Sabuncuoglu Serefeddin Health Science (SSHS)
ISSN: 2667-6338, 2021/Vol.3:2

COMPARISON of the EFFECTS of DIFFERENT FORMS of GREEN TEA on FECUNDITY in *Drosophila melanogaster*

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Research article
Received: 06.07.2021; Accepted: 09.08.2021
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Abstract

Teas are classified into different types depending on the processing techniques of fresh tea, leaves, and sprouts. One of them is the green tea form prepared without fermentation. This type of tea, which has gradually started to replace black tea, also has different forms such as powder, leaf, and flour. The aim of the study was to compare different forms of green tea with green tea flour and to evaluate its effect on the egg yield of *Drosophila melanogaster*. The Oregon wild strain of *D. melanogaster*, an important organism, was used in the study. The 3rd stage larvae of *D. melanogaster* were grown in standard media and media containing different forms of green tea. The egg yield of female individuals obtained from here was calculated for 3 days by performing their 1♀ x 3♂ crossing. When the results were examined, the mean egg yield of female individuals chronically fed with green tea flour was found to be 34±1.15. The egg yield obtained from female individuals grown in a standard medium as the control group was found to be 21±1.15. The egg yield of female individuals fed with tea extract prepared with powder and green tea leaves was found to be 24.3±0.33 and 24.6±0.66, respectively.

As a result of our study, it was concluded that green tea flour (Matcha tea), which had a higher substrate content than other forms, was more effective on fecundity.

Key Words: *Camellia sinensis, Drosophila melanogaster*, Fecundity, Green Tea Flour, Matcha Tea.

Özet

Yaş çay, yaprak ve filizlerinin işlenme tekniklerine bağlı olarak çaylar farklı tiplerde sınıflandırılmaktadır. Bunlardan birisi de fermente edilmeden hazırlanan yeşil çay formudur. Giderek siyah çayın yerini almaya başlayan bu çay tipinin de toz, yaprak ve un gibi farklı formları bulunmaktadır. Çalışmanın amacı yeşil çayın farklı formlarını yeşil çay unu ile karşılaştırarak *Drosophila melanogaster*'in yumurta verimi üzerine etkisini değerlendirmektir. Çalışmada önemli bir organizma olan *D. melanogaster*'in Oregon yabanıl soyu kullanılmıştır. *D. melanogaster*' in 3. evre larvalarının standart besiyeri ve yeşil çayın farklı formlarını içeren besiyerlerinde gelişimleri sağlanmıştır. Buradan elde edilen dişi bireylerin 1♀ x 3♂ çaprazı yapılarak dişi bireylerin 3 gün boyunca yumurta verimleri hesaplanmıştır. Sonuçlar incelendiğinde yeşil çay unu ile kronik olarak beslenen dişi bireylerin ortalama yumurta verimi 34±1,15 olarak tespit edilmiştir. Kontrol grubu olarak standart besiyeri ile yetiştirilen dişi bireylerden elde edilen yumurta verimi ise 21±1,15 olarak tespit edilmiştir. Toz ve yeşil çay yaprağı ile hazırlanan çay ekstraktı ile beslenen dişi bireylerin yumurta verimi ise sırasıyla 24.3±0.33 ve 24.6±0.66olarak tespit edilmiştir. Çalışmamız sonucunda substrat içeriği diğer formlara göre daha yüksek olan yeşil çay ununun (Matcha çayı) fekundite üzerine de daha etkili olduğu sonucuna varılmıştır.

Anahtar Kelimeler: *Camellia sinensis, Drosophila melanogaster,* Fekundite, Matcha çayı, Yeşil Çay Unu.

1. Introduction

Tea is obtained by processing the leaves of *Camellia sinensis* grown in tropical and subtropical regions in the world. The tea plant belongs to the Theaceae family and has two species.

The one of Chinese origin is called *Camellia sinensis*. *Camellia sinensis* is a small-leaved and bushy plant and grows in many countries with a cold climate in Northeast Asia.

Tea obtained from the leaves of the plant known as *Camellia sinensis* is nowadays defined as a healthy beverage due to its bioactive molecules and high antioxidant capacity (Gonzalez et al., 2009). The scientific therapeutic potential of tea has been revealed in recent years. Tea, which is consumed by two-thirds of the world's population, is the most important beverage after water and positively affects the human body in many ways, contains more than 4000 chemical substances (Yang & Landou, 2000).

The differences between teas are usually due to processing, growing conditions, and geographical characteristics. According to their processing or harvesting, teas are divided into four groups: black tea (fermented), green tea (not fermented), oolong tea (semi-fermented), and white tea. Green tea is produced from young tea leaves. After withering, steaming or pan-frying, drying, and classification, teas are packed and presented for sale for consumption without fermentation. Pan-frying is required to prevent the fermentation of tea leaves with natural enzyme activity. While black tea is processed, the tea leaves are fermented for several hours before the processes of either smoke fire, flame fire, or steaming. Withering occurs as the evaporation of water, and a natural fermentation process takes place. The chemical structure of tea leaves changes during fermentation. Unblended teas are named according to their country of origin or region (Darjeeling, Assam, China, etc.). Blended teas are named as type-specific (Earl Gray, Irish Breakfast, etc.) rather than the teas they contain. Unprocessed raw green tea is used as the raw material in the preparation of Pu-erh tea consumed by the Chinese, and the preparation of this tea requires a long fermentation process (Wang et al., 2008).

Oolong and green teas contain high amounts of EGCG (Epigallocatechin gallate) (50% to 80% of total catechin) and EGC (epigallocatechin). Fresh green tea leaves have catechin by 30%-40% in dry weight. Green tea contains approximately 70% catechin (monomeric flavonoids), 10% minor flavanols (mostly quercetin, kaempferol, myricetin, and their glycostructures), and 20% polymeric flavonoids because some oxidation events occur during the withering process. Of the total flavonoids in green tea, 20% and 30% can be oxidized to polymers in black tea. Tea contains many amino acids. However, L-theanine (γ-ethylamine-L-glutamic acid) is the most abundant amino acid in the tea plant as plant-specific and constitutes approximately 50% of the total amino acid level. Volatile substances in tea contain more than 600 different molecules. Furthermore, tea contains carbohydrate, caffeine, adenine, gallic acid, tannin, gallotannin, quercetin glycosidase,

carotenoids, tocopherols, vitamins (A, K, B, C), low amounts of aminophenyl, and a yellow essential oil, which is solid at 25 °C and has strong aromatic smell and taste (Jayabalan et al., 2008).

Tea, which is consumed at medium temperature and in certain amounts, can be defined as a beverage that positively affects health and has no acute or chronic toxic effects. According to studies, it was indicated that people who drank tea regularly also had a healthy lifestyle (Schwarz et al., 2009; Weisburger & Chung, 2002).

The tea composition varies by climate, seasons, tea varieties, and the age of the tea leaf. Furthermore, tannins or phenolic substances containing catechin (flavanol) and gallic acid units are also present at very high rates (5-27%) in tea (Leung & Foster, 1996).

During the fermentation of fresh tea leaves, some catechins are oxidized or condensed to larger polyphenolic molecules (dimers and polymers) such as theaflavins (theaflavin, theaflavin-3-gallate, theaflavin 3' gallate, and theaflavin 3-3' gallate) (3-6%) and thearubigins (12-18%). These polymers provide a bitter taste and a dark color in black tea. Black tea generally contains thearubigin (70%), theaflavin (12%), flavanol (10%), and catechin (8%). The total polyphenol contents of black and green teas are similar. However, this similar content consists of different types of flavonoids depending on the oxidation that occurs during the process (Stangl et al., 2006). When a cup of tea prepared with a liter of water and 10 g of tea is taken, this tea contains approximately 300 mg of solid matter. While 30-40% of this solid matter is catechin, 3-6% is caffeine (Khan & Mukhtar, 2007).

The studies have demonstrated that tea has various pharmacological effects such as antioxidative, anti-inflammatory, antimutagenic, anticarcinogenic, antiangiogenic, apoptotic, antiobesity, hypocholesterolemic, antiatherosclerotic, antidiabetic, antibacterial, antiviral, and anti-aging effects (Çelik, 2006).

It is observed that green tea, the effect of which was investigated in our study, is consumed in different forms in the market. They are in the form of powdered tea, leaf tea, tea bags. Unlike these forms, green tea flour, which started to be produced in our country in 2016, constitutes the main theme of our study. Tea flour is the finely ground powder of specially grown tea leaves (*Camellia sinensis*). Tea plants are grown under 90% shade for flour production, and then the leaves are ground using stone mills. On the other hand, for green tea production, tea is ground under shade less sunlight, and the dried leaves are extracted with water to prepare green tea (Weiss & Anderton, 2003). L-theanine, epigallocatechin gallate (EGCG), caffeine, vitamins, and

amino acids are the main components of *Camellia sinensis* and have been reported both in vitro and in humans. Each component has useful functions, such as the suppression of tumor growth and mood-enhancing effects (de Souza et al., 2019; Higashiyama et al., 2011; Schröder et al., 2019; Smith, 2002). Matcha tea powder, which contains all the components of *Camellia sinensis* leaves, is expected to have effects such as improving blood flow and improving mood and various physiological properties such as skin whitening in humans (Dietz, Dekker & Piqueras-Fiszman, 2017). Furthermore, green tea flour is believed to have more beneficial physiological effects than the hot water extract of green tea. However, there are only a few scientific studies evaluating the effects of tea flour itself (Bonucelli, Sotgia & Lisanti, 2018; Unno et al., 2018).

The model organism we used in the study was *D. melanogaster*. *D. melanogaster,* which was first used in experimental studies by Thomas Morgan in 1911, has many advantages in terms of use (Gui & Grant, 2008).

These advantages are as follows:

• It is a eukaryotic organism with a short generation time (10 days at approximately 25 °C and 40-60% relative humidity.)

• Since they are small organisms, they are very easy and economical to produce in large numbers in culture media in the laboratory.

• They are holometabolic creatures. Namely, they develop by complete metamorphosis.

• They have a wide variety of genetically controllable morphological characters and mutant strains.

• The salivary gland cells of its larvae contain easily recognizable giant chromosomes. They are ideal structures for cytogenetic studies. They allow for chromosomal maps and chromosomal function analyses.

• Many substances that are carcinogenic to humans also show positive results in *Drosophila* tests. Furthermore, there is also no need for metabolic activation for testing promutagens and procarcinogens. As is known, many compounds do not have a direct mutagenic or carcinogenic effect. These compounds can be converted to carcinogens or mutagens in mammalian metabolism. Such compounds are called promutagens or procarcinogens (Hoffman, 2011).

The aim of the study is to evaluate the effect of *D. melanogaster* on egg production by comparing different forms of green tea with green tea flour.

2. Material and Methods

In this study, the Oregon (R) wild type of *Drosophila melanogaster* was used for egg yield. The *Drosophila melanogaster* Oregon R strain is a wild-type strain with normal round, red eyes, and no mutant character (Figure 2.1). *Drosophila* stock cultures are kept alive in bottles containing standard *Drosophila* medium (SDM) in hot cabins with heating and cooling systems with continuous dark conditions at 40%-60% relative humidity and 25±1°C temperature (Figure 2.2).

Figure 2.1. Male (♂) (right) and female (♀) (left) individuals of the Oregon stock of *Drosophila melanogaster*

Figure 2.2. *Drosophila melanogaster* (SDM) stock cultures

During our studies, the instant medium was used in the storage, reproduction, and crossing of *Drosophila* cultures. The instant medium is prepared using 1.5 g of instant medium and 5 ml of distilled water in 50 ml falcon tubes (Figure 2.3).

Figure 2.3. *Drosophila melanogaster* (instant medium) stock cultures

The green tea leave, powdered green tea, and tea flour used in the study were obtained from a private company serving in Çayeli district of Rize located in the Black Sea Region of Turkey. The tea obtained is the product of the same region, and the difference between the teas is due to the way the tea is processed (Figure 2.4).

Figure 2.4. Different forms of 5 g green tea used in the study

The brewing process was carried out by taking two grams of dry tea, boiling it and placing it in 200 ml of distilled water at an average of 98 ºC and resting it for 5 minutes at room temperature (25 °C) with the mouth closed (Cavlak & Yağmur, 2016) (Figure 2.5).

Figure 2.5. Weighing, brewing, and stocking of teas used in the study

In our study, the 3rd-stage larvae obtained by crossing Oregon male (♂) and female (♀) individuals were added to the prepared medium containing Drosophila tea extract, 100 pieces each (Figure 2.6).

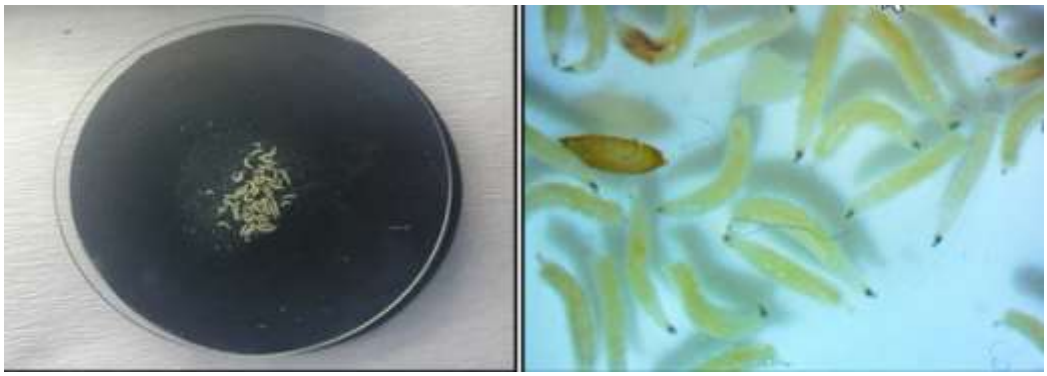

Figure 2.6. Larvae added to *Drosophila* instant medium

The female flies obtained in this way were placed as 1♀ X 3♂ in Petri dishes prepared to determine egg yield (Figure 2.7). Results were presented as mean ± standard deviation (SD) or percentages, when applicable. To be able to determine the statistical significance of the results, Duncan's one-way range test was applied. Data were analyzed using SPSS (ver. 15 for Windows™) software.

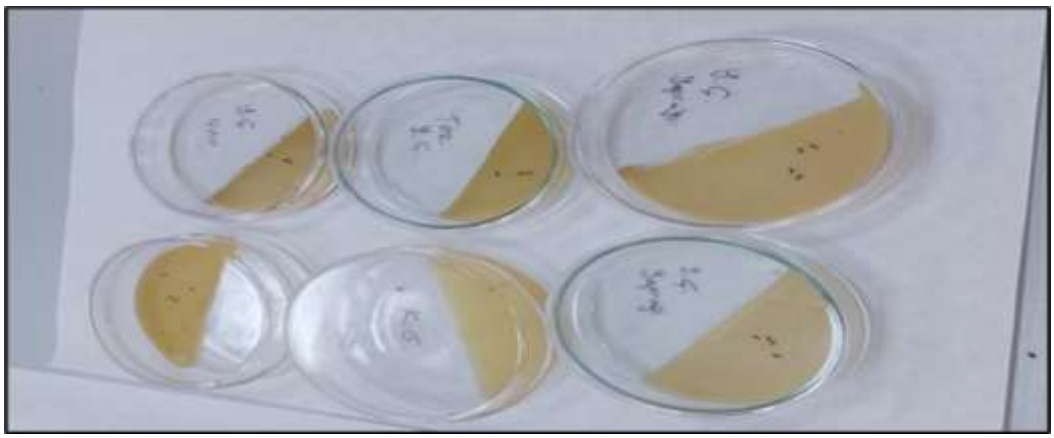

Figure 2.7. Petri dishes placed with *Drosophila* as 1♀ X 3♂

3. Results

The egg yield of the flies in the standard medium prepared in Petri dishes to examine the egg yield was counted under the stereomicroscope. In our study, the survival percentages of *Drosophila melanogaster* fed with different forms of green tea were first examined, and the number of individuals that transformed from larva to pupa and from pupa to adult (Figure 3.1) was also calculated (Table 3.1)

Figure 3.1. Determination of the metamorphosis rates of *Drosophila melanogaster* larvae fed with different forms of green tea

As is seen in Table 3.1, it was determined that green tea forms had no toxic effect at any rate. When the survival percentages were examined, it was observed that the highest survival percentage was 0.01mg/ml in all tea varieties.

Table 3.1. Transformation from larva to pupa, pupa to adult, and survival percentage in *Drosophila melanogaster* at different concentrations of three different forms of green tea

		Number of larvae	**Transformation from larva to pupa**	**Transformation from pupa to adult**	**Survival percentage**
Control Group		100	94	94	93%
Green Tea Flour	0.01mg/ml	100	98	97	97%
	0.005mg/ml	100	95	95	95%
	0.0025mg/ml	100	95	94	94%
Green Tea Leaf	0.01mg/ml	100	95	94	94%
	0.005mg/ml	100	94	94	94%
	0.0025mg/ml	100	92	91	91%
Powdered Green Tea	0.01mg/ml	100	93	92	92%
	0.005mg/ml	100	93	92	92%
	0.0025mg/ml	100	90	90	90%

Therefore, this ratio was also used in the egg yield study. The eggs of female individuals kept alive in media containing different forms of green tea for 3 days were counted daily, and this process was repeated for three days (Figure 3.2) (Table 3.2).

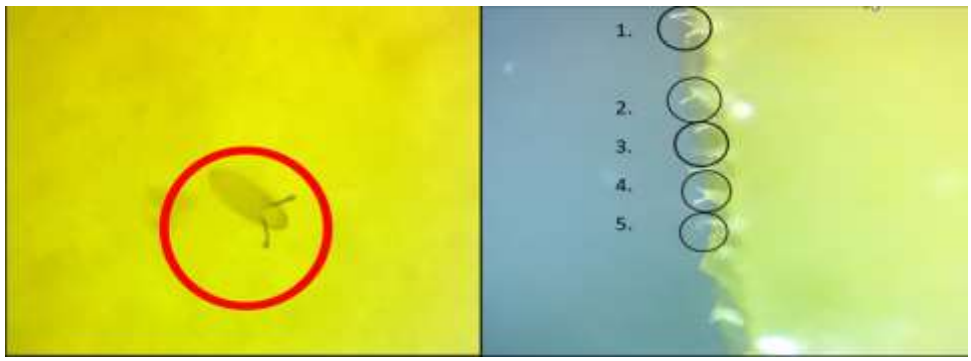

Figure 3.2. Egg counting process in the media of the control group and teas

Table 3.2. Average data obtained as a result of 3-day egg count of *Drosophila melanogaster*

Teas (0.01mg/mL)	1st-day egg yield	2nd-day egg yield	3rd-day egg yield	Average ± S.E
Control Group	21	23	19	21±1.15[a]
Green Tea Flour	36	32	34	34±1.15[c]
Green Tea Leaf	26	24	24	24.6±0.66[b]
Powdered Green Tea	25	24	24	24.3±0.33[b]

SE: Standard Error; Statistical evaluations of the difference between the groups were made within the group. Values shown with different letters in the same column are significant at the level of $p<0.05$.

Female individuals of the Oregon strain of *Drosophila melanogaster* were used in the egg yield study. Male and female individuals of the Oregon (wild type) strain of *Drosophila melanogaster* were crossed to obtain female individuals of the same age. Unmated female individuals obtained from here were used in the egg yield study. The female individuals were fed chronically in media containing 0.01mg/ml of tea.

4. Discussion and Conclusion

In the study, the effect of three different forms of green tea on survival percentage was first investigated. It was determined that all three tea forms generally revealed close results compared to the control group. It was determined that the larvae fed with tea flour extract by 0.01 mg/ml had a survival percentage of 97%. It was determined that the larvae grown in the medium prepared with green tea leaf extract by 0.01 mg/ml had a survival percentage of 94%. A survival percentage of 92% was found in the larvae grown in media containing 0.01 mg/ml powdered green tea extract. When the survival percentage study was examined, it was observed that all three tea forms did not have any toxic effects. When Table 3.1 was examined, it was observed that 0.01 mg/ml was the highest survival percentage in three different concentrations used.

Of these three different concentrations, 0.01 mg/ml was preferred in the egg yield study. The egg count was performed under the stereomicroscope in the media prepared in Petri dishes to determine the egg yield of female individuals of the same age.

The data obtained in our study showed that the use of green tea in different forms had an effect on egg yield in female individuals. While the average number of eggs obtained as a result of applying powdered green tea to female *Drosophila melanogaster* individuals was 24.3, the average number of eggs obtained as a result of applying green tea leaves was 24.6. The number of eggs obtained as a result of the application of green tea flour was 34. In accordance with these results, it was observed that green tea flour increased egg yield. It was determined that all three experimental groups had higher egg yield compared to the control group.

All teas except for green tea flour are extracted (brewed) in hot water, and this extract is drunk. In this way, only the substances that pass from tea to hot water are taken into the body during consumption. However, in green tea flour, the leaf is consumed as a whole because the leaf is turned into powder. Therefore, not only the bioactive substances that can be extracted into the water but also all bioactive components are taken into the body. A study conducted by Wang et al. (2000) determined that more EGCG (Epigallocatechin gallate) could be taken with the consumption of green tea flour than other types of green tea. Tea flour, which is generally known for its ability to accelerate metabolism and increase lipolysis, is a gluten-free flour, and due to L-theanine it contains, it increases the ability to concentrate, supports brain health, and is an important magnesium store. In the studies, it was also revealed to help treat cholesterol, blood pressure, atherosclerosis, and muscle diseases (https://ikedamatcha.com/blogs/tea-news/gluten-free-matcha).

Furthermore, green tea flour is a healthy product since it is a food additive rich in natural and bioactive components, and it is rich in vitamins B1, B2, and C. Green tea powder, which is also rich in minerals, is a source of manganese and fluorine. There is a very limited number of studies that directly examine the effect of green tea flour on health. However, it has been reported that green tea has antioxidant, anti-inflammatory, antimutagenic, anticarcinogenic, antiangiogenic, apoptotic, anti-obesity, hypolipidemic (lowering cholesterol), antiarteriosclerotic (preventing atherosclerosis), antidiabetic, antibacterial, antiviral, and anti-aging effects and that these effects are generally caused by the catechins in the composition of green tea (Koo and Cho 2004). It is clear that green tea flour, which is even richer in catechins, has the same functional effects.

5. Recommendations

The green tea we used in our study is used in many forms around the world. The use of tea flour, which is one of the forms of green tea, is very common, especially abroad. However, it is not known enough in our country. This product should be promoted more with the studies to be conducted. Due to the high caffeine content of different types of tea, excessive consumption may lead to some problems in egg yield. As it was observed in the study, the fact that the powder form of green tea increases egg yield may pave the way for more frequent and safe consumption of this product, together with other studies to be conducted. Furthermore, since it is gluten-free, we consider that it can be used instead of gluten-containing flour used to produce carbohydrate-based foods, especially for patients with gluten sensitivity.

References

Bonuccelli, G., Sotgia, F., Lisanti, M. P. (2018). Matcha green tea (MGT) inhibits the propagation of cancer stem cells (CSCs), by targeting mitochondrial metabolism, glycolysis and multiple cells signalling pathways. *Aging (Albany NY),* 10(8), 1867.

Cavlak, S., and Yağmur, C. (2016). Bazı Poşet Çayların Toplam Fenolik Madde Ve Antioksidan Aktivitelerinin Belirlenmesi [Determination of Total Phenolic Substance and Antioxidant Activities of Some Tea Bags].

Çelik, F. (2006). Çay (*Camellia sinensis*); İçeriği, Sağlık Üzerindeki Koruyucu Etkisi ve Önerilen Tüketimi [Tea (*Camellia sinensis*); Content, Protective Effect on Health, and Recommended Consumption]. Turkiye Klinikleri *Journal of Medical Science*, 26, 642-648.

De Souza, C. P., Gambeta, E., Stern, C. A. J., Zanoveli, J. M. (2019). Posttraumatic stress disorder-type behaviors in streptozotocin-induced diabetic rats can be prevented by prolonged treatment with vitamin E. *Behavioural brain research*, 359, 749-754.

Dietz, C., Dekker, M., Piqueras-Fiszman, B. (2017). An intervention study on the effect of matcha tea, in drink and snack bar formats, on mood and cognitive performance. *Food research international*, 99, 72-83.

Gonzalez de Mejia, E., Ramirez-Mares, M.C., Puangpraphant, S. (2009). Bioactive components of tea: Cancer, inflammation and behavior. *Brain, Behavior, and Immunity* 23(6), 721-731.

Gui, Y., Grant, A. (2008). Join effects of density dependent and toxicant exposure on *Drosophila melanogaster* populations, *Ecotoxicology and Environmental Safety*, 70, 236-243.

Higashiyama, A., Htay, H. H., Ozeki, M., Juneja, L. R., & Kapoor, M. P. (2011). Effects of L-theanine on attention and reaction time response. *Journal of Functional Foods*, 3(3), 171-178.

Hoffman, G.R. (2011). Casarett and Doull's Toxicology the Basic Science of Poisons, Pergaman Press, New York, 201-217.

https://ikedamatcha.com/blogs/tea-news/gluten-free-matcha

Jayabalan, R., Subathradevi, P., Marimuthu, S., Sathishkumar, M., Swaminathan, K. (2008). Changes in free-radical scavenging ability of kombucha tea during fermentation. *Food Chemistry*, 109, 227-234.

Khan, N., Mukhtar, H. (2007). Tea polyphenols for health promotion. *Life Science*, 81, 519-533.

Koo, M.W.L. and Cho, C.H. (2004). Pharmacological Effects of Green Tea on the Gastrointestinal System. Eur. *J Pharm*., 500:177-185.

L., Marahrens, P., Koch, J. G., Heidegger, H., Vilsmeier, T., Phan-Brehm, T., Richter, D. U. (2019). Effects of green tea, matcha tea and their components epigallocatechin gallate and quercetin on MCF-7 and MDA-MB-231 breast carcinoma cells. *Oncology Reports*, 41(1), 387–396.https://doi.org/10.3892/or.2018.6789.

Leung, A. Y., Foster, S. (1996). Encyclopedia of common natural ingredients used in food, drugs, and cosmetics (2nd ed.). John Wiley and Sons Inc., pp. 489-491, New York. Schröder, of pu-erh tea on LDL oxidation and nitric oxide generation in macrophage cells. *Food Science and Technology*, 41, 1122-1132.

Schwarz, B., Bischof, H.P., Kunze, M. (1994). Coffee, tea and lifestyle. *Preventive Medicine*, 23, 377-384.

Smith, A. (2002). Effects of caffeine on human behavior. *Food and Chemical Toxicology*, 40(9), 1243–1255. https://doi.org/10.1016/S0278-6915(02)00096-0.

Stangl, V., Lorenz, M., Stangl, K. (2006). The role of tea and tea flavonoids in cardiovascular health. *Molecular Nutrition & Food Research*, 50, 218-228.

Wang, B.S., Yu, H.M., Chang, L.W., Yen, W.J. and Duh, P.D. (2008). Protective effects

Wang, D. F., Li, J., Wang, C. H., Zhao, G. W., Jin, Y., Chen, D. D., & Ye, S. (2000). Study on the component and immune activity of polysaccharides from tea. *Journal of Tea Science*, 20, 45–50.

Weisburger J.H., Chung, F.L., (2002). Mechanisms of chronic disease causation by nutritional factors and tobacco products and their prevention by tea polyphenols. *Food Chemical Toxicology*, 40, 1145-1154.

Weiss, D. J., & Anderton, C. R. (2003). Determination of catechins in matcha green tea by micellar electrokinetic chromatography. *Journal of Chromatography A*, 1011(1-2), 173-180.

Yang, C.S., Landau, J.M. (2000). Effects of tea consumption on nutrition and health. *Journal of Nutrition*, 130, 2409-2412.