Study on Percentage of Tannin and Caffeine in Libyan Green Tea Beverages and their Nutritional Effect on Human Health

(Original Research Article)

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Recieved on: 12 September 2021 **Accepted on: 29 October 2021**

Abstract

This study is to determinate the percentage of caffeine and tannin in green tea by three Libyan traditional methods and the effect of heating time. Also, it aimed to study the nutritional healthy benefits and side effects of these constituents. Three samples of vert chine chunmee (NAPT) of green teas were analyzed. The first sample was raw, the second sample was boiled for 30 minutes and the third sample was boiled for 5 minutes. The heating temperature was at 100C˚. The percentage of tannin and caffeine were estimated in each sample. Caffeine percentage in the three samples was 0.021, 0.022, 0.012, respectively. Tannin participation in green tea prepared by second Libyan traditional method (green tea boiled for 30 minutes) has 83mg/100ml of tannin. This value is higher than raw and third Libyan traditional method (green tea boiled for 5 minutes), which it was 10.9 mg/100 ml and 49mg/100ml, respectively. This study reveals that the heating time affects on the percentage of caffeine and tannin in green tea; as the heating time increases, the percentage of caffeine and tannin increases**.** But the percentage of tannin in green tea prepared by traditional Libyan methods was higher than caffeine. The rise in tannin percentage in green tea may lead to genetic defect; therefore, the third Libyan traditional method (boiling time for 5 min) may nutritionally consider the best for improving human health, followed by the first

Libyan method. They provide the smallest percentage of caffeine and tannin in Libyan green tea beverages.

Keywords: Tannin, Caffeine, Libyan Traditional Methods, Heating Time, Nutritional Healthy Benefits, Libyan Green Tea Beverage.

Introduction

Tea represents one of the majority consumable beverages in the world. Tea can be classified into three main types, depending on the level of oxidation including green tea, oolong tea and black tea.Green tea is mainly the healthiest form because it has the maximum amount of polyphenol about 30-40%.While, the other teas types represent about 3-10%. Nutritionally, tea has been believed a medicine and a healthful beverage since ancient times. But, newly it has received a big deal of attention because tea polyphenols are powerful antioxidants. [1-5] Proximate analysis of green tea dried leave demonstrated that it has 37% polyphenols, 25% carbohydrates, 3.5% Caffeine, 15% protein, 4% soluble amino acids, 6.5 % lignins, 1.5% organic acids, 2% lipids, 5% Ash and 0.5% chlorophyll. The polyphenols in green tea are flavan-3-ol derivatives commonly known as catechins. These mainly consist of four compounds, (–)–epicatechin (EC); (6.4% approximately), [6] (–)-epigallocatechin (EGC) (19% approximately); EC gallate (ECG) (13.6% approximately) and EGC gallate (EGCG) that represents approximately 59% of the total of catechins [7-8] which possesses strong antioxidative activity. [9] The method of extraction of tea has an effect on its phenolic and caffeine contents and its biological activity.

A previous study in 2008 in USA reported that there was nothing of the speed at which the caffeine is extracted, since tea was steeped for 3 minutes, not fewer. It does not negate at all the idea that most of the caffeine is extracted during the first 30-45 s. It guesses that speed of extraction during the first 30 s. All studies illustrate that even after a 3-4 minutes infusion, there is still between 10-25% of caffeine left since that is what come out in a second infusion. A report by Pakistani researchers , about effects of temperature and water steeping duration on antioxidant activity and caffeine content of tea, the result showed that the total phenolic contents of green tea prepared with cold water steeping are higher than those of any other teas prepared in hot. It is suggesting that some phenolic compounds in green tea might not be heat resistant and degraded at higher temperatures. Another study in Addis Ababa , Athiopia, 2011 by Tadelech Atomssa and A.V.G holop explained that green tea extract decreased the absorption of non-heme iron by 25%. However, vitamin C enhances non-heme iron absorption, so it should squeeze lemon into tea or eat other vitamin-C rich foods such as broccoli, with the meal. [10-14]

Nutritionally, green tea is a popular beverage and can be used as food supplements. It has been described by dietitian for assist patients with clogged arteries, endometrial and ovarian cancer, low blood pressure, bone health (osteoporosis), changes in cervical cells due to human papiloma virus (HPV), white patches in the gums, the prevention

of Parkinson's disease. It decreasing cholesterol and hypertension and diabetes. Oral health, weight loss, antiaging, Asthma, immunity, liver diseases, flu and cold. Other uses included a range of cancers (bladder, esophagus, pancreas, breast, colon, stomach, leukemia, mouth, prostate, and lung); acne, heart disease, diabetes, infertility, heart health (high blood pressure, respiratory infections, improvement of athletic performance and wrinkles. [15-23]

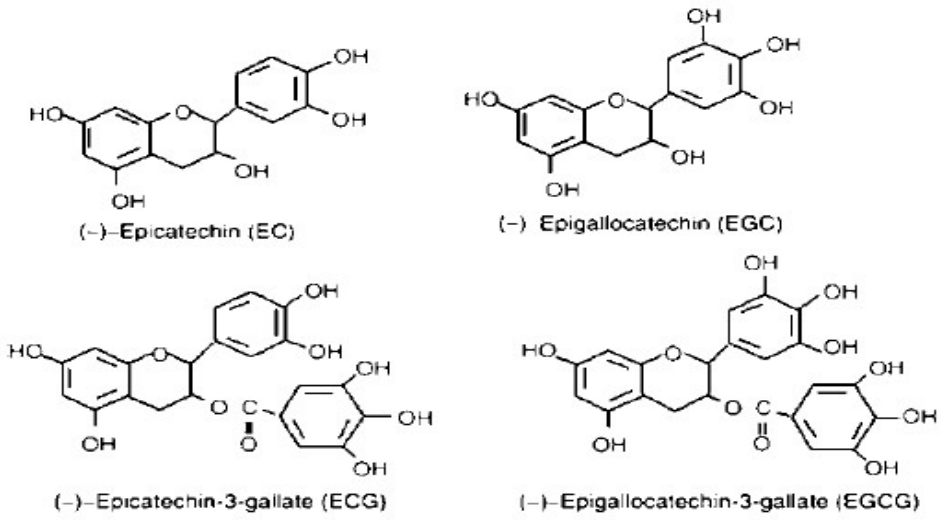

Figure 1. Polyphenols in Green Tea.

High-tannin tea has also been shown to diminish the need for blood removal from people with iron overload, or hemochromatosis. Hemochromatosis arises from a genetic defect that permits uncontrolled absorption of iron. [2-3] Caffeine is recognized as xanthine derivative which come from plants are possibly the oldest known stimulants. It is the most potent ability to enhance alertness, put off sleep and to develop attention. The bigger amounts of caffeine can cause difficulties. These ranges are from headaches and anxiety to irritability and insomnia. [2, 6] In addition, green Tea consumption has an effect on drugs. [24-25] It may inhibit the actions of adenosine, raise the effectiveness of beta-lactam antibiotics by decreasing bacterial resistance to treatment, diminish the sedative effects of benzodiazepines enhance blood pressure in people taking propranolol and metoprolol, reduce *Clozapine* if taken less than 40 minutes after drinking it. When taken together with ephedrine; green tea may lead to insomnia, weight loss, agitation, and tremors. It also decreases blood levels of lithium. Oral contraceptives can prolong the amount of time caffeine stays in the body and may raise its stimulating effects, with Phenylpropanolamine can cause mania and a severe rise in blood pressure. It should not be taken with *warfarin* because it has vitamin K and, thus, can render warfarin ineffective and it should not be mix *aspirin* with green tea together because increase the threat of bleeding. They both prevent platelets from clotting. [5, 19, 21] Therefore, studying of polyphenolic and caffeine contents in green

teas beverages prepared by Libyan traditional methods will be high attention merit. The aim of the present study is to determine the percentage of tannin and caffeine in most common green tea performed by common Libyan methods. It is also to study nutritional and healthy benefits and side effects of these constituents in each method.

Materials and Methods

Materials

Green tea samples, Analytical balance, hot water, 100 volume flasks, cooler, separating funnels, filter papers, crucible, drier, and chemical solution such as ammonia solution, chloroform, Qunine sulphate, 1N $H_2SO_{4,}$.

Sampling

The present study analyzes three samples of green tea from the vert chine chunmee (NAPT), which is the most type of green tea used in Libya. First sample was raw green tea, while, the second sample of green tea was boiled for 30 minutes. The third sample of green tea was boiled for 5 minutes. The boiling temperature was 100 ˚C.

Extraction by Common Libyan Method for Caffeine

For manually separation of caffien, it takes 5 grams or 5 ml tea in 400ml hot water. Put it in 100 volume flask + 5ml craz I+ 5ml craz II & complete to volume by hot water, cool it, then put all in separating funnel then add 10 ml ammonia solution & separate by 25 ml chloroform for 5 times & collect in crucible.

Extraction by Common Libyan Method for Caffeine

For manually separation of tannin, it takes 5 grams or 5 ml of green tea in 400 ml water, make it boiling for 1 hour then filtrate it. Add 1 gram Qunine sulphate to 25 ml water then add 2.5 ml 1N H_2SO_4 then add all to filtrate. The precipitate is formed by known weight then dries it.

Statistical Analysis

Descriptive statistics were performed using SPSS Statistics Software Program (version20, Inc., Chicago, Illinois, USA). Independent T Test was used to assess the significance of the association between percentages of caffeine and tannin according to temperature time of separation. In all tests, $\alpha < 0.05$ was regarded statistically significant. All confidence intervals (CIs) were calculated at the 95% level of statistical significance. Graphs of percentages of caffeine and tannins were constructed using Microsoft office excel 2016 program. The percentage formulas were calculated based on the Libyan National Center for Standards in the Nutrition Laboratory, Al-Arab Medical University, Benghazi, Libya.

Results and Discussion

Effect of Heating Time

In the present chemical analysis, there was a significance difference between percentages of caffeine in the three sample preparations. The average percentage of caffeine in the samples 1, 2, and 3 was 0.021%, 0.022%, 0.012%, respectively. In comparison, there also was a significance difference between percentages of tannins in the three sample preparations. The average percentage was 0.0109 %, 0.049 % and 0.083%, respectively. This indicates that caffeine and tannin contents increase with heat, as the percentage increases by increasing the heating time.

Table 1: Average Percentages of Caffeine and Tannin in Green Tea Samples.

Tannin %	Caffeine %	Green Tea Sample
0.0109	0.021	Raw Green Tea (Sample 1)
0.083	0.0224	Green Tea Boiled for 30 minutes (Sample 2)
0.049	0.0125	Green Tea Boiled for 5 minutes (Sample 3)

According to the Libyan National Center for Standards, the Libyan standard criteria ratio for caffeine in raw green tea steeped in boiled water was 1.5 %. While, the Libyan standard criteria ratio for tannin in raw green tea was 10.5 %. This chemical analysis demonstrated that the method of the preparation may affect on the percentage of caffeine and tannin in green tea. For example, in tannin separation, the increase in the temperature of green tea will raise the participation of tannin. The green tea prepared by second Libyan traditional method contains 83mg/100ml of tannin. This value is higher than the raw green tea sample and the sample prepared by the third Libyan traditional method. It was 10.9 mg/100 ml and 49mg/100ml in raw green tea and green tea prepared by third traditional Libyan method, respectively.

The Extraction Method that Nutritionally Benefit for Human Health

The second Libyan preparation considers the highest sample containing caffeine, which has 22.4 mg/100 ml of caffeine. The third traditional method contains 12.5 mg/1ooml of caffeine whereas, the first traditional methods (raw green tea) contains

2.1mg/100 of caffeine (Figures 2, 4). All these three samples prepared at a constant time. In this study the second traditional Libyan method has higher percentage of tannin than the first (raw green tea) and the third common method (Figures 3, 4). Polyphenols are touting as excellent antioxidants. High-polyphenols tea has also been shown to reduce the need for blood removal from people with iron overload [10-14]. .In our study, the sample boiled for 30 minutes contains higher caffeine percentage than other Libyan tradition preparations, which contains 22.4 mg/100ml of caffeine.

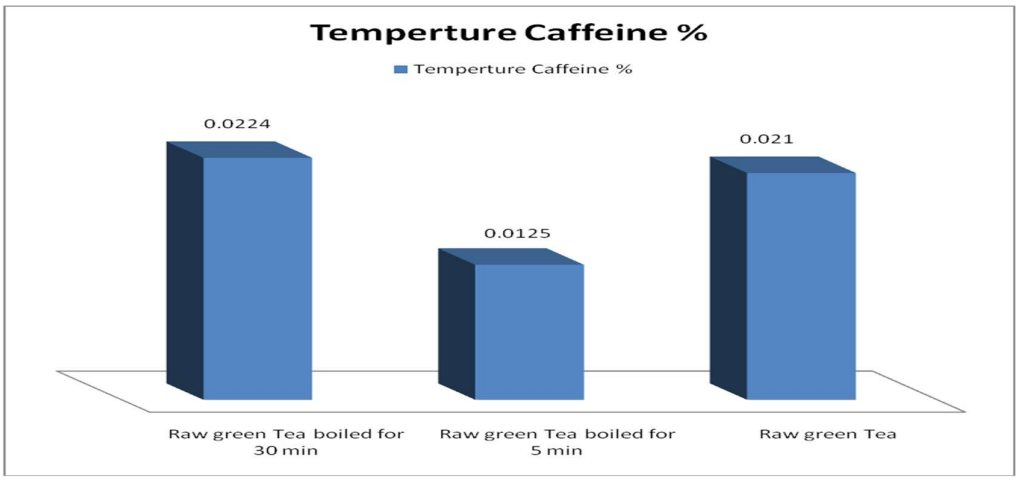

Figure 2. Caffeine Percentage in Green Tea Boiled for 30 min, Green Tea Boiled for 5 min and Raw Green Tea.

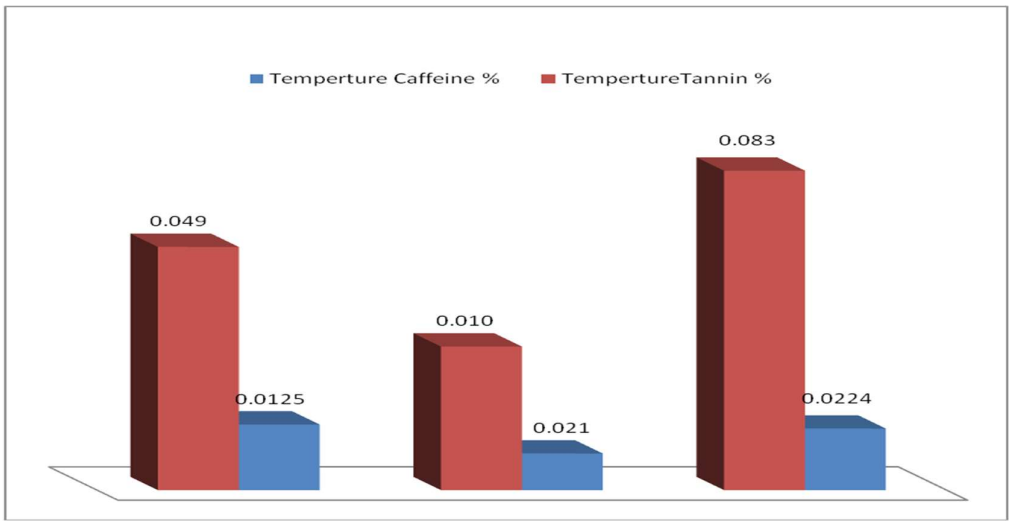

Figure 3 . Percentage of Caffeine and Tannin in Green Tea Boiled for 5 min, Raw Green Tea and Green Tea Boiled for 30 min.

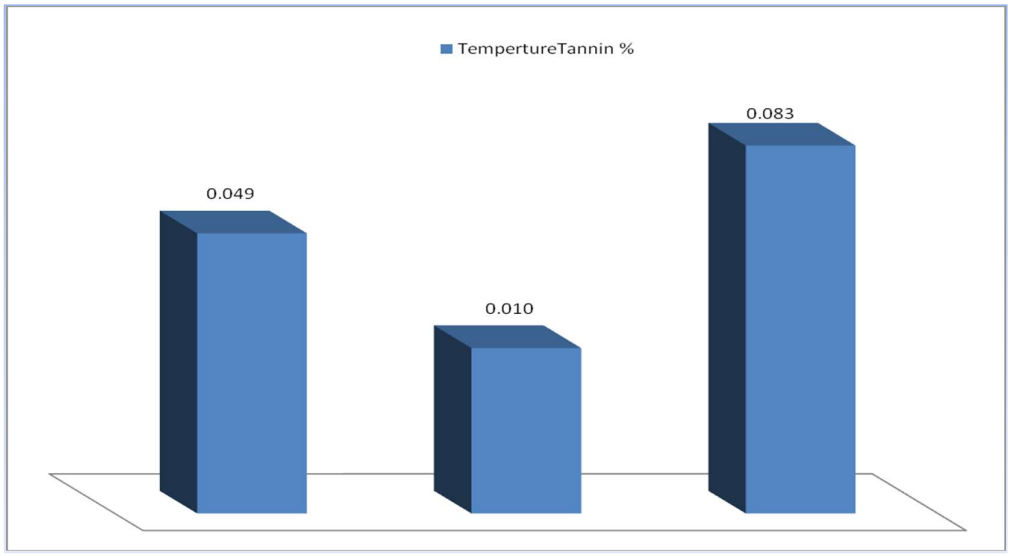

Figure 4. Tannin Percentage in Green Tea Boiled for 5 min, Raw Green Tea and Green Tea Boiled for 30 min.

The results illustrated that there was statistically significant difference between percentages of caffeine and tannin according to their heating times (P= 0.026). This implies that tannin and caffeine percentages increase with heat. Also, tannin percentages in the three green tea samples prepared by the Libyan traditional methods were higher than caffeine percentages. Tannin may lead to genetic defect, abnormal absorption of iron and other health difficulties [2,3]. Therefore, the boiling time in green tea prepared by Libyan traditional method should be short to have a minimum amount of tannin. This points to that the third Libyan traditional method (boiling time for 5 min) is nutritionally the best for human health because it provides a healthy percentage of caffeine and tannin, followed by the first Libyan traditional method.

Conclusion

In alternative medicine, green tea strongly has the ability to improve alertness, put off sleep and to enlarge attention. However, consuming large amount of caffeine may cause negative side effects such as headaches and anxiety to irritability and insomnia. We can consider that all three Libyan traditional methods performed for green tea samples have health benefits. A 30 minutes boiling traditional method is the most common Libyan method contains the highest polyphenolic and caffeine contents. This indicates that the second traditional Libyan method has higher percentage of tannin and caffeine than raw and the third Libyan common methods.

Also, tannin percentages in the three green tea samples prepared by the Libyan traditional methods were higher than caffeine percentages. The rise in tannin percentage in green tea may lead to genetic defect, abnormal concentration of iron, and other health problems. Consequently, the boiling time in green tea prepared by Libyan

traditional method should be at short time. This picks out that the third Libyan traditional method (boiling time for 5 min) may nutritionally consider the best for improving human health, followed by the first Libyan traditional method. They provide a small percentage of caffeine and tannin in green tea beverage.

Competing Interests

We (authors) declare that we have no conflict of interest.

Acknowledgments

Authors thank everyone who contributed with us and helped us to complete this research.

References

1. NHLBI and Bethesda. (1998). Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults: the evidence report. *Obes Res.*, 6 Suppl 2:S51–209.

2. Murase T, Nagasawa A, Suzuki J, Hase T, Tokimitsu I. (2002). Beneficial effects of tea catechins on diet-induced obesity: stimulation of lipid catabolism in the liver. *Int J Obes Relat Metab Disord*, 26: 1459 – 64.

3. Wolfram S, Wang Y, Thielecke F. (2006). Anti-obesity effects of green tea: from bedside to bench. *Mol Nutr Food Res.*, 50: 176 – 87.

4. [DPP} The Diabetes Prevention Program. (2000). The Diabetes Prevention Program (DPP): description of lifestyle intervention. *(Research Group) Diabetes Care.* 25 : 2165 – 71.

5. {LabCE) Adult Treatment Panel III. (2002). Third report of the national cholesterol education program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel III) final report. *Circulation,* 106:3143–421.

6. Berube-Parent S, Pelletier C, Dore J, Tremblay A. (2005). Effects of encapsulated green tea and guarana extracts containing a mixture of epigallocatechin-3-gallate and caffeine on 24 h energy expenditure and fat oxidation in men. *Br J Nutr.* ;94:432–6.

7. Florian P, Kroesen A.J , Zetz M, Froman M.and Schulzke I.D. (2004). Cytokine-dependent transpcitonal down-regulation of epithelial sodium channel in ulcerative colitis .*Gastroentrol., 126: 1711 - 1720*.

8. Janeiro P, Oliveira B.A.M. (2004). catechin electrochemical oxidation mechanisms. *Analytica Acta, 518: 109 - 115.*

9. Mochizukim , Yamazakis, Kanok, Ikeda T. (2002). Kinetic analysis and mechanistic aspects of autoxidation of catechins. *Biochim Biophys Acta, 1569:35-44.*

10. Pasrij D. and Anandhamakrishnan C,.(2015). Techniques for Extraction of green tea polyphenols: *Areview, 8: 935*.
11. Jeu-Ming P.Yuann, Jun-Yun, Hui-Hsueh Chang, and ji-Yuan Liang (2015). Effects of tempreture and water steeping duration on antioxidant activity and caffeine content of tea, *J Food Sci,* 7: e3.

12. Farhoosh R, Golmovahhed G, Mohammad HHK. (2007). Antioxidant activity of various extracts of old tea leaves and black tea wastes (Camellia sinenisis L.). *Food chem, 100:231-236.*

13. Yang DJ, Hwang LS, Lin JT. (2007).Effects different steeping methods and storage on caffeine, catechins and gallic acid in bag tea infusions. *J chromatogr Ana*,, *13 (1156): 312-320.*

14. Cabrera C, Gimenez R, Lopez C. (2003). Determination of tea components with antioxidant activity. *J Agric Food Chem*, 51: 4427-4435.
15. Graham HN. Green tea composition, comconsumption, and polyphenol chemistry. Preventive Med 1992; 21:334-50.

16. Rietveld A, Wiseman S. (2003). Antioxidant effects of tea: evidence from human clinical trials. *J Nutr, 133: 3285S-92S.*

17. Hirasawa M, Takada K. Multiple effects of green tea catechin on the antifungal activity of antimycotics aganist candida albicans. (2004). *J Antimicrobial Chemother. 53: 225-9.*

18. Newman J. (2021). Cooking with green tea. Food Guids. Available from: http://www.secretsofgreentea.com/green-tea-diet-review. Accessed on Sep 18, 2021.

19. Bhagwat S, Beecher GR, Haytowitz DB, Holden JM, Dwyer J, Gebhardt SE, Eldridge Al, Agrawal S, Balentine DA. '(2021). Flavonoid composition of tea:

Comparison of black and green teas', USDA Agricultural Research Service. Available from: http//en. Wikipedia.org/wiki/potential effects of tea on health. Accessed on: August 17, 2021.

20. katiyar S,Elmets CA,Katiyar K. (2007). Green tea and skin cancer:phto immunology, angiogenesis and DNA repair. *J Nutr Biochem, 18: 287-96.*

21. Sinija V.R & Mishra H.N. Green tea:Health benefits. Journal of Nutrtional & Environmental Medicine, 2008; 17(4):232-242.

22. Lee MJ, Maliakal P, Chen L, (2002). Pharmacokinetics of tea catechins after ingestion of green tea (-)-epigallocacatechin-3gallate by humans: formation of different metabolites and individual variability. *Cancer Epidemiol Biomarkers Prevent, 11: 1025-32.*

23. Devine A, Hodgson JM, Dick IM, Prince RL. (2007). Tea drinking is associated with benefits on bone density in older women. *AM J Clin Nutr, : 86 (4): 1243-1247.*

24. Salim U.R. Rehman & Kausar A. (2004). Effect of time and tempreture on infusion of tannins from commercial brands of tea. *Internat J Agricul Biolog, 1560 - 8530.*

25. Jenna M. Chin et al. (2008). Caffeine content of brewed teas. J Anal toxicolog,12 : 32610 – 0275