminishing fluoride concentrations due to the progressive washout of readily soluble components, a principle also implicitly supported by Cao et al.'s (2006) work on extraction efficiency from a finite source.

3.3 Effect of size of tea leaves on fluoride concentration of infusion

The figure 3 show that crushed tea leaves yielded significantly higher fluoride levels compared to uncrushed leaves in black and green teas. In contrast, the difference in fluoride content between crushed and uncrushed leaves was less pronounced in oolong and white teas.

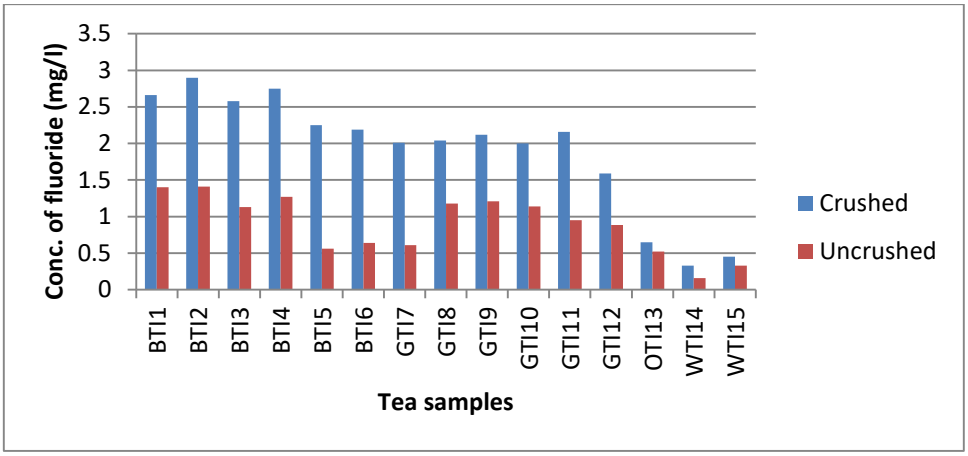

Fig. 3: Concentration of F^- in tea infusions after brewing of crushed and uncrushed tea leaves

The finding that crushed tea leaves yield significantly higher fluoride concentrations than uncrushed leaves in black and green teas is consistent with established principles of extraction and is supported by previous research. Crushing the tea leaves increases the surface area exposed to the hot water, thereby facilitating the more rapid and efficient dissolution and diffusion of soluble compounds, including fluoride ions, from the tea matrix into the infusion (Wong & Tan, 1999; Malinowska et al., 2008). According to WHO, the optimum fluoride intake for humans should range from 2 to 4 mg/day. The level recommended by USEPA is similar, from 2.5 to 4 mg F^-/day for children and adults, respectively (USEPA-US Environmental Protection Agency; 2017). This amount of fluoride can be covered by about 2 – 3 cups (ca. 400 – 600 ml) daily of a popular black tea with F^- concentration about 6 mg/l, even after 15-minute brewing, but without taking into account other sources of fluoride (e.g. toothpaste etc.).

Present results obtained for black tea infusions after 5 min brewing were comparable with the data presented by other authors: 0.6 – 5.5 (Waugh et al., 2016), 1.15 – 6.01 (Cao et al., 2006), 1.47 – 5.45 (Das et al., 2017) and 1.057 – 6.680 mg/l (Satou et al., 2021) The fluoride content at the level of 3 mg/l or lower was also reported: from 0.56 to 3.06 mg/l (Koblar et al., 2012) 0.57 – 3.72 mg/l (Emekli-Alturfan et al., 2009), 0.32 – 2.76 (Malinowska et al., 2008), 1.38 (Esfehani et al., 2018), 0.53 – 2.60 (Mahvi et al., 2006). On the other hand, the studies conducted earlier in Poland by Kłódka et al., (Kłódka et al., 2008) showed significantly lower fluoride concentrations in black tea i.e. from 0.121 to 0.652 mg/l.

The findings revealed substantial amounts of fluoride in all tested tea infusions, with a general trend indicating that black tea infusions contained the highest fluoride levels, followed by green, oolong, and white tea, respectively. It must be emphasized that the amount of fluoride in the analyzed infusions of teas was not high enough to cause a risk of fluorosis, even if left to brew up to 10 min. Nevertheless, a fluoride intake from teas consumed regularly should be taken into account in view of the potential health effects, both positive and negative, especially for children, and pregnant women who often choose plant infusions as healthier replacement of coffee. Above all, in the total fluoride balance, it is necessary to take into account the fluoride content in water used for brewing, which may be significant and variable depending on the country and other sources of fluoride.

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