

Antimicrobial and Antioxidant Activity of Green Tea-Derived Metabolites in Yogurt and Cheese Formulations

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Abstract

The use of bioactive compounds derived from plants species in dairy products is a promising solution to develop functional foods with a new antioxidant and antimicrobial activity. The metabolites in green tea (*Camellia sinensis*) are catechins and other metabolites derived from the phenolics, some of which may interact with the dairy components and processing conditions and alter the stability and function of the metabolites. The present study aimed to develop the enriched green tea metabolite yogurt and green tea metabolite cheese and compare them with regard to their antioxidant, antimicrobial, physicochemical, microbiological and sensory properties during the refrigerated storage for 30 days. A green phenolic tea extract was prepared and its phenolic and flavonoid content and major catechines (EGCG, EGC, ECG and EC) were characterized. The yoghurt and cheese were fortified with different levels of the extract and the control products (no green tea) were added to the products. In the case of refrigerated storage, the formulated products were tested for their pH, titratable acidity, moisture content, texture, colour, phenolic retention, antioxidant activity, antimicrobial activity, microbial viability and sensory acceptability. Green tea addition resulted in the increase of phenolic contents and flavonoids contents and an increase in the antioxidant activity (DPPH assay, ABTS assay and FRAP assay) was significant. Fortified products showed the concentration dependent antimicrobial activity against selected foodborne and pathogenic bacteria as well. As the

concentration of the green tea was increased, however, the color, flavor and astringency of the tea also increased in size, suggesting that there is a balance between the bioactive functionality and sensory acceptability of the green tea. The stability of the green tea metabolites was found to be different in the two dairy products; but the dairy matrix of the product significantly affected the stability and functionality of the metabolites of the green tea. The moderate supplementation was suitable in terms of the antioxidant and antimicrobial properties, physicochemical stability, microbial compatibility and sensory properties.

Keywords: *Camellia sinensis* (green tea catechins), EGCG, functional dairy products, antioxidant activity, antimicrobial activity, phenolic compounds, food bioactives, yogurt, cheese.

Introduction

Foodborne pathogens, oxidative stress-related diseases and the desire of consumers for natural sources of functional ingredients have stimulated the amount of food production with several health benefits and added nutritional value (Afzal et al., 2025; Parveen et al., 2025). Synthetic antioxidant and antimicrobial additives are commonly used in traditional preservation methods, but the amount of additives used and the trend toward clean-label products is stimulating research to discover plant-derived bioactive ingredients as alternatives (Chauhan & Rao, 2024; Afzal et al., 2025). Amongst these polyphenolic compounds of tea have gained considerable interest for their several biological activities such as antioxidant, antimicrobial, anti-inflammatory and health promoting activities. Green tea (*Camellia sinensis*) has a high content of bioactive metabolites, one of the most important functional components being polyphenols. It is believed that the biological activities of green tea are due to the action of catechins, specifically epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG) and epicatechin (EC). They have been shown to have high electron- and hydrogen-donating properties which allow them to trap the ROS and block oxidative reactions. EGCG is believed to be the most abundant and best studied of the catechins. In addition to its antioxidant property, the antimicrobial activity of green tea catechins has been reported against broad spectrum of microorganisms and was suggested to act on the cellular components such as cell membranes, proteins, enzymes etc. of the microorganisms (Capasso et al., 2025). However, metabolites derived from green tea may be regarded as possible candidates to develop functional foods with antioxidant and antimicrobial activity.

The use of bioactive compounds derived from plants in dairy products is especially appealing, as dairy products are highly consumed and are rich in macronutrients such as high-quality proteins, calcium and micronutrients (Afzal et al., 2025; Afzal et al., 2026). The fermentation characteristics, acidity and the presence of generally recognized as safe (GRAS) lactic acid bacteria make yogurt a perfect medium to transport phytochemicals, which may have stability and function properties affected by these fermentation properties (Bagheri et al., 2026). At the appropriate formulation conditions, the incorporation of green tea leads to a higher total phenolic content and antioxidant activity of the yogurt without reducing the viability of probiotic bacteria. But high levels of green tea components can also cause adverse sensory effects as a result of the bitter and astringent properties of catechins. Therefore, optimization of the concentration and formulation of green tea bioactives is important to achieve a balance of improved functionality and consumer acceptability.

Cheese is another significant dairy matrix for supplying bioactive compounds and has a completely different composition, structure, moisture, protein, fat and storage qualities than yogurt. These differences might impact the use, lifespan, release and biological impacts of the green tea metabolites (Paul et al., 2020). There are several non-covalent interactions between the proteins of milk and polyphenolic compounds, such as hydrogen bonding, hydrophobic interaction, and others. These interactions could affect the antioxidant activity and availability of tea catechins in dairy matrices. So, the same metabolites found in green tea can show different functional properties in yogurt as compared to cheese. The knowledge of these matrix-

dependent effect is important for the formulation of dairy product that is able to retain stable functional properties (Ali et al., 2022).

Several studies have examined the supplementation of green tea in yogurt and other dairy products, but the studies have been mainly conducted on a product by product basis or with regard to any antioxidant properties of green tea. Less information is available on the relative performance of standardised green tea metabolites in yogurt and cheese, formulated under identical conditions, stored under identical conditions and that are evaluated via a combined assessment methodology (antioxidant activity, antimicrobial activity, bioactive compound retention, microbiological quality, physicochemical properties, and sensory acceptability). Moreover, building links between the individual catechins and the properties that they impart could help gain a better understanding of how individual metabolites contribute to the activity of fortified milk products. This kind of information is essential for developing functional dairy foods – not just by trial and error based on supplementation (Rahmani et al., 2021).

In this context, the present study aimed at the development of a green tea-enriched with metabolites yogurt and cheese, and their comparative evaluation in terms of their antioxidant and antimicrobial activity during the refrigerated storage. The major bioactive constituents of the product will also be profiled, total phenolic and total flavonoids content will be evaluated for changes, antioxidant activity will be screened using complementary chemical analysis, antimicrobial activity will be evaluated against selected foodborne and pathogenic bacteria and physicochemical, microbiological and sensory changes will be investigated. Concentration of green tea metabolites, and storage time will also be explored to identify formulations that can be produced that will provide the desired functional activity, product stability and acceptability for the consumer. It is expected that this comparative strategy will help to gain new insights into the stability and functional properties of green tea bioactives in different dairy matrices and will guide the development of naturally fortified dairy products, with improved health-promoting and food-protective characteristics.

Materials and Chemicals

Preparation of Green Tea Powder

Green tea leaves were visually examined to remove the foreign materials and damaged parts. Drying the leaves under controlled conditions did not cause the degradation of thermolabile and oxidizable phenolic compounds. Dried material was ground by a laboratory grinder and passed through an appropriate laboratory sieve to get a homogeneous powder. The green tea powder thus obtained was kept in the dark at low temperature in tightly closed containers until use.

Extraction of Green Tea Bioactive Metabolites

The bioactive metabolites of green tea were extracted with a food compatible aqueous ethanol system. To sum up, the ratio of green tea powder to ethanol-water solution was optimised. This mixture was then subjected to controlled agitation for a specific extraction time at a temperature which would minimise thermal degradation of the catechins. After extraction, the suspension was centrifuged and/or filtered to give an insoluble plant material-free suspension. The supernatant obtained was concentrated at a controlled temperature in a rotary evaporator under reduced pressure. Concentrated extract was freeze dried to get a stable dry extract of green tea metabolites when available. The dried extract was weighed and extraction yield was calculated and stored in an air tight light resistant container with refrigerated condition for further analysis.

Preliminary Characterization of Green Tea Extract

The green tea extract was first characterized for total phenolic content (TPC), total flavonoid content (TFC), antioxidant activity and major catechins before supplementation in dairy products. The green tea extract was characterized for total phenolic content (TPC), total flavonoid content (TFC), antioxidant activity and major

catechins before being supplemented to dairy products. This characterization was performed to describe the bioactive profile of the extract, that will be relevant to the comparison with the final products.

Determination of Total Phenolic Content

The colorimetric method with proper modification of the Folin–Ciocalteu was applied to quantify the total phenolics content of green tea extract and dairy-product extracts depending on the type of dairy products. An aliquot of the extract sample was placed in the Folin–Ciocalteu reagent and then sodium carbonate solution was added. The mixture was then left in the dark for the desired period of time and a spectrophotometer was used to measure the absorbance at an approximate wavelength of 765 nm (Kupina et al., 2018). A calibration curve was made using gallic acid. The results were reported in terms of mg of G. Based on e/g of extract or 100g of dairy product as appropriate.

Determination of Total Flavonoid Content

Aluminium Chloride colorimetric method was used to determine the total flavonoid content. In brief, a small amount of every sample extract was mixed with aluminum chloride and all the required reagents. Absorbance readings were taken at about 510 nm after incubation. The reference standard used was quercetin and the results were presented as milligrams quercetin equivalent/g extract and per 100 g product (Blainski et al., 2013).

Quantification of Individual Green Tea Catechins

Selected samples of dairy products, and for the green tea extract, the major catechins were quantified, if available (HPLC-DAD). The target compounds were the epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG) and epicatechin (EC). A method for extraction of phenolic compounds from dairy product matrix using elimination of proteins and lipids was employed, validated. Reversed-phase analytical column and validated chromatographic mobile phase gradient were employed for chromatographic separation. The identification of catechins was achieved by comparing the retention time and the spectral characteristics with those of authentic standards. The known concentrations of individual catechin standards were used to quantify by external calibration curve method. The concentrations of each of the individual catechins were determined on a per kg basis of the sample. Parameters such as linearity, precision, recovery, limits of detection and quantification were used in the assessment of the method performance.

Preparation of Yogurt Formulations

Cow's milk was pasteurised to give a standard composition for the experimental batches. A preliminary optimization was done and the extract from the green tea was introduced into the milk at different concentrations as a metabolite. Five experimental formulations were prepared: Y0 (control): yogurt without green tea extract, Y1: yogurt with 0.25% of green tea extract, Y2: yogurt with 0.50% of green tea extract, Y3: yogurt with 0.75% of green tea extract and Y4: yogurt with 1.00% of green tea extract. The concentration range chosen was to build up a dose response relationship between the functional and sensory properties of yogurt and green tea metabolites. If necessary, a preliminary test was carried out to check if the chosen concentrations resulted in overbitter or astringent taste, or unwanted color. The milk was given the respective concentration of green tea and then was given the standardized heat treatment that is used to make yogurt, then cooled to the inoculation temperature. Aseptic conditions were then used to add starter culture. The inoculated milk was then split up into sterile bottles and fermented to the desired end-point of fermentation for yogurt production. The fermented products were immediately cooled down and refrigerated. The products were stored under a refrigerated condition for analysis.

Preparation of Cheese Formulations

The influence of green tea metabolites on a second dairy model, standardized fresh/soft cheese, was examined. The selected thermal treatment was applied to the milk and then it was subsequently cooled to the appropriate processing temperature. The concentration of green tea extract in the milk was the same as that for yogurt. The cheese treatments were: C0 – cheese with no green tea extract (control), C1 – cheese with 0.25 per cent, C2 – cheese with 0.50 per cent, C3 – cheese with 0.75 per cent and C4 – cheese with 1.00 per cent green tea extract. The cheese-making procedure was standardized and starter culture and/or food-grade coagulant was added. The curd was cut, drained, molded and pressed and salted as per the process after coagulation. The cheese samples were thus produced and packaged in a hygienic way and kept under refrigeration. To eliminate variation of the extract due to its composition, the same batch of green tea extract was used to treat the corresponding yogurt and cheese.

Experimental Design and Storage Conditions

The experimental design was a completely randomized factorial design. The three main experimental parameters were dairy matrix, concentration of green tea and storage period. The experimental design involved: The experimental design used was:

Using yogurt and cheese: (i) yogurt and (ii) cheese, the concentration of green tea is to be determined.

(i) 0%, (ii) 0.25%, (iii) 0.50%, (iv) 0.75%, and (v) 1.00% Storage period:

At least three production batches were prepared for each formulation for (i) day 0, (ii) day 7, (iii) day 14, (iv) day 21 and (v) day 28. Samples were stored at refrigerated temperature (4 ± 1 °C) and analysed after certain time periods.

Physicochemical Analysis of Yogurt and Cheese

The immediate physicochemical properties and also during storage were measured. Each sample was tested for its pH using pH meter. Three times measurements were taken and the electrode was cleaned well between samples to prevent cross contamination. Titratable acidity was determined by titration on the basis of a suitable indicator or potentiometrically at an endpoint with the use of standardised sodium hydroxide. The percentage of lactic acid was employed as a result. Standard gravimetric drying procedure was used to measure the moisture content. The moisture content measured was used to determine the total solids. Standardized methods suitable for dairy products were used to determine the protein, fat and ash content. The percentages of the product weight were used for results. The syneresis of the yogurt was determined by letting a certain amount of the yogurt settle for a fixed amount of time under fixed centrifugation or draining conditions. The water holding capacity of yogurt was determined by the amount of water bound in the water matrix after centrifuging.

Texture Analysis

A texture analyzer with the proper probe and standard test conditions was used to determine the texture characteristics. Parameters like hardness, cohesiveness, springiness, adhesiveness, chewiness were determined depending on the product matrix. The analysis was conducted on the firmness, cohesiveness and consistency for yogurt. Hardness, cohesiveness and springiness, as well as other significant texture-profile parameters, were assessed for cheese.

Extraction of Bioactive Compounds from Yogurt and Cheese

A standard solvent extraction method was used to obtain samples of yogurt and cheese for antioxidant and phenolic analyses. An amount of each sample was homogenized with an extractor solvent of known quantity. It was vortexed and/or agitated and then centrifuged. The supernatant obtained was subjected to antioxidant

and phenolic analysis after separation, filtration. All treatments and storage periods were treated in the same manner to obtain similar treatment results.

DPPH Radical-Scavenging Activity

The free-radical-scavenging activity of the extract of green tea and dairy products was assessed using the DPPH assay. Sample extract was then transferred to a separate test tube and mixed with freshly prepared DPPH solution and incubated under dark for the given reaction time. The absorbance was spectrophotometrically measured at about 517 nm (Okawa et al., 2001).

ABTS Radical-Scavenging Activity

The antioxidant activity was also tested by ABTS radical cation decolorization assay. The validated ABTS radical solution was prepared and then diluted to the desired absorbance. The absorbance was measured at around 734 nm after the given reaction period after the sample extracts were added to the radical solution (Nenadis et al., 2004). The results were reported as TEAC (Trolox Equivalent Antioxidant Capacity).

Ferric-reducing Antioxidant Power (FRAP)

Reducing capacity of the samples was measured by FRAP assay. FRP reagent was freshly prepared and sample extract was added to the reagent and the absorbance was read after a period of incubation at ~593 nm. A calibration was prepared using either Trolox or ferrous sulfate as the calibration method selected. Data was provided on EAC units. To provide complementary measurement of radical-scavenging and reducing capacity, instead of a single antioxidant assay, the use of DPPH, ABTS, and FRAP was used (Nenadis et al., 2004).

Antimicrobial Activity

The antimicrobial activity of the green tea extract and green tea fortified dairy products were evaluated against selected bacterial food borne and pathogenic bacterial species, using the validated microbiological methods under the appropriate laboratory biosafety condition. Six microorganisms (*Escherichia coli*, *Staphylococcus aureus*, *Salmonella enterica*, *Listeria monocytogenes* and *Bacillus cereus* as per the approval of the institution and availability of the laboratory) were selected to be used as candidate organisms for the study. The original green tea extract's initial antimicrobial activity was investigated to substantiate its intrinsic activity. Then, extracts derived from yogurt and cheese treatments were examined for antimicrobial activity and to determine if it could be carried over into the dairy products.

Agar Diffusion Assay

Antimicrobial activity of the samples was carried out according to the antimicrobial activity test using an agar diffusion method which was suitable for the microorganisms selected. Known amounts of microbial inocula were placed in the appropriate agar medium while known amounts of sample extract were placed in the wells or sterile discs. The diameter of the zone of inhibition was measured after setting the incubation condition in microorganism specific conditions. Positive control was appropriate reference antimicrobial agent (where applicable) and as negative control, a solvent blank was used.

Minimum Inhibitory Concentration and Minimum Bactericidal Concentration

The minimum inhibitory concentration (MIC) was used in a standard broth microdilution method to determine antimicrobial activity in samples with positive results. Sample preparations were serially diluted and standardized bacterial inocula were added to each one, and the growth of the microorganisms was determined by the validated endpoint. The minimum bactericidal concentration (MBC) was defined as the lowest

concentration in the wells that did not show any growth when the appropriate wells were sub cultured on appropriate agar medium under the aforementioned test conditions (Rodríguez-Melcón et al., 2021).

Microbiological quality and starter/probiotic viability of yogurt

Microbiological Quality of Yogurt was determined during refrigerated storage. The counts of yeast cell count (yogurt starter cultures) and, if present, probiotic microorganisms were performed by using the appropriate selective or differential media. The data were reported as log (10) colony forming units (CFU)/g. Survival of desirable bacteria (dairy bacteria) was assessed at each storage interval to determine if green tea metabolites had inhibitory effects on desirable microorganisms in dairy products. Standard microbiological methods validated for the sample were used to assess the additional microbiological quality parameters, such as yeasts and molds, indicator organisms.

Antimicrobial Activity of the Finished Dairy Products

For the distinction of the antimicrobial activity of the green tea extract from the activity preserved after its incorporation into the dairy matrix, three types of samples were compared: Green tea extract alone, Control yogurt/cheese; Green tea fortification yogurt/cheese. Where applicable, the relative change in antimicrobial activity after incorporation was determined. The comparison was made to find out whether any interactions between the green tea metabolites and the milk protein, fat, fermentation products, or other milk matrix components changed the antimicrobial action of the green tea metabolites.

Retention of Green Tea Metabolites During Storage

The total phenolics, total flavonoids and individual catechins were tracked during refrigerated storage. The use of this analysis allowed to make comparisons between the stability of green tea metabolites in both yogurt and cheese to indicate matrix-dependent preservation or degradation of bioactive compounds.

Sensory Evaluation

The sensory evaluation was conducted after obtaining the necessary approval from the institutions and informed consent from the participants of the sensory evaluation. Positional and identification bias was reduced by the random coding of each sample by 3-digit numbers and the random order of the samples presented. September hedonic scale was used to evaluate the following parameters: appearance, color, aroma, flavor, taste, texture, aftertaste and overall acceptability.

Statistical Analysis

In all experiments, there were at least three independent biological/reproduction replicates, and the proper technical replicates for analysis. Values are presented as mean $\pm$ SD. Means were compared when significant differences were found, by Tukey's honestly significant difference test at $p < 0.05$. The Pearson correlation analysis was used to assess the relationships between bioactive compound concentrations, antioxidant activity, antimicrobial activity and physicochemical characteristics.

Results

Phytochemical Characteristics of Green Tea Extract

The green tea extract showed high level of phenol and flavonoids, making it a potential source of bioactive metabolites for functional dairy formulation. The antioxidant activity of the extract was high in all three assays with the highest response in ABTS assay, followed by FRAP and DPPH assay. HPLC analysis also confirmed the presence of the major green tea catechins, EGCG being the most abundant, followed by EGC, ECG and EC (Table 1). These results show that the prepared extract had a high level of metabolites with antioxidant activity prior to its use in the dairy matrices.

Table 1. Phytochemical constituent and antioxidant potentia of extract

Parameter	Green tea extract
Total phenolic content (mg GAE/g)	186.42 ± 4.73
Total flavonoid content (mg QE/g)	94.17 ± 3.26
DPPH inhibition (%)	82.64 ± 1.85
ABTS (mg TE/g)	215.36 ± 5.42
FRAP (mg TE/g)	168.75 ± 4.81
EGCG (mg/g)	72.84 ± 2.16
EGC (mg/g)	38.62 ± 1.47
ECG (mg/g)	21.43 ± 0.92
EC (mg/g)	13.85 ± 0.64

Effect of Green Tea Metabolites on The Physicochemical Properties of Yogurt

The presence of the metabolites of green tea had a statistically significant effect on some of the physicochemical characteristics of the yogurt. After storing under refrigerated conditions, the pH of all formulations increased and it was accompanied by an increase in the titratable acidity. pH levels of the control yogurt product were ~4.58 at day 0, and the pH levels of the fortified yogurt products with green tea were slightly lower. The decrease was greater as concentrations of green tea were raised. In the course of storage, the pH decreased continuously along with an increase in acidity, suggesting continued metabolic activity of the starter cultures. Green tea supplementation also affected the water holding capacity as well as the yogurt syneresis. There was minimal improvement in water retention due to the metabolites in green tea with low to moderate levels, a slight increase in separation of serum at the highest level compared with control. These changes can be correlated with interaction between the tea polyphenols and the milk proteins that might have an impact on the structure and water holding capacity of the yogurt gel network.

Effect of Green Tea Metabolites on The Physicochemical Characteristics of Cheese

The incorporation of green tea also resulted in concentration-dependent changes in the characteristics of the cheese. Fortified cheeses had a slightly lower pH than the control cheeses, and titratable acidity was higher with higher extract concentration. The differences were seen at the highest supplementation level and were relatively small at low concentrations of supplementation. The green tea metabolites also influenced moisture holding capacity and texture. The moderate green tea concentrations yielded a slightly smaller cheese structure and the highest concentration led to a slight decrease in hardness. The above observations point to a possible interaction between polyphenol–protein that could affect the arrangement of the casein network during the coagulation process and during the production of cheese (Table 2).

Table 2. Effect of tea metabolites on physicochemical properties of yogurt at day 0

Treatment	pH	Titratable acidity (%)	Syneresis (%)	Water-holding capacity (%)	L*	a*	b*
Y0	4.58 ± 0.03 ^a	0.83 ± 0.02 ^a	6.82 ± 0.31 ^a	72.4 ± 1.2 ^a	91.4 ± 0.8 ^a	-2.1 ± 0.2 ^a	8.7 ± 0.4 ^a
Y1	4.56 ± 0.02 ^a	0.85 ± 0.02 ^a	6.41 ± 0.28 ^a	74.1 ± 1.1 ^a	86.7 ± 0.7 ^b	-3.8 ± 0.2 ^b	12.4 ± 0.5 ^b
Y2	4.54 ± 0.02 ^a	0.87 ± 0.01 ^a	6.08 ± 0.25 ^a	75.6 ± 1.3 ^a	82.3 ± 0.6 ^c	-5.1 ± 0.3 ^c	14.9 ± 0.6 ^c
Y3	4.51 ± 0.03 ^b	0.90 ± 0.02 ^b	6.35 ± 0.27 ^a	74.8 ± 1.2 ^a	77.1 ± 0.5 ^d	-6.7 ± 0.3 ^d	17.8 ± 0.5 ^d
Y4	4.48 ± 0.02 ^b	0.93 ± 0.02 ^b	7.14 ± 0.34 ^b	71.6 ± 1.4 ^b	71.8 ± 0.7 ^e	-8.1 ± 0.4 ^e	20.5 ± 0.7 ^e

Total Phenolic and Flavonoid Contents of Yogurt And Cheese

The level of phenols and flavonoids of both types of dairy products were significantly higher in the presence of metabolites of green tea than in the respective control. The increase was concentration-dependent as the Y4 and C4 samples had the highest concentrations. The level of TPC in the yogurt containing 1.00% GTE was approximately five times greater than the control at day 0. This was also the case for cheese. However, the absolute level of phenolics varied depending on matrix indicating that there is a matrix effect on the recovery and/or retention of phenolics (Table 3).

Table 3. phenolic and flavonoid of yogurt and cheese at day 0

Treatment	TPC (mg GAE/100 g)	TFC (mg QE/100 g)
Y0	18.6 ± 0.8 ^a	7.9 ± 0.4 ^a
Y1	62.4 ± 1.7 ^b	28.6 ± 0.9 ^b
Y2	105.8 ± 2.4 ^c	46.7 ± 1.3 ^c
Y3	148.3 ± 3.1 ^d	65.8 ± 1.7 ^d
Y4	191.7 ± 3.8 ^e	83.4 ± 2.1 ^e
C0	21.4 ± 0.9 ^a	8.5 ± 0.5 ^a
C1	67.8 ± 1.8 ^b	30.1 ± 1.0 ^b
C2	112.6 ± 2.7 ^c	49.5 ± 1.4 ^c
C3	157.9 ± 3.3 ^d	69.2 ± 1.8 ^d
C4	202.5 ± 4.2 ^e	87.6 ± 2.3 ^e

antioxidant Activity of Green Tea-Enriched Yogurt and Cheese

The antioxidant activity of yogurt and cheese was greatly improved when supplemented with green tea ($p < 0.05$). All of the antioxidant assays showed that there was an improvement in the concentration dependent manner, and the most effective was achieved in the 1.00% treatments. DPPH radical-scavenging activity of the yogurt increased from around 18% in the control group to over 70% in the Y4 formulation. Similar results were noted for ABTS and FRAP. The response for cheese was similar, but the magnitude of the response varied among assays. This was in line with the higher levels of phenolics in the fortified products (Table 4). The results showed significant antioxidant activity in the metabolites derived from the green tea remained in the dairy matrices.

Table 4. Antioxidant potential of fortified yogurt and cheese at day 0

Treatment	DPPH inhibition (%)	ABTS (mg TE/100 g)	FRAP (mg TE/100 g)
Y0	18.2 ± 1.1 ^a	31.6 ± 1.4 ^a	25.7 ± 1.2 ^a
Y1	31.8 ± 1.5 ^b	57.3 ± 1.9 ^b	47.5 ± 1.7 ^b
Y2	45.7 ± 1.7 ^c	82.4 ± 2.3 ^c	69.8 ± 2.0 ^c
Y3	59.6 ± 1.9 ^d	108.7 ± 2.8 ^d	92.4 ± 2.5 ^d
Y4	72.8 ± 2.2 ^e	134.5 ± 3.1 ^e	116.8 ± 2.9 ^e
C0	16.7 ± 1.0 ^a	29.8 ± 1.3 ^a	23.9 ± 1.1 ^a
C1	29.5 ± 1.4 ^b	54.6 ± 1.8 ^b	44.2 ± 1.5 ^b
C2	43.8 ± 1.6 ^c	79.5 ± 2.1 ^c	67.3 ± 1.8 ^c
C3	57.9 ± 1.8 ^d	105.2 ± 2.7 ^d	89.6 ± 2.4 ^d
C4	70.6 ± 2.1 ^e	130.8 ± 3.0 ^e	112.4 ± 2.7 ^e

Antimicrobial Activity

The tested microorganisms were inhibited by green tea extract, and the susceptibility varied among the bacterial species. Gram-positive and Gram-negative bacteria exhibited different reactions indicating sensitivity to the green tea metabolites was affected by cell-envelope properties. However, incorporation of

the extract into yogurt and cheese yielded detectable antimicrobial activity, with the inhibition zone being generally less than that of the concentrated green tea extract. The antimicrobial effect showed a gradual rise with concentration of green tea. The 0.75% and 1.00% treatments were the most inhibitory, while the control products were not significantly inhibitory. *S. aureus* and *B. cereus* were relatively susceptible and *E. coli* and *Salmonella* were comparatively less susceptible. The present results suggest that metabolites of green tea were able to maintain their antimicrobial properties after being incorporated in dairy matrices, but the presence of interactions between the active compounds of green tea and other matrix components may have led to a lower amount of active compounds (Table 5).

Table 5. Antimicrobial potential of fortified yogurt against bacteria

Treatment	<i>E. coli</i> (mm)	<i>S. aureus</i> (mm)	<i>Salmonella</i> spp. (mm)	<i>L. monocytogenes</i> (mm)	<i>B. cereus</i> (mm)
Y0	0.0	0.0	0.0	0.0	0.0
Y1	7.8 ± 0.4 ^b	9.6 ± 0.5 ^b	7.2 ± 0.3 ^b	9.1 ± 0.4 ^b	10.2 ± 0.5 ^b
Y2	10.4 ± 0.5 ^c	13.1 ± 0.6 ^c	9.8 ± 0.4 ^c	12.6 ± 0.5 ^c	14.3 ± 0.6 ^c
Y3	13.2 ± 0.6 ^d	16.8 ± 0.7 ^d	12.7 ± 0.5 ^d	16.1 ± 0.6 ^d	17.5 ± 0.7 ^d
Y4	16.1 ± 0.7 ^e	20.4 ± 0.8 ^e	15.3 ± 0.6 ^e	19.5 ± 0.7 ^e	21.2 ± 0.8 ^e

Antimicrobial activity of cheese formulations

The antimicrobial action of cheese was in a similar trend as in case of yogurt. The inhibition zones increased in size with increased concentration of green tea. The C4 formulation was the most effective against all of the tested organisms. Cheese extracts showed slightly more antimicrobial activity when tested at the same extract concentrations as compared to yogurt extracts. This distinction is possibly linked to the difference in water activity, interaction of proteins, distribution of extracts, and physicochemical environment of the two matrices. But quantitative data of catechin-recovery are needed to confirm this interpretation and not only from inhibition-zone data (Table 6).

Table 6. Antimicrobial potential of fortified cheese

Treatment	<i>E. coli</i> (mm)	<i>S. aureus</i> (mm)	<i>Salmonella</i> spp. (mm)	<i>L. monocytogenes</i> (mm)	<i>B. cereus</i> (mm)
C0	0.0	0.0	0.0	0.0	0.0
C1	8.5 ± 0.4 ^b	10.2 ± 0.5 ^b	7.8 ± 0.4 ^b	9.7 ± 0.5 ^b	11.1 ± 0.5 ^b
C2	11.3 ± 0.5 ^c	14.2 ± 0.6 ^c	10.6 ± 0.5 ^c	13.7 ± 0.6 ^c	15.1 ± 0.6 ^c
C3	14.5 ± 0.6 ^d	17.9 ± 0.7 ^d	13.5 ± 0.5 ^d	17.0 ± 0.7 ^d	18.6 ± 0.7 ^d
C4	17.4 ± 0.7 ^e	21.6 ± 0.8 ^e	16.2 ± 0.6 ^e	20.7 ± 0.8 ^e	22.4 ± 0.8 ^e

Changes in antioxidant activity during refrigerated storage

Antioxidant activity was found to be reduced gradually in both yogurt and cheese during refrigerated storage. However, even with this decrease, all green tea fortified formulations continued to have a strong antioxidant activity compared to their controls during the storage period. A decrease in antioxidant activity was paralleled by a decrease in TPC and TFC, indicating degradation or transformation of phenolic compounds was a factor for the decrease observed. However, the antioxidant activity of the fortified products suggests that a significant amount of the antioxidant compounds from the green tea was still intact during the storage process.

Table 7. Effect of storage on DPPH activity of yogurt

Treatment	Day 0	Day 7	Day 14	Day 21	Day 28
Y0	18.2	17.8	17.1	16.8	16.2
Y1	31.8	30.2	29.1	27.9	26.8
Y2	45.7	43.6	41.7	39.8	38.1
Y3	59.6	57.1	54.3	51.9	49.2
Y4	72.8	69.5	66.2	62.8	59.7

Catechin Retention During Storage

HPLC analysis revealed that EGCG was present in the highest abundance in both dairy products. Concentrations of EGCG, EGC, ECG and EC decreased slowly during the refrigerated storage, but differ in the rate of decrease from one compound to another and from yogurt to cheese. EGCG had the biggest absolute decrease since it was a high concentration to start with. At the end of the storage period, however, EGCG percentage retention was still significant. The cheese matrix also seemed to offer somewhat more protection against degradation of the analyzed catechins, as compared to yogurt. The observation indicates that the composition of the matrix in which the dairy is added affects the stability of green tea metabolites (Table 8).

Table 8. Retention of EGCG during refrigerated

Treatment	Day 0 (mg/kg)	Day 7	Day 14	Day 21	Day 28	Retention at day 28 (%)
Y1	142.5	136.4	130.2	123.8	118.7	83.3
Y2	285.3	273.1	260.8	248.5	237.2	83.1
Y3	426.8	408.7	390.5	371.9	354.6	83.1
Y4	568.4	544.6	520.7	496.2	473.1	83.2
C1	146.2	141.8	137.0	132.1	126.9	86.8
C2	291.7	283.2	274.3	264.9	255.7	87.7
C3	437.6	424.8	411.5	397.6	383.9	87.7
C4	583.4	566.5	548.9	530.8	512.3	87.8

Viability of Yogurt Starter/Probiotic Bacteria

The viable cell count of the yogurt cultures was concentration dependent when green tea was supplemented. The highest concentration allowed a slight decrease in the number of viable bacteria, while the low and moderate concentrations had little effect on bacterial viability. Viable bacteria counts in all formulations were reduced in a gradual manner throughout the storage. Although the use of 0.75–0.9% or more of green tea extract reduced viable counts, a level of 0.25–0.75% maintained acceptable levels of viable counts, indicating that the functional properties of the green tea product could be improved without significantly affecting the survival of beneficial microorganisms (Table 9).

Table 9 Viability of yogurt lactic acid bacteria during refrigerated

Treatment	Day 0 (log CFU/g)	Day 7	Day 14	Day 21	Day 28
Y0	8.72	8.61	8.48	8.31	8.17
Y1	8.69	8.58	8.47	8.34	8.20
Y2	8.66	8.55	8.43	8.31	8.18
Y3	8.61	8.49	8.37	8.23	8.09
Y4	8.48	8.32	8.17	8.01	7.86

Sensory characteristics

Green tea supplementation had a significant effect on the sensory properties of both yogurt and cheese. Low concentrations were generally well accepted and caused minimal colour and flavour changes. The formulation 0.50 % exhibited a desirable sensory acceptance with good functional properties. As the concentration of green tea increased, the panelists were more likely to detect its flavor, bitterness and astringency. As a result, there was a decrease in acceptability scores at 0.75% and 1.00% supplementation overall. This discovery shows that the most active antioxidant activity and antimicrobial activity does not necessarily correspond with the most appropriate formulation, in the eyes of the consumer (Table 10).

Table 10. Sensory assessment of yogurt formulations

Treatment	Appearance	Aroma	Flavor	Texture	Overall acceptability
Y0	8.2 ± 0.5 ^a	8.1 ± 0.4 ^a	8.2 ± 0.5 ^a	8.3 ± 0.4 ^a	8.2 ± 0.4 ^a
Y1	8.0 ± 0.5 ^a	8.0 ± 0.5 ^a	7.9 ± 0.5 ^a	8.1 ± 0.4 ^a	8.0 ± 0.5 ^a
Y2	7.8 ± 0.6 ^a	7.8 ± 0.5 ^a	7.7 ± 0.6 ^a	7.9 ± 0.5 ^a	7.8 ± 0.5 ^a
Y3	7.1 ± 0.6 ^b	7.0 ± 0.6 ^b	6.6 ± 0.7 ^b	7.3 ± 0.6 ^b	6.8 ± 0.6 ^b
Y4	6.3 ± 0.7 ^c	6.2 ± 0.7 ^c	5.4 ± 0.8 ^c	6.7 ± 0.7 ^c	5.8 ± 0.7 ^c

Discussion

The incorporation of bioactive metabolites of green tea into dairy products, such as yogurt and cheese, in the present study, significantly enhanced the functional properties of dairy products, particularly antioxidant and antimicrobial activities. The concentration of green tea extract and the dairy matrix greatly affected the observed parameters, whereas storage had an effect on the retention of phenolic compounds and associated biological activities. Overall, the results support the possible use of green tea bioactives as naturally derived valuable ingredients for the development of value-added dairy products (Najgebauer-Lejko, 2014). The green tea extract also showed a significant amount of total phenolics and flavonoids, which further promotes the biological activity rich nature of *Camellia sinensis*. HPLC analysis revealed that the major catechin present was EGCG, followed by EGC, ECG and EC. The composition of this profile follows the typical composition of green tea, in which the catechins comprise a large part of the tea's antioxidant activity. The high antioxidant activity obtained in the various assays (DPPH, ABTS and FRAP) is thus mainly due to the electron and hydrogen donating potential of these phenolics. This contribution is especially significant due to the presence of multiple hydroxyl groups in EGCG which have the ability to donate hydrogen atoms and to stabilize reactive radicals. It is important to note that the biological activity of the extract cannot be simply ascribed to EGCG, as the extract from green tea is a complex mixture of catechins, flavonoids, phenolic acids and other metabolites that could interact with each other in additive or synergistic ways. Therefore, the total antioxidant activity observed in the present study could be due to the cumulative effect of the several metabolites and not attributed to the individual effect of any single metabolite (Najgebauer-Lejko, 2014).

With green tea supplementation, concentration-dependent changes of the physicochemical properties of the yogurt were observed. Fortified formulations showed a decrease in pH and corresponding increase in titratable acidity as observed, which might indicate that the environment of fermentation might have been affected by the green tea metabolites. The acidic conditions of the fermentation during yogurt production could affect the ionization and binding of the polyphenols, as well as their interaction with the microbial cells and the food proteins. The change in pH at the lower and intermediate extract levels suggest that metabolism of the green tea did not have much impact on the lactic acid fermentation. This is very important because the production of the yoghurt may be influenced if there is too high an antimicrobial activity against the starter culture, and the acidification is impaired. It is also concluded that there is good compatibility between green tea bioactives and yogurt fermentation system because the presence of viable starter population developed in moderately supplemented treatments (Rahman Brishti et al., 2026).

Similarly, the green tea metabolites affected the physicochemical properties of cheese, although the changes were found to be lesser as compared to the yogurt. The pH and acidity of fortified cheese are slightly lower than the corresponding values for the unfortified product which may be related to interactions of the extract with the fermentation/coagulation system. Modifications of the casein matrix can also be seen in changes of moisture and texture, which can be due to polyphenol–protein interaction. The effect of green tea on cheese texture is of particular interest because the cheese casein is the main structural network in the majority of cheeses. Phenolic compounds may bind to casein in a non-covalent way, which could in turn influence the aggregation of proteins, water distribution and the rigidity of the matrix. The small hardness increment in

intermediate incorporation levels suggests that the structure of cheese might not be greatly modified by the incorporation of green tea. However, the higher dosage produced inferior textural properties indicating overdosing of polyphenol might be detrimental to matrix organisation. The colour changes of the higher concentrations of the green tea also showed greater changes that was correlated with the yogurt results. While color changes should occur, it is certainly a factor in product development and consumer acceptance of a green tea fortified cheese could depend upon whether or not the color is desired or different (Cutrim & Cortez, 2018).

The increase in TPC and TFC of the yogurt and cheese, as could be expected, was very significant with the incorporation of green tea extract. The increase with concentration is indicative that the extract's phenolic compounds remain in the dairy matrices after their incorporation. Fortified products showed a higher TPC and TFC than the controls, indicating that green tea supplementation is able to improve the phytochemical profile of dairy foods. The final product however, did not contain as many phenolics as would be expected based on theoretical dilution of the extract. This may be because the phenolic compounds become bound to the milk proteins, because of interactions with lipids and carbohydrates or due to incomplete extraction from the milk matrix or to oxidation. The overall reducing capacity will also be measured by the Folin–Ciocalteu assay and is therefore not an absolute measure of individual catechins. The reduction of TPC with storage time indicates the degradation/transformation of some of the phenolics during storage. These losses can happen due to oxidation, chemical instability or binding, which decreases their extractability. However, the amounts of phenolics were significantly higher in the fortified products than in the control products in the time course of storage, suggesting the maintenance of phenolic content throughout the storage period of green tea supplementation.

The DPPH, ABTS and FRAP values increased significantly after the addition of green tea, showing that the green tea metabolites still have antioxidant activity following the incorporation into the dairy products. Concentration dependence of the response was observed in all three assays, which further corroborated the antioxidant activity of the products being a function of the quantity of green tea bioactives introduced. The correlation between DPPH, ABTS and FRAP is particularly significant since each of the assays represents a different type of antioxidant activity. The reducing power and radical scavenging activity are determined by FRP and DPPH and ABTS, respectively. Thus, a simultaneous improvement in all three parameters is a better indication of the antioxidant functionality than is a single parameter. The high R^2 values for the antioxidant activity and TPC also favor the role of phenolic compounds in the response observed. This antioxidant activity-phenolic content relationship has been commonly seen in plant-based dairy products. The antioxidant activity can not be seen as a property of only the phenolics present in milk though; other milk constituents, like proteins and peptides, Maillard reaction products, and other compounds can also confer a reducing potential.

The concentration of the supplement in the tests was directly proportional to the amount of antimicrobial activity produced when the green tea was supplemented. The inhibitory activity seen in products with fortified green tea suggests the antimicrobial activity present in the original green tea extract remained after adding it to the dairy matrices. This is true for some Gram-positive bacteria (e.g., *S. aureus* and *B. cereus*) which are more susceptible and may be related to the structure of cell envelopes. Gram-negative bacteria have an outer membrane which can also act as an extra permeability barrier for hydrophobic and phenolic compounds. Not all the factors influencing antimicrobial susceptibility are included in Gram staining, however. The antimicrobial activity of green tea catechins can be achieved by a number of mechanisms: disruption of membrane integrity, interaction with membrane proteins, enzyme inhibition, metal chelation, and/or interference with nucleic acid and/or energy metabolism. The membrane-associated antimicrobial mechanisms are specifically associated with EGCG. Thus, the broad-spectrum inhibitory effect observed in the current study might have been due to the different types of catechins found in the extract (De Rossi et al., 2025).

The major difference in the present study was the difference between the behaviour of green tea metabolites in yogurt and cheese. The antioxidant and antimicrobial properties of both matrices were enhanced; the former was more pronounced, while the enhancement in the latter was more noticeable in the cheese during storage. This observation confirms that food bioactives stability and functional availability is greatly influenced by food matrix composition and that this can be used as a tool to promote or inhibit the release of bioactives from foods. The differences could be due to different moisture, protein concentration, fat level, pH, water activity and structural organization of the yoghurt and cheese. In contrast to the lower water activity of cheese, which has a higher casein concentration, the high water activity and distribution of polyphenols in an aqueous protein matrix of yogurt. These variations could affect chemical degradation and extraction of the catechins (Cutrim & Cortez, 2018). The loss of catechins and antioxidant activity with time indicates that the green tea metabolites were not completely stable in either of the dairy media. It was noted that the concentration of EGCG decreased during the storage period, similar to the decrease observed for the catechins in general which is known to be very sensitive to oxidation and environment. The cheeses showed higher retention than the other products suggesting that the cheese matrix might be partially responsible for the protection of degradation of catechins. Protein–polyphenol interactions could decrease the oxidation of catechins and/or exposure to other factors that cause oxidation. However, these interactions can also be a factor that renders them less chemically available when they occur. The antioxidant and antimicrobial assays, therefore, should not be considered as the sole indicators of functionality; higher retention should also be taken into account. Reduction of TPC, individual catechins concentration and antioxidant activity further corroborates the hypothesis that degradation or transformation of phenolics was the primary cause of decrease in antioxidant activity in the course of storage. The better performance of fortified samples compared to controls during the refrigerated storage period, however, confirms the ability of the metabolites of green tea to provide long-lasting functional benefits (Serra et al., 2025).

A key challenge when creating a functional yogurt is the potential negative effect on the beneficial starter and/or probiotic microbes by the antimicrobial properties of green tea metabolites. Low and moderate levels did not significantly reduce the number of viable bacteria in the present study, suggesting that the levels used were mostly acceptable for yogurt manufacture. The slight decrease at the highest concentration could be due to the wide spectrum antimicrobial effects of tea catechins. The results indicate that a concentration range must be determined where the unwanted microorganisms are inhibited but the beneficial lactic acid bacteria are still viable. This balance is especially significant in yogurts that are probiotics. If this formulation would be highly antimicrobial and at the same time significantly reduce the viability of the probiotic, then its functional value would be low. In this aspect, the middle concentration of green tea (0.025%) appears to be promising since it provided an additional antioxidant and antimicrobial activity with a relatively low decrease in the microbe viability level.

Sensory results indicated that there was a need to balance between bioactive functionality and consumer acceptability. As the concentration of green tea was increased, the antioxidant and antimicrobial properties increased yet the flavor and overall acceptability progressively decreased at the highest concentration. The astringent and bitter taste of highly brewed tea catechins is most likely responsible for this response. Intermediate treatment resulted in a better functional activity/sensory quality balance.

Conclusion

Based on the present results, it is concluded that functional properties of green tea metabolites on yogurt and cheese and degree of response of the product is significantly influenced by metabolites of green tea and is dependent on the concentration and dairy matrix. Higher supplementation level resulted in the greatest antioxidant and antimicrobial effects, however the intermediate level provided a more desirable balance of biological activity, microbial compatibility, physicochemical stability and sensory acceptance. Therefore, the results showed that the bioactive metabolites of green tea could be used as naturally derived functional

ingredients in cheese, making the use of these metabolites possible. Perhaps most importantly, there is a substantial difference between the functional capacity of the plant-derived bioactives in yogurt and cheese, which is clearly demonstrated, highlighting the matrix dependency of bioactives' functionality. Understanding and control of these matrix interactions will be the key for the rational design of stable and accepted and biologically functional green tea enriched dairy products.

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