

# Controlling of Auto Oxidation Process of Soft Dough Biscuits Using Flavonoids Extracted from Green Tea (*Camellia sinensis*)

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**Abstract:** Since consumer attitude towards the artificial anti oxidant used in controlling of auto oxidation of fat in food products is gradually waning, finding out of alternative plant base anti oxidant sources for food products are important. Hence, aim of this study is to explore the possibility of using flavonoids, extracted from green tea in controlling of auto oxidation process in biscuits. 300g of green tea at 3.0% moisture content was taken and ground to get 150 micron particles. 25g of ground tea was subjected to decoction process to extract flavonoids. Since preliminary test results revealed that flavonoids concentration beyond 500ppm causes to disturb the taste profile of biscuits, three treatments of biscuits were prepared using 200ppm, 300ppm and 400ppm flavonoids to a standard biscuits recipe along with a control treatment without incorporating anti oxidant. Biscuits obtained from four treatments were stored under normal atmospheric condition in triple laminate pouches for a period of 6 months. Samples were drawn from each treatment monthly including just after preparation too to determine changes occurrence on peroxide value, free fatty acid level and organoleptic properties pertaining to five sensory stimuli such namely, aroma, crunchiness, texture and over all acceptability. All treatments were replicated thrice and results obtained from 4 treatments were finally compared with a commercial sample too. Results revealed that peroxide value of commercial biscuits, 200ppm, 300ppm and 400ppm flavonoids incorporated biscuits and biscuits in control treatment were increased from the initial value of 1.5 to 2.1, 2.9, 3.0, 3.5 and 10.5meq respectively during the period of 6 months. However, peroxide value of all treatments was remained below the standard value of SLSI (Sri Lanka Standards Institution) 10meq except the control treatment. In the case of free fatty acid level, which was also increased from the initial value of 0.25% to 0.4%, 0.6%, 0.65%, 0.8% and 1.6% in the same treatment order of above during 6 months. Free fatty acid level of all treatments was also remained below the standard value of SLSI (1.0%) except the control treatment. Sensory evaluation revealed that there was no significant difference between 300ppm and 400ppm flavonoids incorporated biscuits samples and the commercial product. Therefore, incorporation of 400ppm of flavonoids extracted from green tea is a viable option in controlling of auto oxidation process of soft dough biscuits.

**Keywords:** Flvonoids, soft dough biscuits, green tea, auto oxidation, peroxide value, free fatty acid, decoction or hot water extraction

## 1. Introduction

Biscuit is a delicious food product that usually caters to all segments in the social cross profile of the society irrespective of their social status; is generally made out of soft wheat, sugar, Shortening, leavening agent, water and other essential micro constituents. Since biscuit is a hygroscopic food product, that generally tends to absorb water vapor from surrounding environment and susceptible for fat degradation process by auto oxidation (Duncan, 2003). Therefore, the producers deliberately compelled to use antioxidants along with a high moisture barrier packing material to combat fat degradation process in order protect their product in the market. There are three types of antioxidants available in the commercial environment. They are natural, nature identical and artificial antioxidants. The antioxidants most frequently used are synthetic phenols such as butylated hydroxy toluene (BHT), butylated hydroxyl anisole (BHA) and propyl gallate. However, safety of these synthetic antioxidants and preservatives has been questioned due to toxicity, liver damage and carcinogenicity. Therefore using of safer antioxidants from natural sources is of interested because, consumers believe that natural antioxidants caused to least harmful effect on human health (Nandhitha. et al., 2009 and Erwin et al., 2004). Moreover, health conscious consumer may willfully tend to purchase food products formulated with natural antioxidants. Hence, aim of this study is to explore the possibility of using natural antioxidant (polyphenoids) extracted from green tea (*Camellia sinensis*) in controlling of auto oxidation process of soft dough biscuits.

## 2. Materials and Method

CTC (cutting, tearing and curling) green tea was purchased from a super market and moisture content of which was measured using laboratory moisture analyzer (Shimadzu MOC63u). All chemicals were used in this experiments were also of analytical grade.

### 2.1 Decoction or hot water extraction of flavonoids

The method described by Dominique and Qian 2010, was followed in extracting of flavonoids from green tea. Therein, three hundred grams of green tea at 3.0% moisture content were taken and ground to get particle size 150 micron using laboratory grinder and Endecott sieve shaker. Thereafter, 25g of ground tea at 150 micron was taken and subjected to flavonoids extraction process by keeping it in 750 ml of distilled water at 77°C temperature for 30 minutes in a water bath. The extract was cooled to room temperature (28°C) and filtered using Whatman No.40 filter paper under vacuum. Filtration was taken and further concentrated by using rotary evaporator until powdered form of flavonoids was obtained. Finally, powdered form of flavonoids were packed in triple laminated pouches and stored at 4 °C for subsequent use of the study.

### 2.2 Preparation of soft dough biscuits with flavonoids

Flavonoids obtained from rotary dryer in powdered form were incorporated into 3 treatments of a standard biscuits recipe which contained 592g of shortening and 3 levels of

flavonoids at 200ppm, 300ppm and 400ppm separately. Thereafter, three stage mixing was followed by initially mixing shortening (596g), powdered sugar (572g), cane syrup (50g), malt extract (35.8g), lecithin (12g) and green tea flavonoids using laboratory mixer for 3 minutes. Two kilo grams of wheat flour was added into the tub of mixer and mixed for further 3 minutes. 3.6g, 14.2g and 17.8g of sodium bicarbonate, ammonium carbonate and salt respectively were dissolved in 600ml of water and the solution was added to the mixer and mixed for 1 minute at slow mixing speed. The resultant soft dough was allowed to rest for 30minutes and raw biscuits thereafter were made using rotary molding cutter. Raw biscuits were baked in a hot air oven until moisture content reached to  $3.5 \pm 0.5$ . Baked biscuits were packed in triple laminate pouches and stored in normal atmospheric condition (28-30°C and RH 75-80%) for a period of 6 months. A control treatment was also prepared without using any anti oxidant and finally all treatments were compared with a commercial sample too. All treatments were replicated thrice.

### 2.3 Determination of peroxide value, Free Fatty acid level and organoleptic properties of biscuits

Samples were drawn from each treatment monthly including just after preparation too to determine changes occurrence

on peroxide value (AOAC method 41.1.13), free fatty acid level (AOAC method 41.1.21) and organoleptic properties pertaining to 5 sensory stimuli such as taste, aroma, crunchiness, texture and over all acceptability. Data obtained for peroxide value and free fatty acid levels as well as from the sensory evaluation pertaining to 5 point unipolar hedonic scale with respect to 30 numbers of respondents were analyzed statistically using parametric and non parametric (Kruskall-Wallis) ANOVA.

## 3. Results and Discussion

### 3.1 Development of peroxide value of biscuits

Major scope of this study is to determine efficacy of tea flavonoids in controlling of auto oxidation process in soft dough biscuits. Therefore, detection of peroxide value is very important as which gives the initial evidence of rancidity in unsaturated fats and oils. So also it gives a measure of the extent to which an oil sample has undergone primary oxidation. Hence, development of peroxide value of biscuits prepared from three levels of green tea flavonoids along with the control and commercial product are showing in figure 1.

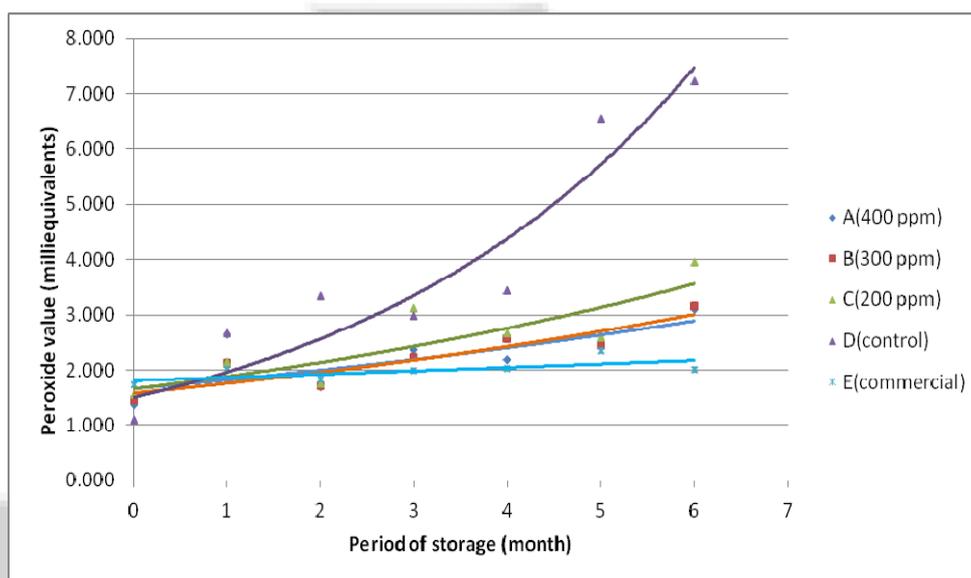


Figure 1: Development of peroxide value of biscuits

The graphs in figure 1 clearly indicate that peroxide value of control treatment is steadily increasing over the period of 6 months. However increment of peroxide value of biscuits prepared with green tea flavonoids as well as commercial product are slowly progressing over the period. Moreover, best treatments comparatively commercial product were 300ppm and 400ppm flavonoids incorporated samples because, peroxide values of these two treatments were increased by about 1.5meq during 6 months period of storage. Therefore, flavonoids extracted from green tea can be used as a best source in controlling of autoxidation process of biscuits as well as a good natural antioxidant for health consciousness consumer. Nevertheless, peroxide value of all of these treatments remained below the stipulated value of SLSI (SLS 251 Specification for biscuits - second Edition 2010) 6meq even after 6 months of shelf

life; except the control treatment, which surpass the value 6meq when age of the biscuits reached to 5 months.

Reason for this phenomenon is that most of the polyphenols in green tea are flavonoids known as catechins. Catechins constitute about 25% of the dry weight of the tea leaf (Yuenyuan et al. 2005). The major catechins are: (-)-epigallocatechin-3-gallate (EGCG), (-) – epigallocatechin (EGC), (-)-epicatechin-3-gallate (ECG) and (-)-epicatechin (EC). The previous studies have shown that total Phenolic content (TPC) of green tea was higher than that of black and oolong tea due to reduction of catechins during fermentation and that also affected radical scavenging activity of the tea (Yokozawa et al., 1998) and confirmed by Atouiet al. (2005). Therefore, flavonoids in green tea (Catachins) can act as a strong oxygen scavenging agent or an antioxidant in

a substrate. Moreover, during green tea production, the main polyphenols (the catechins) remain relatively intact with the process, this is due to the enzymes, which catalyze their oxidative polymerization, are deactivated by heat treatment soon after plucking. Data obtained from this study were further analyzed statistically using parametric ANOVA and results revealed that calculated F value (5.66) for treatments is higher than the table value ( $F_{24}^4 2.78$ ). Therefore there is a significant difference between the treatments on peroxide values. Further analysis of results was done to determine whether there is a significant difference between the treatments pertaining to the test method "least significant difference" (LSD). Results revealed that there is no significant difference between tea flavonoids incorporated treatments and commercial sample except the control treatment. Hence, flavonoids extracted from green tea are

capable to control progressing of peroxide value of soft dough biscuits during shelf life.

### 3.2 Development of Free Fatty Acid level of biscuits

When oils are under gone oxidation process, variety of chemical compounds such as peroxides, aldehydes and free fatty acids (FFA) are created. Hence FFA level in an oil can be used as a yard stick to measure degree of degradation. While considering this fact, development of free fatty acids in three levels of flavonoids incorporated biscuit samples along with the control and commercial product are given in figure 2.

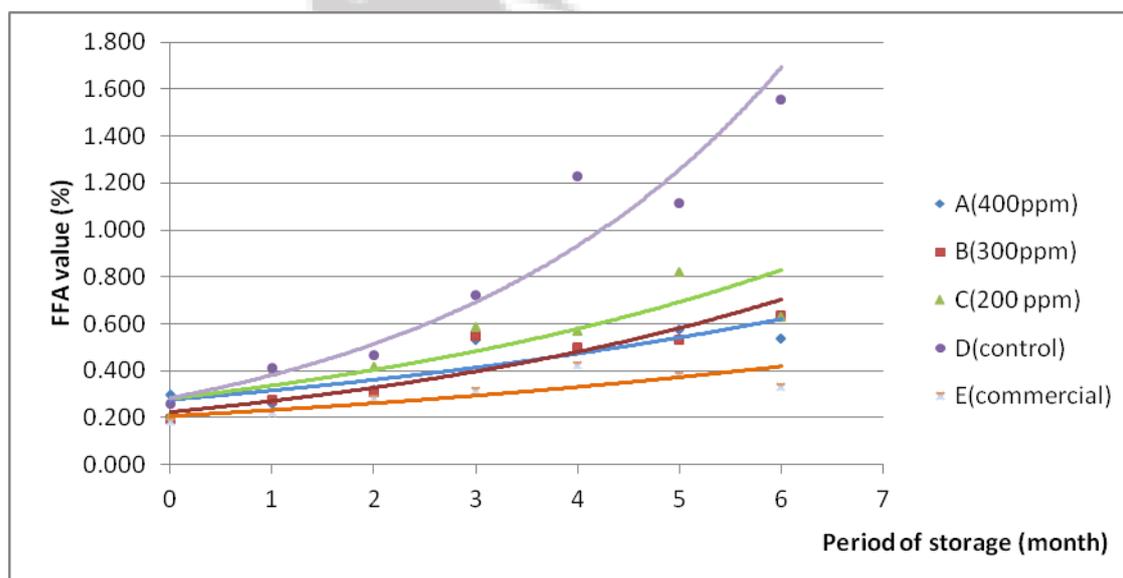


Figure 2: Development of Free Fatty Acid level of biscuits during six months of storage

The graphs in figure 2 indicate that free fatty acid level of the control treatment is steadily increasing because, there is no oxygen scavenging agent in the substrate comparatively commercial and flavonoids incorporated biscuits. Since, flavonoids extracted from green tea enriched with catechins and catechins having strong affinity with oxygen, biscuits prepared with flavonoids is safe for fat degradation process. Therefore, increment of free fatty acid levels in flavonoids treated biscuits were progressing slowly however at a descending manner with the increment of flavonoids level. In the case of commercial biscuit, as which has been treated with an artificial antioxidant, development of FFA was taking place at a slow pace. Nevertheless, development of FFA in all treatments except the control treatment remained below the stipulated value of SLS 1.0% (SLS 251 Specification for biscuits - second Edition 2010) during the testing period of six months. Hence, flavonoids extracted from green tea are capable to control formation of free fatty acids in the soft dough biscuits.

Data obtained from the study were further analyzed statistically using parametric ANOVA and results revealed that calculated F value (10.6) for treatments is higher than the table value ( $F_{24}^4 2.78$ ). Therefore there is a significant difference between the treatments on development of free fatty acid levels. Further analysis of results pertaining to the

least significant difference (LSD) revealed that there is no significant difference between tea flavonoids incorporated treatments and the commercial sample except the control as well as 200ppm flavonoids treated samples. Hence, 300ppm and 400ppm levels of flavonoids extracted from green tea are capable to control development of free fatty acid level in soft dough biscuits.

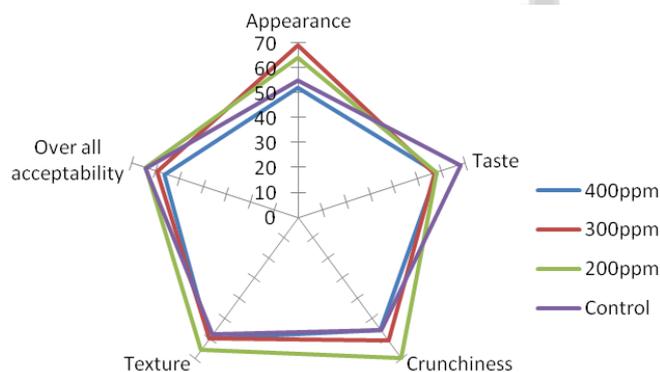
### 3.3 Organoleptic properties of Biscuits prepared with green tea flavonoids

Organoleptic properties of biscuits prepared with 200ppm, 300ppm and 400ppm green tea flavonoid extracts were compared with the control treatment pertaining to the five sensory stimuli and results were analyzed according to Kruskal-Wallis non parametric ANOVA for just after and six months after preparation.

### 3.4 Sensory properties of biscuits just after preparation

The non parametric data obtained from the sensory evaluation were analyzed statistically and result revealed that calculated H value pertaining to the five sensory stimuli such as appearance, taste, crunchiness, texture and over all acceptability 4.68, 2.16, 3.25, 0.89 and 1.22 respectively were lower than the table value ( $X_{0.9}^2 = 7.82$ ). Therefore,

there is no significant different between the sensory stimulus of each treatment at 0.05 significant level. Reason for this consequence is sensory organs of the respondents are not sharp enough to discriminate sensory profile of each treatment enrich with such a low amount of flavonoids, which is the only foreign constituent available in the standard recipe of the soft dough biscuits. To illustrate outcome of this conclusion further, sensory profiles pertaining to rank mean sum of each treatment were drawn and which are given in figure 3.



**Figure 3:** Sensory profiles of flavonoid incorporated biscuits along with the control treatment

Sensory profiles depicting in the figure 3 also indicating that there is no significant difference between the sensory stimuli of each treatment of soft dough biscuits just after preparation.

### 3.5 Sensory properties of biscuits after 6 months of preparation

Data obtained from the sensory evaluation again analyzed statistically and results revealed that there is no significant difference between treatments at 0.05 significant level; because calculated H values pertaining to the five sensory stimuli such as appearance, taste, crunchiness, texture and over all acceptability 1.09, 3.13, 0.80, 2.60 and 1.17 respectively were lower than the table value ( $X^2_{0.9} = 7.82$ ). Hence, flavonoids of green tea are incapable to disturb sensory profile of soft dough biscuits even after six month of shelf life.

## 4. Conclusion

Flavonoids extracted from green tea can be used as an important antioxidant source in controlling of autoxidation process of soft dough biscuits. The desirable level of flavonoids in controlling of fat oxidation process is in between 300 and 400ppm, because above 500ppm level is capable to disturb sensory profile of biscuits up to some extent. Moreover, this flavonoids level is capable to preserve organoleptic properties of biscuits for more than six months. Hence tea flavonoids are a better alternative to replace artificial antioxidants in biscuits and also it is good news for health consciousness consumers in the dynamic market.

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