

Antioxidant Activity and Preservative Effect of Black Tea and Oolong Tea Extracts in Homemade Cookies

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Abstract

The use of natural antioxidants in food preservation has gained increasing attention due to safety concerns and negative consumer perception associated with synthetic antioxidants. Synthetic preservatives such as BHA and BHT have been linked to potential toxic and carcinogenic effects when consumed long term. Consequently, growing demand for clean-label and health-oriented food products has driven interest in natural antioxidants, which offer effective oxidative protection with improved safety and nutritional benefits. Preservation using natural antioxidants in food has been receiving growing interest because of the safety of synthetic food preservatives. The study aimed to determine the possibility of *Camellia sinensis*, from black tea and oolong tea extract, being used as natural antioxidants and substitute preservatives in homemade cookies and to compare the performance of these extracts with a synthetic antioxidant, butylated hydroxyanisole (BHA). Black tea and oolong tea were used to purify antioxidants through the Soxhlet method with isopropanol as a solvent. These extracts were then analysed using various methods, which included the DPPH assay, total phenolic content (TPC), peroxide value (PV), and the antioxidant activity (AOA). The DPPH test showed that the free radical scavenging activity was the highest in BHA (93.01 %), then black tea extract (50.42 %), and oolong tea extract (6.05 %). TPC analysis revealed that black tea extract contained more phenolic compounds as compared to the oolong tea extract. Both tea extracts were found to be effective when used in homemade cookies in reducing lipid oxidation in 14 days of storage, with the control sample experiencing the most PV increase. The results of the AOA have shown that black tea and oolong tea extracts had a value of 48.05 % and 48.90 %, respectively, which was higher than BHA (21.59 %). These results indicate that even though BHA has a good in vitro antioxidant activity, the tea extracts are more effective in antioxidant protection in food systems. Overall, oolong tea extract and black tea extract have good perspectives of effective and safe natural food preservatives.

1. Introduction

The loss of product quality in fat-rich confectionery, especially cookies, is largely attributed to lipid oxidation in the fat, particularly since cookies are made with polyunsaturated fats and oils. The oxidation of lipids is caused

by the beginning of free radical chains, of which hydroperoxides are primary oxidation products. Afterward, these hydroperoxides, as unstable compounds, yield secondary oxidation products, which include aldehydes, ketenes, and organic acids. These compounds are the actual cause of the off-rancid flavours and odours, as well as the colour loss, texture deterioration, degradation in the nutritional quality of the food product, and noticeably shortened the shelf life and acceptability of the food product [1]. Furthermore, oxidized lipids cause sensory quality loss and are harmful to health by causing and/or producing cellular and genetic toxicity from prolonged consumption, further justifying the importance of the management of lipid oxidation in food products [2].

Within the food sector, there is considerable application of the synthetic antioxidants, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). These additives are used to reduce oxidative degradation. These compounds impact lipid oxidative deterioration by acting as chain-breaking antioxidants. By donating H atoms to the lipid radical, and thereby, acting as a chain-breaking antioxidant, they interrupt the advancement phase of lipid oxidation. Because of the additives' potentiveness and thermal stability, they are ideal for use in the baked food items. However, the long-term use of synthetic antioxidants and potential adverse health effects, such as liver damage and cancer, have been documented in studies and as a result, there are concerns over the toxicological evidence, and these concerns are growing [4]. As a result, the food industry is responding to these consumers and is now producing clean-label products using extracts from plants that also offer antioxidant benefits and prevent the deterioration of food products.

The presence of polyphenols with antioxidant properties, such as the capacity to quench free radicals, metal bonding, and preventing the peroxidation of lipids, has led to the increasing scholarly focus on plant-sourced natural antioxidants. Among tea leaves from *Camellia sinensis*, strong antioxidant qualities have earned it commercial recognition. Antioxidants in tea are shaped by the fermentation processes in the production of the tea. The black tea, with its full fermentation, contains notable theaflavins and thearubigins, products of the oxidation of catechin, which serve as the black tea's staple and heat-stable strong antioxidants, and the other notable sensory characteristics of the black tea. This activity of black tea makes it applicable in heat-processed food products like baked items [5].

Oolong tea is partly fermented and contains a mild fermentation of catechins and partly oxidized constituents of the flavins. This combined phenolic has been described to deliver effective radical scavenging, while it may well reduce the unwanted sensory effects when applied to the food matrices [6]. Consequently, oolong tea is a potential source of natural antioxidants to be used in bakeries. Although there has been some research on the possible health benefits of consuming tea, there has not been as much research on the health benefits of tea as a natural preservative, especially in cookie systems that are very prone to lipid oxidation. Further, the bulk of the research surrounding tea preservation has been with powdered tea and water tea extracts, which are purported to be less stable and have lower extraction efficiency. Soxhlet extraction has been theorized to have a higher recovery of numerous stronger double-bonded phenolic compounds, which have been theorized to increase antioxidant efficacy. There has not been much research on the usage of Soxhlet-extracted tea in liquid form in food products.

Therefore, the purpose of this study is to examine and compare the antioxidant properties of Soxhlet-extracted black tea and oolong tea as natural preservatives in homemade cookies, where BHA is used as a synthetic control. Antioxidant properties were estimated through in vitro testing, which looked at DPPH free radical scavenging, phenolic content, and the cookie matrix to track oxidative stability through the analysis of peroxide and free radical scavenging upon sequestration. This analysis provides a clearer understanding of tea extracts as preservatives in baked products that can replace synthetic preservatives.

2. Methodology

2.1 Materials

Dried black tea and oolong tea leaves (*Camellia sinensis*) were obtained from a local supplier. The synthetic antioxidant butylated hydroxyanisole (BHA) was used as a reference. Analytical-grade isopropanol, ethanol, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent, gallic acid, sodium carbonate, potassium iodide, sodium thiosulfate, chloroform, acetic acid, and starch indicator were used for analytical procedures. All reagents were of analytical grade.

2.2 Preparation of Tea Extracts

A total of 20 g of each tea sample was weighed using an analytical balance and ground into a coarse powder using a clean and dry grinder. This grinding process was done to increase the surface area and efficiency during extraction. The ground tea powder was then stored in labelled amber glass bottles and placed in a cool and dry place until use. Black tea and oolong tea extracts were extracted using the Soxhlet extraction method to obtain liquid extracts for food application. The powdered tea was then packed into a thimble and placed in the Soxhlet

chamber. A total of 200 mL of 70 % isopropanol (v/v) was used as the solvent and added to a 250 mL round-bottom flask. The Soxhlet extraction process was carried out at 70 °C for 6 hours, allowing repeated cycles of isopropanol to extract phenolic and antioxidant compounds effectively [8][9]. The isopropanol-based extract was cooled and then concentrated using a rotary evaporator at 40 °C under reduced pressure to remove the solvent. The resultant semi-solid residue was reconstituted with 10 mL of distilled water to get a standardized tea extract in liquid form. The extract was kept in an amber bottle at 4 °C to avoid degradation and was used for antioxidant and cookie applications.

2.3 Fourier Transform Infrared (FTIR) Analysis

Fourier Transform Infrared analysis was performed using attenuated total reflectance (ATR) mode to confirm the absence of residual solvent in the tea extracts. Spectra were recorded in the range of 4000–400 cm^{-1} with a resolution of 4 cm^{-1} . Functional groups corresponding to polyphenolic compounds were identified and compared with reference spectra [10].

2.4 Determination of Antioxidant Activity

2.4.1 2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Assay

The antioxidant activity of tea extracts and BHA was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical method based on a procedure modified from Gramza-Michałowska & Korczak (2023) [11]. DPPH solution was prepared by dissolving 0.1 mM DPPH in ethanol. A total of 1 ml of tea extract was mixed with 2 ml of DPPH solution and stirred gently. The solution was mixed gently, and the flask was covered with aluminium foil to protect the DPPH solution from the light. To enable the reaction between the antioxidant chemicals and the DPPH radicals, the mixture was incubated for 30 minutes at room temperature (around 25 °C) in a dark place. The absorbance was measured at a wavelength of 517 nm using a UV-Vis spectrophotometer. Controls were prepared by replacing the tea extract with 95 % ethanol. The following formula was used to determine the radical scavenging activity (RSA), also known as the percentage inhibition:

$$\text{DPPH Scavenging Activity (\%)} = \left(\frac{A_0 - A_1}{A_0} \right) \times 100 \quad (1)$$

Where,

A_0 = Absorbance of control (DPPH + ethanol)

A_1 = Absorbance of sample (DPPH + extract)

2.4.2 Total Phenolic Content (TPC)

Total phenolic content (TPC) was measured following the Folin–Ciocalteu assay method outlined by Shannon et al. [12]. Sodium carbonate solution (Na_2CO_3) 2mL was added to a test tube containing 100 μL of the corresponding tea sample. The resulting mixture was vortexed. After 2 minutes of vortexing, 100 μL of Folin–Ciocalteu reagent was added, which was mixed thoroughly. After 30 minutes of 25°C light-protected incubations, the reaction mixture went on to undergo absorbance measurements at 720 nm with a UV-Vis spectrophotometer. The reaction mixture was placed in a water bath set to 25°C for 30 minutes to help the reaction proceed at a faster rate before absorbance measurements were taken. Subsequently, the absorbance of the solution was recorded at 720 nm with a UV-Vis spectrophotometer.

A standard calibration curve was prepared from six differing concentrations of gallic acid. The quantity of total phenolic content was determined from the prepared gallic acid standard curve in the form of equivalent gallic acid per extract millilitre (mg GAE/L).

2.5 Preparation of Homemade Cookies

With a few adjustments from the methodology of Pędziwiatr et al. (2024) [13], black tea extract, oolong tea extract, and butylated hydroxyanisole (BHA) were added to handmade cookies. For all replicates in a run, a mixture of unsalted butter (2/3 cup) and granulated sugar (1/2 cup) was whipped in a stand mixer for a few minutes until a creamy, homogeneous butter mixture was formed. Yolk of 1 egg and 1/2 tsp of black tea extract, oolong tea extract, or BHA solution were added, and the mixture was blended for 1/min to achieve a solution blend. For the blank (control) cookies, no antioxidants were added to the mixture. Another bowl was used to sift the dry ingredients, which included wheat flour (2 1/2 cups), 1 tsp of baking powder, and 1/8 tsp of salt. These were then added to the wet mixture in parts to achieve a smooth, soft dough. The cookie dough was wrapped in

aluminium foil and placed in a refrigerator for 75 minutes at 4 degrees Celsius. The dough pieces were arranged on a baking tray lined with baking paper and baked in a preheated conventional oven at 180 °C for 15 minutes using upper and lower heating. The baked cookies were cooled at room temperature (25 °C) for 30 min and stored in airtight containers at room temperature for 14 days.

2.5.1 Peroxide Value (PV) Test

Peroxide value (PV) was determined to evaluate lipid oxidation in cookie samples during storage following the method with modifications [14]. Chloroform, acetic acid, saturated potassium iodide (KI), 0.01 N sodium thiosulfate, 1 % (w/v) starch solution, and distilled water were used.

Firstly, the 5.00 (±0.05) grams of sample were accurately weighed into a 250 ml glass-capped Erlenmeyer flask, and the weight was recorded to two decimal places. Next, 30 ml of acetic acid and chloroform solution were added using a measuring cylinder. Then, the flask was warmed carefully on the hotplate and swirled the flask until the sample was completely dissolved. After that, 0.5 ml of saturated potassium iodide solution was added to the flask using a 1 ml Mohr pipette. The flask was covered with a stopper and swirled for exactly one minute.

30 mL of distilled water was added using a measuring cylinder. Then, the flask was stoppered again and shaken vigorously to release iodine from the chloroform layer. The burette was filled with 0.1 N sodium thiosulfate solution for the titration process. If the solution started as a deep orange-red, the titration was carried out slowly while shaking until the colour became lighter. If the initial colour was bright yellow, the titration was continued without this step. 1 ml of starch solution was added to the solution as an indicator. The solution was titrated, then the addition was continued slowly until the greyish-blue colour in the aqueous layer completely disappeared. In fat-rich cookie samples, titration was continued until the bottom chloroform layer appeared milky white or cloudy. Finally, the volume of sodium thiosulfate used in the titration process was recorded accurately to two decimal places to calculate the peroxide value (PV) in meq/kg. The peroxide value can be calculated using the following equation:

$$\text{Peroxide value: } \frac{(S-B) \times N \text{ thiosulfate} \times 1000}{\text{weight of sample}} \quad (2)$$

Where,

S = Titration of sample

B = Titration of blank

2.5.2 Inhibition of Lipid Oxidation Analysis

The inhibition of lipid oxidation in cookie samples was evaluated using peroxide value (PV) measurements during storage. This method assessed the effectiveness by comparing the lipid oxidation degradation rate, expressed as peroxide value (PV), between the control cookies and cookies treated with black tea, oolong tea, and BHA. The level of antioxidant activity was measured by comparing the rate of lipid oxidation (peroxide value) in the control (without antioxidants) and in cookies with added antioxidants. The equation used to analyse the AOA was:

$$\text{AOA} = \frac{\text{Degradation rate of control} - \text{degradation rate of sample}}{\text{Degradation rate of control}} \times 100 \quad (4)$$

3. Results and Discussion

3.1 Fourier Transform Infrared Spectroscopy (FTIR) Analysis

Fourier Transform Infrared (FTIR) analysis was used to determine the primary functional groups in extracts of black tea and oolong tea. The presence of bioactive compounds, particularly phenolic compounds that makes the extract antioxidant. was confirmed with the help of FTIR spectra obtained. FTIR is an established method used to determine the oxidation-related changes in molecules and antioxidant-lipid. food systems interactions [15]. The maximum absorption of black tea extract and oolong tea extract is given. in Figures 1 and 2.

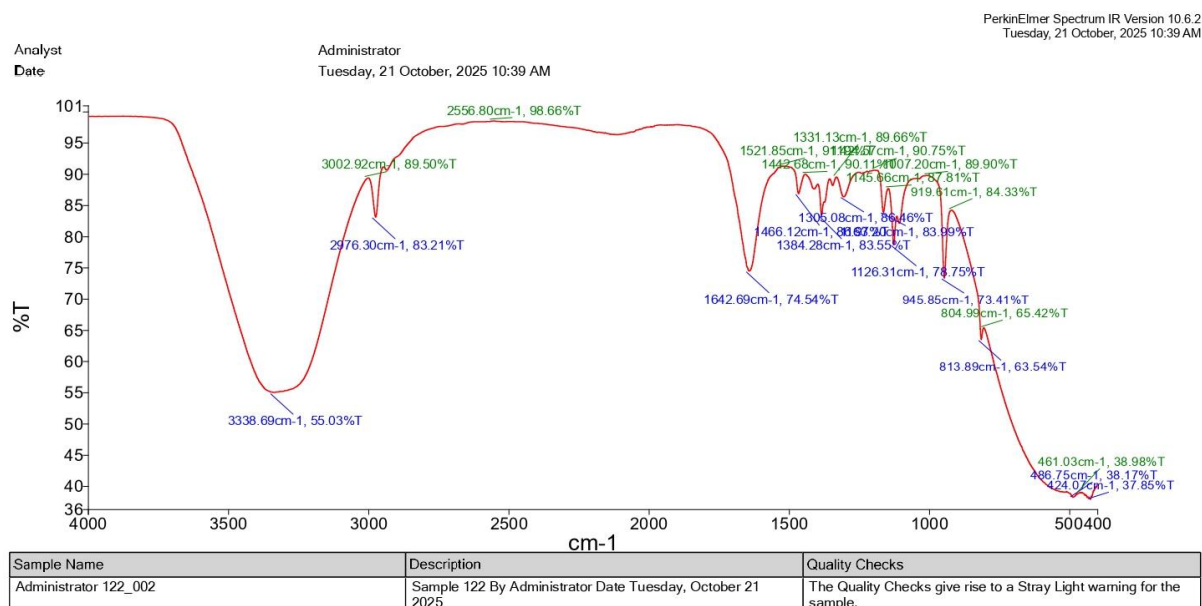


Fig. 1 FTIR spectrum of black tea extract

The FTIR spectrum of black tea extract (Fig. 1) had a broad absorption at around 3339 cm^{-1} that is associated with OH stretching frequencies of phenolic elements. Such a peak proves the existence of polyphenols, theaflavins, and thearubigins, which are the products of tea fermentation and cause the presence of hydrogen-donating antioxidant action [16]. The intermolecular hydrogen bonds are strong in this band, which means it is broad, and this helps in the stabilization of phenoxy radicals formed during oxidation reactions.

C=O stretching and aromatic C=C were attributed to an absorption band at 1643 cm^{-1} , which is usually linked to conjugated polyphenolic structures. This region is also sensitive to oxidation due to the potential formation of carbonyl compounds during the phenolic oxidation [15]. Nevertheless, the presence of such bands in the extracts indicates the presence of structurally stable oxidized polyphenols and not highly oxidized lipids.

Several characteristic peaks were observed in the $600\text{--}1500\text{ cm}^{-1}$ fingerprint region, which is highly informative for evaluating oxidation-related structural changes. Bands at $\sim 1466\text{ cm}^{-1}$ and $\sim 1384\text{ cm}^{-1}$ correspond to $-\text{CH}_2-$ and $-\text{CH}_3$ aliphatic groups, respectively. These bands have been linked to lipid-associated structures and interactions between antioxidants and lipid matrices in food products [17]. Additionally, absorption peaks at $\sim 1163\text{ cm}^{-1}$ and $\sim 1007\text{ cm}^{-1}$ were attributed to C–O stretching vibrations of phenols and flavonoids, which are key functional groups involved in metal ion chelation and inhibition of lipid peroxidation [18].

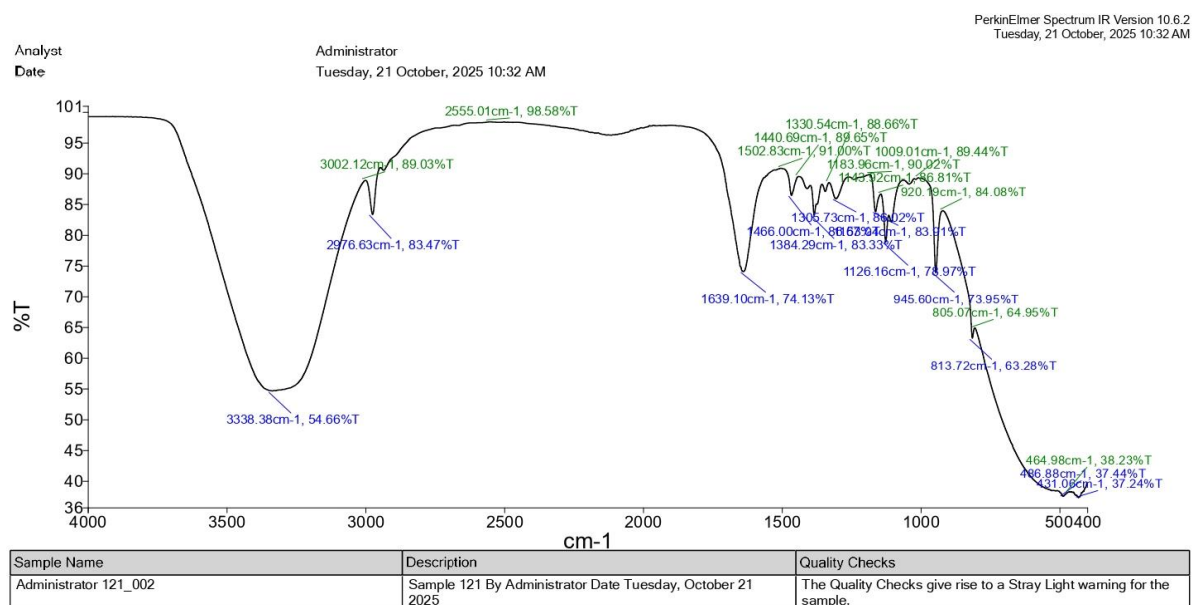


Fig. 2 FTIR spectrum of oolong tea extract

The oolong tea extract (Fig. 2) showed a comparable FTIR profile with some notable differences. A broad band at $\sim 3338\text{ cm}^{-1}$ was also observed, indicating the presence of hydroxyl-rich polyphenols, mainly catechins and partially oxidized phenolic compounds characteristic of semi-fermented tea [19,20]. The absorption band at $\sim 1639\text{ cm}^{-1}$ suggests conjugated aromatic structures with a slightly lower degree of oxidation compared to black tea, which is consistent with the processing method of oolong tea. Similar absorption features were present in the $600\text{--}1500\text{ cm}^{-1}$ region, including bands related to aliphatic and phenolic functional groups. The retention of these peaks indicates that oolong tea extract maintains antioxidant-related structures capable of interacting with lipid components and reducing oxidative degradation.

Overall, the FTIR analyses indicate that black and oolong tea extracts possess important functional groups such as hydroxyl, aromatic, and carbonyl groups, which are responsible for the antioxidative potential. The slight discrepancies in peak positions and intensities are a result of differences in the composition of the phenolics as well as the extent of oxidation of the two tea types. These FTIR findings are consistent with the observed reduction in peroxide values and antioxidant activity results, suggesting a strong association between the antioxidant-related functional groups and lipid oxidation inhibition.

3.2 DPPH Radical Scavenging Analysis

The next step after extracting the antioxidants involved testing using the DPPH radical scavenging assay. The antioxidant activity of BHA, black tea extract, and oolong tea extract, measured from the DPPH radical scavenging assay, is recorded in Table 1. The BHA sample had the greatest scavenging activity of $93.01 \pm 0.025\%$ which demonstrated its ability to donate hydrogen and is most likely the most effective synthetic antioxidant standard. Black tea extract had the next highest antioxidant activity at $50.42 \pm 0.017\%$ and oolong tea extract had the lowest at $6.05 \pm 0.737\%$.

Table 1 Analysis results showing the comparison of antioxidant activity in percentage of each sample, $n=3$

Sample	DPPH Scavenging Activity (% inhibition)
BHA	93.01 ± 0.025
Black tea	50.42 ± 0.017
Oolong tea	6.05 ± 0.737

The DPPH scavenging activity of BHA is exceptionally high due to its precise chemical structure and mechanism of action. As a specially designed synthetic phenolic antioxidant, BHA is readily transferred to or from hydrogen in a reaction mechanism, donating its hydrogen to a DPPH radical. BHA transfers a hydrogen atom to the radical, which stabilizes the radical and thus terminates the free radical chain reaction. The high inhibition percentage in the assay is due to the rapid reaction. The low standard deviation of ± 0.025 indicates the low variation, even distribution, and consistency of BHA, which maintains its role as a positive control antioxidant. The phenolic hydroxyl group and sterically hindered configuration account for the strong radical scavenging efficiency of BHA [21].

By comparison, black tea extract had a moderate DPPH scavenging activity of 50.42% , which is still substantial and suggests effective natural antioxidant compounds are present. Polyphenolic black tea's antioxidant potential within the DPPH assay is strongly affected by the fermentation process, however. Through full fermentation, the monomeric catechins are oxidized and polymerized through a series of enzymes to theaflavins (TFs) and thearubigins (TRs), which, although both at least somewhat maintain antioxidant potential, are larger in molecular structure. The structural complexity of these compounds increases, and the hydroxyl groups become less available, which can slow the reaction kinetics of DPPH radical scavenging. Consequently, black tea extract has a high total phenolic content but often still shows less DPPH scavenging activity than synthetic antioxidants or unfermented tea extract. This has been reported to be a result of polymerized phenolic compounds reacting less efficiently with the DPPH in radical scavenging single radical assays [19].

Among all samples, oolong tea extract showed the lowest value of DPPH inhibition, at $6.05 \pm 0.737\%$. Oolong tea falls into the category of semi-fermented tea, which means its polyphenolics comprise the less oxidized natural catechins, theaflavins, and intermediate theaflavins, which are only partially oxidized. Because of this heterogeneous composition, the Oolong tea sample would likely have less radical scavenging efficiency in the DPPH compared to the fully formulated synthetic antioxidants. Partially oxidized polyphenols have a reputation of being less effective hydrogen donors, which could have contributed to the relatively lower scavenging capacity in the DPPH assay, as is also suggested by the reference [19]. Furthermore, the relatively

high standard deviation observed in DPPH inhibition of the two tea extracts also suggests a greater fluctuation in their radical scavenging capacity due to a variable degree of oxidation of the tea leaves, efficiency of the extraction process, or inconsistent composition of the extract. The observed sequence in antioxidant DPPH inhibition in our study (BHA > black tea extract > oolong tea extract) indicates a marked difference in activity between synthetic and natural antioxidants, demonstrating fully characterized DPPH radical scavenging behaviour. The DPPH assay has a quick hydrogen-donating capacity to a single, stable radical systems. Therefore, the DPPH method does not fully portray the complex array of oxidation reactions occurring in real food systems.

A lower value of DPPH scavenging activity does not suggest a lack of antioxidant activity in lipid-oxidizing food matrices such as cookies. Lipid oxidation in food matrices is a slow process and includes several steps, such as initiation, propagation, and termination of radicals, as well as metal ions and oxygen interactions. Tea polyphenols have been said to have antioxidant effects via alternative mechanisms such as metal ion chelation, pro-oxidant enzyme action inhibition, lipid radical stabilization interfaces, and direct radical scavenging. While having lower DPPH values, tea polyphenols can confer antioxidant activity in food systems despite having lower DPPH values. So, whilst black and oolong tea extracts had lower antioxidant activity than BHA in the DPPH assay, this does not mean they are not potential natural preservatives for homemade cookies. Their impact in the subsequent peroxide value (PV) and antioxidant activity (AOA) analysis is anticipated to provide a better indication of their actual antioxidant activity in food systems.

3.3 Total Phenolic Content (TPC)

Total Phenolic Content (TPC) analysis was conducted to quantify the concentration of phenolic compounds present in BHA, black tea extract, and oolong tea extract. Phenolic compounds are key contributors to antioxidant activity and oxidative stability. The results of the TPC are presented in Table 2 and Fig. 3.

Table 2 Analysis results showing comparison of antioxidant activity in percentage of each sample, $n=3$

Sample	Total Phenolic Content (mg GAE/L)
BHA	1.901 ± 0.036
Black tea	4.282 ± 0.013
Oolong tea	3.926 ± 0.005

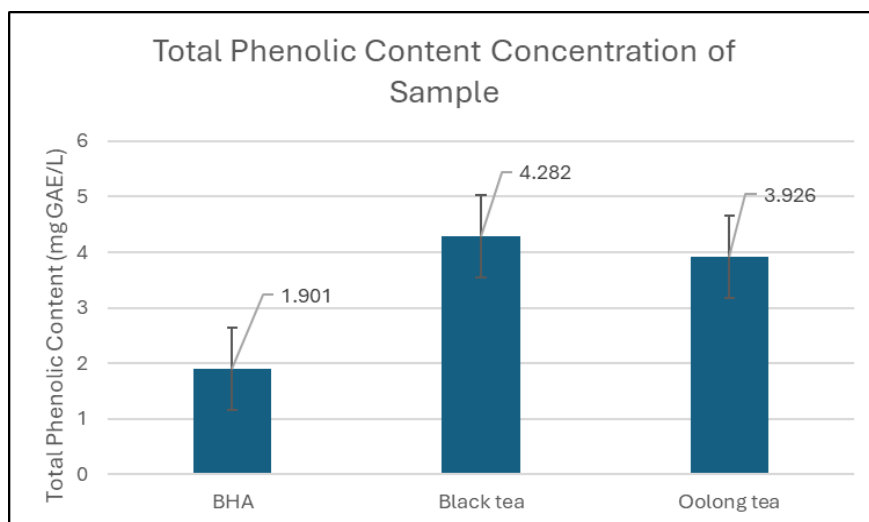


Fig. 3 Total Phenolic Content Concentration of Sample, $n = 3$

The total phenolic content (TPC) of BHA, black tea extract, and oolong tea extract in mg gallic acid equivalent per litre (mg GAE/L), which can be also seen in Table 2 and Fig. 3. The concentration of phenolic compound in the samples, in the order of highest to lowest, was black tea extract 4.282 ± 0.013 mg GAE/L, oolong tea extract 3.926 ± 0.005 mg GAE/L, and BHA 1.901 ± 0.036 mg GAE/L. The values of tea extracts shown in Table 2 and Fig. 3 are significantly higher than the synthetic antioxidant BHA, which means the tea extracts are of higher value natural antioxidants.

This elevated TPC in black tea extract is the result of full fermentation and oxidation of catechins with the polymerisation of theaflavins and thearubigins. The polymerised polyphenols can donate electrons readily and

resonate to form stable free radical complexes. The overall neurological density and the complex phenolic compounds result in the high TPC extract value. This is like other findings and fully fermented for polymerised phenolic black tea total phenolic antioxidant level is higher than that of partially fermented tea polymerised phenolics. [23].

Oolong tea extract showed a slightly lower TPC as compared to black tea, but it was still much higher than BHA. This finding agrees with a semi-fermented property of the oolong tea, which keeps a mixture of unoxidised catechins as well as partially oxidised polyphenolic intermediates. The oolong TPC value is the result of unfermented tea oxidation as catechin structure of polyphenolic oxidised intermediates is mixed with partially fermented tea. The phenolic profile of oolong tea is between the profiles of green and black tea, which is the reason there is a low standard deviation (± 0.005). This also indicates deep reproducibility in extraction with phenolic oolong tea, which is the high stability extraction efficiencies and an equally homogenous oolong tea sample preparation, contained which is the low standard deviation.

On the other hand, BHA contained the lowest TPC value from the other samples, which was expected as BHA is not a phenolic-rich compound but a low-molecular-weight synthetic antioxidant. Its antioxidant activity is the result of the donation of hydrogen atoms to free radicals, producing a relatively stable radical. Unlike the plant extracts, BHA lacks a broad spectrum of phenolic compounds, which collectively contribute to the TPC values. However, BHA and BHT, which are synthetic antioxidants, are still used as they are effective at low concentrations. Nevertheless, consumer concerns regarding the possible toxicity of synthetic antioxidants and the regulatory constraints have increased the use of natural antioxidants, which are abundant phenolic compounds and have better safety profiles [24].

The difference in TPC among tea extracts and BHA, respectively, demonstrates the higher content of phenolics in black and oolong tea and their possible benefits in the protection of food from spoilage. Phenolic compounds are vital in the mitigation of oxidative spoilage because they scavenge free radicals and complex with pro-oxidant trace metal ions and disrupt the pathways of lipid oxidative degradation. These actions are the basis for the improvement of the oxidative stability of food and consequently prolong the shelf life of food [25].

This feature is especially significant for lipid-rich baked food products like cookies because the fats in cookies are easily prone to oxidative rancidity during shelf storage. Foods with higher phenolic content have been strongly associated with improved rancidity control because the formation of lipid peroxides, the first product of lipid oxidation, is delayed and, consequently, the food quality remains high for a longer period [26]. Therefore, the TPC values of black and oolong tea extracts are higher TPC values than the other extracts, indicating their greater potential in controlling oxidative rancidity of cookies, which is confirmed in other assays, like peroxide value (PV) and antioxidant activity (AOA) is confirmed. In summary, with the highest TPC suggesting the highest phenolic antioxidants, black tea extract is shown to have, followed by oolong tea extract, and the lowest phenolic BHA content.

3.4 Application in Homemade Cookies

3.4.1 Peroxide Value (PV) Analysis

The Peroxide value (PV) analysis was conducted to evaluate lipid oxidation in cookies during storage. The PV results for control and antioxidant-treated samples are shown in Table 3 and Fig.4.

Table 3 Analysis results showing PV value of tea extract and BHA after applying to homemade cookies

Sample	PV Value (meq/kg)		
	Day 1	Day 6	Day 14
Control	23.0	36.0	48.0
BHA	18.4	31.0	38.0
Black Tea	14.0	15.6	27.0
Oolong Tea	17.2	18.0	30.0

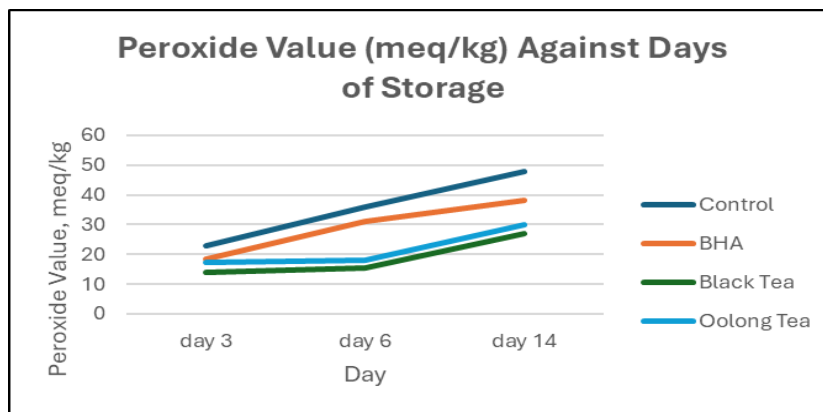


Fig. 4: Peroxide value against days of storage for 14 days

Table 3 and Fig. 4 show how the peroxide value (PV) of the cookies changes over time, which indicates how far the lipids have oxidized. A trend of increasing PV was evident in all samples, demonstrating the formation of primary oxidation products. During the entire time sampled, the control consistently had the most PV. It increased the most (almost doubled), rising from 23.0 meq/kg on Day 1 to 48.0 meq/kg on Day 14. This increase is due to the unquenched free radical cascade reactions (because of no antioxidant protection) that rapidly form and over deplete the lipids in the cookies. Others have reported similar findings, demonstrating that baked products containing significant amounts of fat in shortening or butter oxidatively deteriorate easily due to the presence of oxygen and light [27].

Aside from the control, every sample showed consistently lower PV values throughout the storage period, confirming the ability of antioxidants to slow down the process of lipid oxidation. Antioxidants slow down the oxidation process through free radical scavenging, hydroperoxide stabilization, and interruption of propagation reactions within the lipid oxidation system. Of the treatment samples, black tea had the highest inhibitory effect on the formation of peroxides, as PV values on day 1 of the storage period were 14.0 meq/kg and increased to 27.0 meq/kg past 14 days. Oolong tea extract performed well too, as it increased from 17.2 meq/kg to 30.0 meq/kg during the same period. In contrast, synthetic antioxidants like BHA performed moderately well as they increased over the same time from 18.4 meq/kg on day 1 of the storage period to 38.0 meq/kg on day 14. This shows that BHA is not as effective against oxidation when comparing the two over a prolonged time.

Even though BHA showed the highest DPPH radical scavenging activity at 93.01 %, it was still outperformed by the tea extracts about the extent to which it was able to inhibit lipid oxidation in the cookies during the storage period. Thus, it becomes clear that there exists an obvious contrast to the underlying limitations of in vitro antioxidant analysis. DPPH chemical assays have rapid radical scavenging, while during the analysis of the PV test, it can be observed that the radical preserves its antioxidant role during the analysis of highly complex food matrices.

Different teas contain antioxidant polyphenols of differing structures, which include catechins, theaflavins, thearubigins, flavonoids, and which are also able to act through diverse mechanisms. These antioxidants can stabilize lipid radicals and undergo complexation with. In addition to this, there is also the ability to scavenge free radicals, and the prolonged protection that is gained from oxidative protection is the blockade of pro-oxidant, metallic ions. The presence of the multifunctional heterogeneous active compounds may help explain the level of superiority in the activity of the tea extracts when put side by side with BHA, which is a synthetic antioxidant that acts using a single mechanism. In baked goods and in food systems rich in lipids, the tea polyphenols showed comparable and better antioxidant activity than BHA and BHT [28]. The total phenolic compounds present in the tea polyphenols explain the lower PV values in the cookies. The values suggest that the total phenolic compounds present in the tea explain the significant suppression of peroxide formed during storage.

3.4.2 Inhibition of Lipid Oxidation Analysis

The Antioxidant activity (AOA) was measured to assess BHA, black tea extract, and oolong tea extract to inhibit the oxidation of lipids in cookies over time of storage. AOA, in this case, rather than being fully derived from in vitro assays, was derived from peroxide value (PV) over time, allowing for more of an assessment of the complex food matrix present. The oxidation deterioration rate and the corresponding AOA values for each sample are shown in Tables 4 and 5.

Table 4 *Degradation rate of control and tea extract sample*

Sample	Degradation Rate (%)
Control	2.27
BHA	1.78
Black Tea	1.18
Oolong Tea	1.16

Table 5 *Antioxidant activity (AOA) of BHA and tea extract sample*

Sample	Antioxidant Activity, AOA (%)
BHA	21.59
Black Tea	48.05
Oolong Tea	48.90

The AOA test was derived from real food system data, and the peroxide value (PV) over time of storage. Unlike in vitro assays, AOA provides the most accurate and trustworthy assessment of preservative potential in complex food matrices like cookies. The deterioration rate from Table 4 is the lipid oxidation deterioration rate for each sample over the 14 days of storage. The control cookies, which did not have any antioxidants, had the greatest deterioration rate (2.27 %), indicative of rapid and ongoing lipid oxidation. This was matched with the significant elevation in PV values previously recorded (23.0 to 48.0 meq/kg), confirming that the lipid fraction of the cookies is indeed susceptible to oxidative deterioration in the absence of antioxidants.

Regarding the control, cookies treated with BHA had a lower deterioration rate, 1.78 %, indicating that BHA, to some degree, was effective in delaying the formation of hydroperoxides. However, the extent of reduction was rather moderate, indicating that perhaps it is not that effective in the long run. This is because BHA, through a single antioxidant mechanism of hydrogen donation to free radicals, may become less effective the longer the storage time due to depletion of antioxidants, volatility, or instability that are antioxidants in the system of the baked food [21]

On the other hand, cookies incorporated with the natural extracts of tea had noticeably lower rates of deterioration. With 1.18 %, black tea extract had a deterioration rate that was lower than the 1.16 % rate of oolong tea extract, which had the lowest rate overall. Both values represented a significant reduction when compared to the control and BHA alone, illustrating the effective antioxidant stabilization of the lipid matrix tea extracts in storage, as well as the continuous control of peroxide formation over time. The result regarding the tea extracts also showcases their antioxidant effectiveness, being prolonged in actual food systems.

The AOA % values of the samples are compared to their control in Table 5. These values are the percentages at which oxidation was inhibited. The highest AOA was observed in oolong tea extract (48.90 %), then black tea extract (48.05 %), and lastly BHA (21.59 %). These findings suggest that natural tea extracts turned out to be more efficient than BHA in inhibiting lipid oxidation during storage. Although BHA shows higher radical scavenging activity measured by the DPPH assay, it is less effective than tea extracts in slowing the rate of lipid oxidation during storage.

Compared to BHA, which had the lowest AOA, both tea extracts were more than two times more effective in reducing lipid oxidation. The higher AOA values exhibited by both tea extracts were due to their multifunctional tea antioxidants. The tea polyphenol antioxidants include catechins, theaflavins, and thearubigins. These tea polyphenol antioxidants act through several pathways, including the scavenging of free radicals, the chelation of metal ions, and the stabilization of lipid radicals, to provide prolonged protection against oxidation [29]. Therefore, the natural antioxidants obtained from black and oolong tea extracts, BHA, are more effective in retaining the oxidative stability of cookies during real-time storage.

3.4.3 Observation of Homemade Cookies

Visual and textual methods were used to assess the physical and structural quality of cookies during storage. The selection of texture was made due to its correlation with the major quality determinants of moisture content and lipid oxidation in baked goods. Cookie, described as *well-preserved*, maintained a soft texture, intact surface structure, and showed no visible signs of spoilage after 14 days of storage. Specifically, cookies treated with black tea and oolong tea extracts exhibited no fungal or mold growth, no surface cracking, and no oil exudation, indicating effective preservation of the cookie matrix. In contrast, the control samples became visibly harder and brittle, accompanied by noticeable moisture loss, which are common indicators of oxidative and physical degradation in baked products during storage. This evaluation proves the claims of those antioxidants being most effective during the storage period. The results of this evaluation are in Table 6.

Table 6 Analysis results showing observations of homemade cookies after 14 days

Sample	Texture
Control	Hard and brittle
BHA	Slightly soft
Black Tea	Soft and well-preserved
Oolong Tea	Soft and well-preserved

For the evaluation of homemade cookies, texture is one of the most important quality parameters of baked goods, as it reflects both the oxidative deterioration of the cookies during storage and the structural integrity of the cookie matrix. The observation made after two weeks of storage was that the control cookies without antioxidants were visibly and texturally harder and more brittle. The textural deterioration results from lipid oxidation accompanied by moisture loss, which are the main factors contributing to the hardening of cookies over time.

Compared to the control samples, cookies treated with BHA retained a relatively softer texture than the control samples. These findings indicate that BHA helped mitigate some oxidative interactions and possibly moisture loss, leading to less texture change during storage. Although cookies with tea extracts showed some preservation, some degree of texture change remained, suggesting that BHA provided some oxidative protection as well. Cookies with black tea extracts and oolong tea remained soft and maintained their texture after 14 days of storage. The polyphenolic complex reduced some free radical damage and peroxidation. The preserved soft structure of the cookies was also due to moisture retention and less breakdown, primarily thanks to the tea and cookie structural components.

Cookies preserved with black tea maintained their texture longer than those preserved with oolong tea. The differences with black tea were most significant, showing greater oxidative preservation, likely because of its higher phenolic content. Overall, these findings prove the usefulness of tea extracts demonstrated a better ability to preserve the physical structure of baked goods during storage.

4. Conclusion

This study demonstrated that black tea and oolong tea extracts are capable natural antioxidants for inhibiting lipid oxidation in baked food systems. Soxhlet extraction using isopropanol successfully produced phenolic-rich extracts, with black tea showing higher total phenolic content than oolong tea. In the DPPH radical scavenging assay, BHA exhibited the highest antioxidant activity (93.01%), followed by black tea extract (50.42%), while oolong tea extract showed the lowest activity (6.05%). Despite this, peroxide value analysis of cookies revealed that black and oolong tea extracts provided superior long-term oxidative protection during storage (48.05% and 48.90%, respectively) compared to BHA (21.59%). These findings confirm that phenolic compounds in tea extracts contribute more effectively to oxidative stability in complex food matrices than in vitro assays alone suggest. In summary, the results suggest that black and oolong tea extracts have strong antioxidants and that for each different food application, there are additional benefits from each tea. This research contributes to the knowledge of how tea antioxidants can potentially improve the oxidative stability, quality, and longevity of baked goods and supports the growing need for more sustainable and less resource-intensive alternatives for food preservation.

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Author Contribution

This journal requires that all authors take public responsibility for the content of the work submitted for review. The contributions of all authors must be described in the following manner:

*The authors confirm contribution to the paper as follows: **study conception and design:** Nik Nurul Izzah Binti Faroerzy; **data collection:** Nik Nurul Izzah Binti Faroerzy; **analysis and interpretation of results:** Nik Nurul Izzah Binti Faroerzy, Nor Faizah Binti Razali; **draft manuscript preparation:** Nik Nurul Izzah Binti Faroerzy. All authors reviewed the results and approved the final version of the manuscript.*

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