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Oxidative stability of baked products incorporated with *Phyllanthus emblica* seed extract: a functional alternative to synthetic antioxidants

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Lipid oxidation limits shelf-life of bakery products, typically controlled by synthetic antioxidants (BHT, BHA, and TBHQ) with safety concerns. This study aimed to evaluate ethyl acetate extract of *Phyllanthus emblica* seed (PSE) as a natural antioxidant to extend the shelf-life of sponge cakes and biscuits. The extract was assessed for antioxidant activity, thermal stability, antimicrobial activity, and toxicity prior to application. HPLC analysis identified seven major phenolic compounds: gallic, vanillic, sinapic, *p*-coumaric, and ellagic acids, myricetin, and quercetin. PSE showed significantly ($p < 0.05$) higher antioxidant activity than synthetic antioxidants, with lower IC_{50} in DPPH (41.23 μg mL^{-1}) and $ABTS^{\bullet+}$ (29.31 μg mL^{-1}) and higher FRAP (180.34 μg mL^{-1}). PSE retained over 90% antioxidant activity at 180 °C for 2 h, exceeding BHT (62%), BHA (63%), and TBHQ (87%). Toxicity assessment indicated low toxicity, with an IC_{50} of 1025.37 μg mL^{-1} (Caco-2 cells) and an LC_{50} of 1832.71 μg mL^{-1} (zebrafish embryos), and no effects on survival, hatching, or heartbeat were observed at 200 μg mL^{-1}, the bakery application concentration. Sponge cakes and biscuits were treated with PSE (200 μg mL^{-1}) and compared with BHT, BHA, TBHQ, and control (no antioxidant). Sponge cakes with PSE retained peroxide value (PV) and TBARS below thresholds (0.5 meq per kg and 0.3 mg kg^{-1}) for 14 days, while controls exceeded limits within 7 days. In biscuits, PV remained below 1.0 meq per kg for 56 days with PSE, BHT, and TBHQ. PSE extended microbial shelf-life to 12 days in cakes and 84 days in biscuits without affecting sensory quality. Findings highlight PSE as a thermally stable, non-toxic, and effective natural alternative to synthetic antioxidants in bakery products.

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Sustainability spotlight

This research aligns strongly with global sustainability efforts by addressing multiple Sustainable Development Goals (SDGs). Specifically, it supports SDG 3: good health and well-being, by developing a natural antioxidant alternative that reduces reliance on synthetic antioxidants with potential health risks. At the same time, it advances SDG 12: responsible consumption and production, by valorizing *Phyllanthus emblica* seeds, an underutilized by-product that is often discarded as waste, thereby enhancing resource efficiency and minimizing environmental impact. By extending the oxidative stability of baked products with natural antioxidants, this study not only enhances food safety and shelf-life but also promotes more sustainable, eco-friendly food systems that contribute to long-term food security.

1 Introduction

The challenge of food preservation has become increasingly complex in recent years as lipid-rich foods demand effective protection against oxidative deterioration to ensure extended shelf life and quality retention.[1] Lipid oxidation remains one of the most significant causes of quality loss in baked products, which contain high fat levels and are subjected to high processing temperatures (100–200 °C).[2] During oxidation, unsaturated fatty acids react with oxygen to form hydroperoxides (primary products) and aldehydes and ketones (secondary products), leading to undesirable changes in flavor, aroma, texture, and color.[3] Consequently, these reactions limit the storage stability of baked products by accelerating rancidity, nutritional degradation, and reducing consumer acceptability. Thus, controlling lipid oxidation is essential for maintaining product freshness and safety since these products are commonly stored under ambient conditions for long period.

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To mitigate oxidative deterioration, antioxidants are commonly incorporated into bakery formulations. These compounds interrupt free radical chain reactions, decompose peroxides, and chelate pro-oxidant metals, thereby enhancing the oxidative stability and sensory quality of food products.[4,5] The Sri Lankan Food (Antioxidants) Regulation of 2009 limits antioxidant usage in baked products to 200 mg kg^{-1},[6] ensuring product safety while maintaining quality. Widely used synthetic antioxidants include butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), *tert*-butylhydroquinone (TBHQ), and propylgallate (PG).[7] BHA is commonly used in low-fat foods, including cereal products such as breakfast cereals and cake mixtures. BHT is typically used in bakery products at levels of 0.01–0.04%, which is more effective than BHA.[8] TBHQ is considered the best synthetic antioxidant for frying oils, but not for baked products because of high volatility at baking temperatures.[9] Nevertheless, synthetic antioxidants are found to be efficient and inexpensive, but their long-term usage is questionable. Recent research findings revealed that the prolonged usage of synthetic antioxidants adversely affects living beings, such as rodents and monkeys, and has a potential carcinogenic effect.[10] For example, BHA and BHT have been associated with tumor development in humans and rats, particularly affecting the forestomach and liver.[11] Studies have also demonstrated that high doses of BHA and BHT can enhance carcinogenesis in monkeys.[12] Moreover, the long-term usage of TBHQ with high dosage has been found to increase the risk of development of carcinogenicity, cytotoxicity, and potential adverse effects on human health.[13,14] Baran *et al.*[15] observed that exposure to BHA and/or TBHQ can increase the production of reactive oxygen species (ROS), leading to apoptosis and oxidative DNA damage in zebrafish models. Moreover, some synthetic antioxidants such as BHA and BHT are highly volatile at high baking temperatures, making them less suitable for heat-processed foods. These drawbacks have encouraged researchers and consumers alike to seek natural, safe, and thermally stable alternatives from plant sources.

Natural plant extracts have emerged as promising alternatives to synthetic antioxidants due to their abundance of bioactive compounds such as polyphenols, flavonoids, tannins, and carotenoids, which effectively scavenge free radicals and inhibit lipid oxidation.[16] Several studies have demonstrated that natural antioxidants retain higher stability at baking temperature than synthetic ones. For instance, rosemary extract remained active at 200 °C, successfully preventing rancidity in multigrain bread for up to 10 days, whereas BHT and BHA lost over 70% of their activity at 150 °C.[17] Masoud *et al.*[18] reported that incorporating 10% muskmelon seed flour into biscuits significantly enhanced their antioxidant potential, increasing total phenolic content (85.52 to 210.54 mg GAE per g), total flavonoid content (54.84 to 142.28 mg CE per g), and DPPH inhibition (48.63 to 65.54%), while yielding the most acceptable sensory results, confirming its value as a natural antioxidant source. Cakir *et al.*[19] reported that incorporating *Prunus spinosa* powder into gluten-free rice cakes significantly enhanced total phenolic content (35.91 ± 0.25 to 73.39 ± 0.58 mg GAE per g) while reducing the estimated glycemic index. Similarly, *Psidium guineense* Sw. leaf extract incorporated into cakes reduced peroxide and hexanal formation more effectively than BHT, while improving sensory properties.[20] Such findings confirm the potential of plant-based antioxidants to enhance both oxidative stability and product acceptability in bakery systems.

In addition to functional advantages, the use of fruit-processing by-products such as seeds, peels, pomace, and skins offers an environmentally sustainable solution to reduce food waste while recovering valuable bioactive compounds.[21] Studies have shown that substituting refined flour with tomato pomace in bread and muffins extended shelf life by 4–7 days.[22] In another study, Reddy *et al.*[23] investigated that the ethanolic extracts from amla (*Phyllanthus emblica*), drumstick leaves (*Moringa oleifera*), and raisins (*Vitis vinifera*) provided greater antioxidant protection in biscuits than BHA. These examples highlight the dual benefits of natural extracts in food preservation and waste valorization.

Phyllanthus emblica (Indian gooseberry or amla) is a medicinal plant widely recognized for its rich composition of phenolics, flavonoids, and hydrolyzable tannins such as emblicanin A and B, phyllantine, and phyllantidine, which exhibit strong antioxidant and antimicrobial activities.[24,25] While the fruit pulp is extensively used in functional foods and nutraceuticals, the seeds are typically discarded as waste despite their high antioxidant potential. The *Phyllanthus emblica* seed extract (PSE) contains potent phenolic compounds such as resveratrol, gallocatechin, and quercetin, which contribute to its radical scavenging capacity and antibacterial effects.[26] Previous studies have shown that incorporating *Phyllanthus emblica* extracts into meat products effectively reduced lipid peroxidation and maintained desirable sensory qualities during storage. For instance, Bariya *et al.*[27] reported that goat meat patties enriched with *Phyllanthus emblica* fruit and seed coat extracts showed significantly lower TBARS values (below 1 mg malonaldehyde per kg after 21 days) and reduced free fatty acid formation compared to controls, while maintaining superior color, flavor, and overall acceptability during storage. Nevertheless, there is currently no research investigating the application of *Phyllanthus emblica* seed in bakery products, where both thermal stability and oxidative protection are essential.

However, this study is the first to systematically evaluate the effectiveness of the ethyl acetate extract of *Phyllanthus emblica* seed with major synthetic antioxidants (BHT, BHA, and TBHQ) in controlling both oxidative and microbial spoilage in baked products, supported by a comprehensive toxicological profile. The objectives of this research are to determine the antioxidant and antimicrobial activities of *Phyllanthus emblica* seed extract, evaluate its thermal stability at baking temperatures, and assess its toxicological safety through both *in vitro* and *in vivo* assays. Furthermore, the study aims to examine the oxidative and microbial stability of baked products incorporated with PSE during storage, with the ultimate goal of exploring its potential for food application and performance evaluation as a natural antioxidant.

2 Materials and methods

2.1 Materials

Freshly harvested *Phyllanthus emblica* fruits (mature green stage) were obtained from a home garden in Matale, Sri Lanka, and authenticated (Specimen No. 2024/322) by the National Herbarium, Peradeniya. Bacterial cultures (*Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 11778, *Escherichia coli* ATCC 25922, *Salmonella typhi* ATCC 6539) were taken from the Microbial Storage Bank, Department of Biosystems Technology, Faculty of Technology, University of Sri Jayewardenepura. The Caco-2 cell line was obtained from the Institute of Biochemistry, Molecular Biology, and Biotechnology, University of Colombo. Zebrafish (*Danio rerio*) embryos were sourced from the Medical Research Institute, Colombo. Reagents and solvents (HPLC/analytical/food grade) were from Sigma Aldrich (St. Louis, MO) and Merck (Darmstadt, Germany). Chemicals included BHA, BHT, TBHQ, Folin–Ciocalteu reagent, gallic acid, quercetin, methanol, acetonitrile, acetic acid, DPPH, ABTS, fetal bovine serum (sterile-filtered, Capricorn, South America), Dulbecco's Modified Eagle Medium (high glucose 4.5 g L^{-1}, with L-glutamine, sodium pyruvate and phenol red), SRB dye, antichlorine water, thiobarbituric acid, Mueller–Hinton agar (pH 7.3 ± 0.1 at 25 °C), ampicillin, total plate count agar (pH 7.0 ± 0.2 at 25 °C), and potato dextrose agar (pH 5.6 ± 0.2 at 25 °C). Ingredients for baked products (wheat flour, sugar, margarine, eggs, salt, and baking powder) were sourced locally.

2.2 Preparation of phenolic extracts

Phyllanthus emblica seeds were separated manually and dried at 40 °C for 12 h in an oven (LDO-150F, Labtech Co., Ltd, Korea). The dried seeds were milled into fine powder (<180 μm) using a grinder (SL-T2-550, SILIL, Sri Lanka) and stored at 4 °C until use. *Phyllanthus emblica* seeds extract (PSE) was prepared using ultrasound-assisted extraction (UAE) according to the method reported by Irakli *et al.*[28] An aliquot (1.0 g) of dried powder was mixed with 3 mL of 100% ethyl acetate and introduced to an ultrasonicator (LD-LUHS-A16, Labtron, USA) and extracted at 40 kHz for 10 min (<40 °C). Ethyl acetate was chosen as the extraction solvent owing to its moderate polarity, which effectively solubilizes a broad spectrum of phenolic acids and flavonoids of mid-to-low polarity, contributing to extracts with enhanced antioxidant activity.[29] After ultrasonic-assisted extraction, the crude extract of *Phyllanthus emblica* seed was centrifuged at 1500×*g* for 10 min using a centrifuge (Sorvall ST 8R, Thermo Fisher Scientific, Germany, 2021) and the supernatant was filtered through Whatman No.1 paper. Then, the filtrate was evaporated under vacuum at 40 °C on a rotary evaporator (RE-301, Yamato Scientific Co., Ltd, USA), and stored in dark glass vials at −20 °C until further use.

2.3 Total phenolic content

The total phenolic content of PSE was determined using the Folin–Ciocalteu method.[30] An aliquot (0.1 mL) of phenolic extract was mixed with 0.5 mL Folin–Ciocalteu reagent (1 : 10 diluted) and neutralized with 0.4 mL sodium carbonate (7.5%, w/v). After 1 h incubation in the dark at room temperature, absorbance was measured at 765 nm using a spectrophotometer (G10S UV-vis, Thermo Fisher Scientific, USA). The total phenolic content was calculated from a gallic acid standard curve ($y = 0.0121x + 0.0112$, $r^2 = 0.999$) and expressed as g GAE per kg DW.

2.4 Total flavonoid content

The total flavonoid content of PSE was determined using the aluminum chloride colorimetric method.[31] An aliquot (0.5 mL) of phenolic extract was mixed with 1.5 mL of solvent, 0.1 mL of 10% aluminum chloride, and 0.1 mL of 1 M sodium acetate, and the volume was adjusted to 3 mL with solvent. After 30 min incubation in the dark at room temperature, absorbance was measured at 415 nm using a spectrophotometer (G10S UV-vis, Thermo Fisher Scientific, USA). The total flavonoid content was calculated from a quercetin standard curve ($y = 0.0066x + 0.0078$, $r^2 = 0.997$) and expressed as g QE per kg DW.

2.5 HPLC profile

Identification of phenolic compounds and flavonoids was performed by HPLC (high-performance liquid chromatography) according to Aguilar-Hernandez *et al.*[32] The extracts (10 μL) were injected into an HPLC system (Agilent 1260 Infinity, Agilent Technologies, SA) equipped with a photodiode array detector fitted with a C18 reverse-phase column (5 μm particle size, 4.6 mm diameter, 150 mm long; Agilent Eclipse XDB, Agilent Technologies). The isocratic mobile phase consisted of 53% methanol and 2% acetic acid at a flow rate of 0.4 mL min^{-1}. The peak area was detected at 280–365 nm. Compounds were quantified by calibration curves of standards (gallic acid, vanillic acid, sinapic acid, *p*-coumaric acid, ellagic acid, myricetin, and quercetin) at 25–200 μg mL^{-1}. The results were expressed in mg per g DW.

2.6 Antioxidant activity

2.6.1 DPPH radical scavenging assay. The DPPH radical scavenging activity of PSE was measured using the method reported by Hantano *et al.*[33] and compared with synthetic antioxidants (BHT, BHA, and TBHQ). Briefly, an aliquot (0.3 mL) of sample (50, 100, 150, 200, and 250 μg mL^{-1}) was mixed with 1.0 mL DPPH reagent and 1.7 mL of 80% methanol, and then incubated in the dark at room temperature for 30 min. Absorbance was recorded at 517 nm using a spectrophotometer (G10S UV-vis, Thermo Fisher Scientific, USA). The scavenging activity (%) was calculated using eqn (1), where A_0 and A_1 are the absorbance of the control and sample, respectively, and expressed as half-maximal inhibitory concentration (IC_{50} μg mL^{-1}).

$$\text{Scavenging activity (\%)} = [(A_0 - A_1)/A_0] \times 100 \quad (1)$$

2.6.2 Ferric reducing antioxidant power (FRAP) assay. The reducing power of PSE was determined using the method reported by Oyaizu *et al.*[34] and compared with BHT, BHA, and

TBHQ at various sample concentrations (50–250 μg mL^{-1}). A 0.3 mL sample was mixed with 1 mL of sodium phosphate buffer (0.2 M, pH 6.6) and 1 mL of 1% potassium ferricyanide, vortexed, and incubated at 50 °C for 20 min. After adding 1 mL of 10% TCA, the mixture was centrifuged (3000*g*, 5 min). A 1.5 mL of supernatant was combined with 1.5 mL of distilled water and 0.3 mL of 0.1% ferric chloride, and then incubated for 30 min. Absorbance was recorded at 700 nm (G10S UV-vis, Thermo Fisher Scientific, USA). The reducing power percentage was calculated using eqn (2), where A_0 and A_1 are the absorbance of the control and sample, respectively.

$$\text{Reducing power (\%)} = [(A_1/A_0) - 1] \times 100 \quad (2)$$

2.6.3 ABTS radical cation scavenging assay. The $ABTS^{\bullet+}$ scavenging activity of PSE was assessed using the method reported by Re *et al.*[35] and compared with BHT, BHA, and TBHQ. $ABTS^{\bullet+}$ (7 mM) was reacted with potassium persulfate (2.4 mM) at a 1 : 1 ratio and incubated overnight at 4 °C in the dark to form a dark green stock solution. Before use, it was diluted with methanol to get an absorbance of 0.70 ± 0.02 at 734 nm. A 0.2 mL of different sample concentrations (10, 15, 20, 25, 30, 35 μg mL^{-1}) was mixed with 5.8 mL ABTS working solution, and absorbance was recorded after 6 min using a spectrophotometer (G10S UV-vis, Thermo Fisher Scientific, USA). Scavenging activity was calculated using eqn (3), where A_0 and A_1 are the absorbance of the control and sample, respectively, and expressed as IC_{50}.

$$\text{Scavenging activity (\%)} = [(A_0 - A_1)/A_0] \times 100 \quad (3)$$

2.7 Thermal stability

The thermal stability of PSE at different concentrations (50, 100, 150, 200, and 250 μg mL^{-1}) and of the synthetic antioxidants was assessed by measuring the retention of antioxidant activity after heating at 180 °C for 2 h in an oven (LDO-150F, Labtech Co., Ltd, Korea) according to the method reported by Seneviratne *et al.*[36] The stability was expressed as a percentage of the initial (non-heated) FRAP values (Section 2.5.2) and compared with the retained percentages of BHT, BHA, and TBHQ.

2.8 Antimicrobial activity

The antimicrobial activity of PSE was evaluated using the disc diffusion method[37] and compared with BHT, BHA, and TBHQ. Four food-borne pathogenic bacteria were tested: *Staphylococcus aureus* (ATCC 25923) and *Bacillus cereus* (ATCC 11778) as Gram-positive, and *Escherichia coli* (ATCC 25922) and *Salmonella typhi* (ATCC 6539) as Gram-negative. Subcultures were prepared by inoculating 100 μL of each strain into 10 mL of peptone water and standardizing bacterial suspensions to 0.5 McFarland. Mueller–Hinton agar plates were inoculated with the bacterial cultures, and filter paper discs soaked in 100 μL of the sample were placed on the surface. Ampicillin served as the positive control, and 100% ethyl acetate was the negative control. Plates were incubated at 37 °C for 18–24 h, and inhibition zones were measured using calipers. All tests were performed in triplicate.

2.9 *In vitro* cytotoxicity

The cytotoxicity of PSE was assessed using the method reported by Mandim *et al.*[38] and compared with BHT, BHA, and TBHQ. Caco-2 cells were maintained in DMEM supplemented with 10% fetal bovine serum, 2 mM glutamine, and antibiotics (penicillin 100 U mL^{-1} and streptomycin 100 mg mL^{-1}) at 37 °C with 5% CO_2. Cells with 70–80% confluence were treated with antioxidants at different concentrations (50, 100, 200, 400, and 800 μg mL^{-1}), while untreated cells served as controls. After adherence, cells (0.5×10^4 per well) were incubated for 24, 48, and 72 h. The reaction was stopped with 20 μL of 10% (w/v) TCA, incubated at 4 °C for 1 h, washed, dried, and stained with 50 μL of 0.4% (w/v) SRB for 15 min. Excess SRB was removed with 1% (v/v) acetic acid, and adhered SRB was solubilized with 100 μL of 10 mM Tris base. Absorbance was recorded at 540 nm (Synergy HT, Biotek, USA). The cell viability (%) and IC_{50} values were calculated.

2.10 *In vivo* toxicity

Zebrafish (*Danio rerio*) embryos were exposed to PSE, BHA, BHT, and TBHQ following OECD guideline.[39] At 6 hours post-fertilization (hpf), healthy embryos were selected and 10 fertilized eggs per concentration were transferred to individual wells of 6-well plates. Embryos were exposed to five concentrations (50, 100, 200, 400, and 800 μg mL^{-1}) of extracts in 2 mL of distilled water, while the control group was kept with diluted anti-chlorine water. All the treatments were carried out in triplicate at approximately 28 °C. Embryos were observed under a 1600x USB Digital Microscope (China) at 24, 48, 72, and 96 hpf to record survival rate (%), hatching rate (%), mortality rate (%), heartbeat (bpm), and developmental anomalies (pericardial edema, yolk sac edema, spinal curvature, and non-structural deformities). Toxicity was assessed by calculating the lethal concentration (LC_{50}).[40,41]

2.11 Effect of antioxidants on lipid oxidation, microbial shelf-life and sensory quality of baked products

Once the baked products were prepared, their proximate composition, color, and textural properties were analyzed. Throughout the storage period, formation of peroxides and TBARS, microbial activity, and sensory properties were determined to assess the effectiveness of the natural antioxidants in preserving product stability and consumer appeal.

2.11.1 Baked product preparation. Sponge cakes and biscuits were prepared according to the methods reported by Janjarasskul *et al.*[42] and Klunklin and Savage,[43] respectively, with slight modifications. The sponge cake batter included whole-purpose wheat flour (17 g), sugar (13 g), beaten eggs (50 g), butter (10 g), whole milk (24 g), and baking powder (1 g). The biscuit dough mixture consisted of wheat flour (100 g), butter (75 g), sugar (62.5 g), egg (112.5 g), and salt (0.75 g). Both batter and dough were mixed with PSE, BHT, BHA, or TBHQ at about

200 mg kg^{-1} of butter. The control sample was prepared without adding antioxidants. Biscuit shapes were prepared by sheeting the dough to 3 mm thickness and cutting into round shapes with a 5 cm diameter. Finally, the sponge cakes and biscuits were baked in a preheated electric oven (LDO-150F, Labtech Co., Ltd, Korea) at 180 °C for 20 min and 170 °C for 10 min, respectively.

2.11.2 Baked product properties. The proximate composition of the sponge cakes and biscuits, including moisture, ash, fat, fiber, and protein, was determined according to AOAC standard protocols (AOAC 930.15, AOAC 942.05, AOAC 920.39, AOAC 662.09, and AOAC 2001.11, respectively).[44] Textural properties such as hardness, adhesiveness, resilience, cohesiveness, springiness, and chewiness were analyzed using a Texture Profile Analyzer (Brookfield model CT3–50 kg, USA) with a cylinder probe (TA39).[45,46] The analyzer was set to perform two-cycle measurements to produce a two-bite texture profile curve, with a trigger load of 9.00 N and a test speed of 2.5 mm s^{-1}. The color was measured using a digital colorimeter (DS-220, China). After calibrating the instrument, the L^* (lightness), a^* (redness), and b^* (yellowness) values were recorded on three different surfaces of each product. All measurements were performed in triplicate.

2.11.3 Lipid extraction from sponge cakes. Lipid extracts were prepared according to the method described by Nanditha *et al.*,[47] with slight modifications. Each product sample was finely ground and mixed with hexane in 1 : 3 (w/v) ratios. The mixture was then shaken in an orbital shaker for 20 minutes. The lipid extract was decanted, and the extraction process was repeated twice with fresh portions of hexane. All extracts were combined, dried over anhydrous sodium sulfate, and filtered. The hexane was evaporated using a rotary evaporator (RE-301, Yamato Scientific Co., Ltd, USA). The lipid extract was then stored at −20 °C until further analysis.

2.11.4 Peroxide value (PV). The PV of the baked products was determined using the IDF standard method 74A:1991.[48] The lipid extract (0.01–0.30 g) was mixed with 9.8 mL of chloroform : methanol (7 : 3 v/v) and vortexed for 2 s. Ammonium thiocyanate solution (50 μL) was added and vortexed again for 2 s. Then, 50 μL of iron(II) chloride solution was added and mixed well. Incubation was carried out for 5 min in the dark, and the absorbance was measured using a spectrophotometer (G10S UV-vis, Thermo Fisher Scientific, USA) at 500 nm against the blank, which contains all reagents except the lipid sample. To construct the calibration curve, a stock solution of iron(III) with a concentration of 10 μg mL^{-1} was prepared, and the procedure was followed for different concentrations of the working solution (1–10 μg mL^{-1}).

2.11.5 Thiobarbituric acid reactive substances (TBARS). The TBARS value of the baked products was determined according to the method described by Gomez and Almajano,[49] with some modifications. The lipid extract (0.05–0.10 g) was dissolved in 5 mL of chloroform : methanol (7 : 3 v/v), mixed with 5 mL of TBA–TCA solution and vortexed. The resulting solution was boiled at 95 °C for 15 min in a water bath (SWB-C, Biobase Co., Ltd, China), cooled immediately, and the absorbance was measured using a spectrophotometer (G10S UV-vis, Thermo Fisher Scientific, USA). A calibration curve was prepared using 1,1,3,3-tetraethoxy propane as the standard solution. The results were expressed using eqn (4), where A is the absorbance of oil at 532 nm, 72.3 is the molecular weight of malondialdehyde, C is the slope of the calibration curve and W is the sample weight (g).

$$\text{TBARS (mg malondialdehyde equivalent/g of oil)} = (A \times 72.3)/(C \times W) \quad (4)$$

2.11.6 Microbial shelf-life. The aerobic plate count (APC) and yeast and mold count (YMC) of sponge cakes and biscuits were measured at 3-day intervals for 2 weeks and 14-day intervals for 3 months respectively.[50,51] A 1.0 g sample from each product, stored at room temperature, was ground using a mortar and pestle. The samples were then homogenized in 9.0 mL of peptone water, and from each homogenized serial dilution, 1.0 mL was transferred to sterile Petri plates. For APC determination, the pour plate technique was used with plate count agar, and the plates were incubated at 37 °C for 48 h, following standard guidelines. To determine YMC, 1.0 mL of each dilution was transferred in triplicate to sterile Petri plates using the pour plate technique with potato dextrose agar, and the plates were incubated at 25 °C for 4 days, according to ISO 7218.[52]

2.11.7 Sensory quality. The sensory quality of sponge cakes and biscuits was evaluated at three-day intervals for 12 days and 14-day intervals for 84 days, respectively, using 30 semi-trained panelists following the method described by Senanayake *et al.*[53] and Bajaj *et al.*[54] The cakes (3 × 3 × 1.5 cm) and biscuits (5 cm diameter and 3 mm thickness) were baked within the last 10 h and served to each panelist in randomized order. Each panelist was asked to rate according to the sensory properties such as appearance, color, odor, taste, texture (hardness and softness), and overall acceptability using an 11-point hedonic scale (1 – greatest imaginable dislike and 11 – greatest imaginable like).

2.12 Statistical analysis

All samples were analyzed in triplicate unless otherwise specified. All the data were subjected to analysis of variance (ANOVA) using Minitab version 17 statistical package (Minitab, LCC), while the mean separation was performed by Tukey's pairwise test with a significance level of $p < 0.05$. The study results were graphically represented using the OriginPro® 2025 (OriginLab Corporation, Northampton, MA, USA) software.

3 Results and discussion

3.1 Total phenolic and flavonoid content

In the ethyl acetate extract of *Phyllanthus emblica* seed, TPC was 22.72 ± 0.10 g GAE per kg and TFC was 15.62 ± 0.05 g QE per kg seed. The selection of a suitable extraction method and the solvent are very important when considering extraction efficiency. Kaur *et al.*[55] reported that ultrasound-assisted PSE provided the highest TPC (5.82 ± 0.05) compared to maceration (1.71 ± 0.05) and shaking (3.08 ± 0.05) methods due to

structural disruption in plant cell walls, resulting in direct contact with the solvent. In a similar study, Mishra *et al.*[56] found that the extraction yield (%) of total phenolics from *Phyllanthus emblica* seed was significantly higher in ethyl acetate extracts (57.2 ± 2.01) compared to butanol (24.31 ± 1.20) and aqueous extracts (14.88 ± 1.16).

3.2 HPLC profile

HPLC analysis of the PSE identified seven major phenolic acids and flavonoids (Fig. 1). They were detected at different retention times ranging from 3.9 to 12.8 min (Table S1). The identified compounds included gallic acid, vanillic acid, sinapic acid, *p*-coumaric acid, ellagic acid, myricetin, and quercetin. As denoted in Fig. 1, quercetin (30.03 ± 0.24 mg per g DW) was present in the highest concentration, followed by ellagic acid (11.35 ± 0.08 mg per g DW), *p*-coumaric acid (9.99 ± 0.04 mg per g DW), gallic acid (7.79 ± 0.08 mg per g DW), sinapic acid (6.53 ± 0.06 mg per g DW), and myricetin (4.81 ± 0.03 mg per g DW), whereas vanillic acid (3.22 ± 0.14 mg per g DW) was present in the lowest concentration. The high abundance of quercetin and ellagic acid indicates strong potential antioxidant and antimicrobial properties, as both compounds are well recognized for their radical scavenging activity, metal chelating ability, and bioactive effects.[57,58] Gallic acid further contributes to the antioxidant capacity of the extract due to its high redox potential and capacity to neutralize free radicals. Although present in lower concentrations, vanillic acid, sinapic acid, *p*-coumaric acid, and myricetin may provide complementary or synergistic effects, thereby enhancing the overall bioactivity of the extract.[59] Importantly, several of these phenolic acids, such as gallic acid, ellagic acid, and quercetin, have been reported to exhibit relatively high thermal stability. For instance, gallic acid shows limited degradation at moderate to high temperatures (at 180 °C),[60] while ellagic acid retains stability under high temperature conditions (at 200 °C),[61] enabling it to retain bioactivity during thermal treatment. Quercetin, though somewhat sensitive to prolonged heating, can remain functionally active under controlled processing conditions, particularly when present in complex food matrices.[62]

A similar study by Nambiar *et al.*[63] identified the phenolic profile of the methanolic extract of *P. emblica* seeds (1 : 20 w/v). They reported the presence of phenolic compounds such as caffeic acid (0.022 μg mg^{-1}), syringic acid (0.003 μg mg^{-1}), *p*-coumaric acid (0.013 μg mg^{-1}), myricetin (71.280 μg mg^{-1}), and tannic acid (6.461 μg mg^{-1}), which contribute to the total antioxidant activity depending on both their relative concentrations and synergistic interactions. The phenolic profile of *P. emblica* seed extract obtained in this study differed markedly from that reported by Nambiar *et al.*[63] While their methanolic extract was dominated by myricetin (71.28 mg g^{-1}), our ethyl acetate extract contained comparatively low levels of myricetin (4.81 mg per g DW) but a high abundance of quercetin (30.03 ± 0.24 mg per g DW), which was not detected in their study. These variations can be primarily attributed to the differences in solvent polarity and extraction techniques. Methanol, being highly polar, facilitates efficient extraction of polar flavonols such as myricetin, whereas ethyl acetate, with moderate polarity, is more selective for less polar flavonoids and free phenolic acids, thereby increasing quercetin elution. In addition, the UAE applied in the present study may have induced partial hydrolysis of glycosidic conjugates, liberating free quercetin that would otherwise remain undetected in conventional maceration.

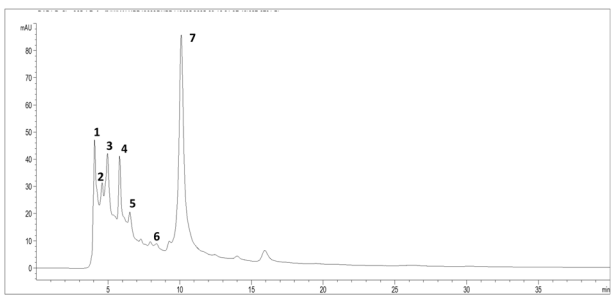

Fig. 1 HPLC chromatogram of phenolic compounds and flavonoids identified in the ethyl acetate extract of *Phyllanthus emblica* seed: (1) gallic acid, (2) vanillic acid, (3) sinapic acid, (4) *p*-coumaric acid, (5) ellagic acid, (6) myricetin, and (7) quercetin.

3.3 Antioxidant activity

The antioxidant activity was evaluated using three different assays: DPPH radical scavenging activity, ferric reducing antioxidant power (FRAP), and $ABTS^{\bullet+}$ scavenging activity (Fig. S1). A lower IC_{50} value corresponds to higher antioxidant activity, as it reflects the concentration required to inhibit 50% of the free radicals. In the DPPH assay (Table 1), PSE demonstrated the strongest antioxidant activity because its IC_{50} was significantly ($p < 0.05$) lower (41.23 ± 0.29 μg mL^{-1}) compared to BHT (213.76 ± 0.47 μg mL^{-1}), BHA (295.43 ± 1.40 μg mL^{-1}) and TBHQ (44.35 ± 2.15). Similarly, in the ABTS assay (Table 1), PSE exhibited significantly ($p < 0.05$) higher antioxidant activity (29.31 ± 0.27 μg mL^{-1}) than BHT (65.62 ± 0.55 μg mL^{-1}), BHA (54.21 ± 0.16 μg mL^{-1}), and TBHQ (30.67 ± 0.38 μg mL^{-1}). The FRAP assay (Table 1) further highlighted the highest reducing power percentage of PSE (180.34 ± 1.03%) compared to BHT (45.36 ± 1.21%) and BHA (36.76 ± 1.24%), with TBHQ (153.31 ± 4.06%) again showing the highest antioxidant activity. Jaiboonma *et al.*[29] reported that the antioxidant activity of PSE depends on the polarity of the extraction solvent. Ethyl acetate, with moderate polarity, exhibited the highest activity by efficiently extracting a balanced mix of phenolic compounds and flavonoids, whereas non-polar hexane mainly extracted lipophilic compounds with low antioxidant potential and highly polar butanol primarily extracted non-antioxidant sugars. Thus, the optimal solvent selectivity makes it more effective in enhancing antioxidant activity across DPPH, FRAP, and $ABTS^{\bullet+}$ assays. Comparatively, as reported by Jaiboonma *et al.*,[29] ethyl acetate extracts showed the highest FRAP activity (751.92 ± 5.22 mg AAE per g extract) and the strongest $ABTS^{\bullet+}$ scavenging activity (IC_{50} = 31.53 ± 0.36 μg mL^{-1}). A similar study was conducted by Mishra *et al.*,[56] in which PSE was extracted using water, diethyl ether, butanol and ethyl acetate, and the maximum DPPH free radical scavenging activity (61.07 ±

Table 1 Antioxidant activity of phenolic extract and synthetic antioxidants[a]

	DPPH$^{\bullet}$ IC_{50} (μg mL^{-1})	ABTS$^{\bullet+}$ IC_{50} (μg mL^{-1})	FRAP reduced percentage (%)
PSE	41.23 ± 0.29^{c}	29.31 ± 0.27^{c}	180.34 ± 1.03^{a}
BHT	213.76 ± 0.47^{b}	65.62 ± 0.55^{a}	45.36 ± 1.21^{c}
BHA	295.43 ± 1.40^{a}	54.21 ± 0.16^{b}	36.76 ± 1.24^{d}
TBHQ	44.35 ± 2.15^{c}	30.67 ± 0.38^{c}	153.31 ± 4.06^{b}

[a] Each data point represented mean ± SD ($n = 3$). Different lowercase letters showed a significant difference in the same column ($p < 0.05$). IC_{50}: half maximal inhibitory concentration, PSE: *Phyllanthus emblica* seed extract, BHT: butylated hydroxytoluene, BHA: butylated hydroxyanisole, and TBHQ: *tert*-butylhydroquinone.

1.63%) was achieved by the ethyl acetate fraction. The DPPH radical scavenging activity of the aqueous fraction (0.098 ± 0.98%) and butanol fraction (12.99 ± 2.25%) remained lower compared to the ethyl acetate fraction. The reason for the poor DPPH free radical scavenging activity of the aqueous extract of the seed may be that the major portion of phenolic acids and flavonoids present in the seed has poor solubility in water and hence could not be partitioned into water. According to our study, the antioxidant activity of the tested compounds followed the ranking order: PSE > TBHQ > BHT > BHA, which showed that PSE exhibited stronger antioxidant potential than the commonly used synthetic antioxidants BHT, BHA and TBHQ. The antioxidant activity of PSE is likely due to its content of flavonoids and phenolic compounds including myricetin, tannic acid, syringic acid, and coumaric acid.[64] However, TBHQ exhibited significantly ($p < 0.05$) higher antioxidant activity among other synthetic antioxidants. The highest activity of TBHQ can be attributed to its highly reactive structure, which enhances hydrogen donation and radical scavenging efficiency.[65] Phenolic compounds and flavanoids present in PSE are well known for their strong antioxidant potential, primarily due to their ability to neutralize free radicals and chelate metal ions.[66] These findings demonstrate that PSE is a highly effective natural antioxidant, with activity comparable to and in some cases exceeding that of synthetic antioxidants such as BHT and BHA.

The strong antioxidant activity of PSE is likely due to the combined and complementary effects of its phenolic compounds, including gallic, vanillic, sinapic, *p*-coumaric, and ellagic acids, as well as myricetin and quercetin. The hydroxybenzoic and hydroxycinnamic acids (such as gallic, vanillic, *p*-coumaric, and sinapic acids) act as fast hydrogen donors, neutralizing free radicals at both water-based and lipid interfaces.[67,68] Flavonols such as quercetin and myricetin, on the other hand, have strong chain-breaking abilities and can stabilize free radicals through resonance, thereby helping to protect lipid membranes from oxidative damage.[69] Ellagic acid enhances this protection by effectively binding metal ions, thereby reducing the formation of highly reactive radicals through Fenton-type reactions.[70] Moreover, the smaller phenolic acids can regenerate flavonols through redox interactions and extending the overall antioxidant activity of the mixture.[68] These combined mechanisms of phenolic acids and flavonoids create a synergistic effect that provides greater antioxidant protection than any single compound alone.[71] This synergy explains why the crude extract shows superior performance compared to individual synthetic antioxidants under real food storage conditions.

Extraction is a critical step in unlocking the potential of phenolic antioxidants from plant matrices, and recent advances in extraction technologies have enabled greater efficiency, reduced solvent consumption, lower energy usage, and shorter processing times. Among these, ultrasound-assisted extraction (UAE) has emerged as a "green" technique that employs acoustic cavitation to disrupt plant cell walls, enhance mass transfer, and improve the yield of target bioactive compounds. Studies have consistently shown that UAE significantly increases total phenolic content (TPC), total flavonoid content (TFC), and overall antioxidant activity compared to conventional solvent extraction. For instance, Yi *et al.*[72] demonstrated that sonication is more efficient than reflux or Soxhlet extraction, and that 80% methanol yields higher recovery of phenolic compounds than ethanol or other aqueous solvent mixtures, highlighting the importance of both solvent selection and extraction parameters. Similarly, for the leaves of *Phyllanthus emblica*, UAE achieved a phenolic yield of 56.82% compared to only 16.78% using conventional vortex mixing, illustrating the superior efficiency of ultrasound-assisted techniques. The high yield, reduced processing time, and lower solvent requirement make UAE highly suitable for further research studies.[73] The scalability and compatibility with green extraction principles further enhance its applicability for sustainable utilization of plant by-products and the development of natural antioxidant-enriched food systems.

3.4 Thermal stability

The thermal stability of antioxidants is crucial in determining their suitability for incorporation into baked products at baking temperatures, as antioxidants lose their stability due to their volatility at high temperatures. In this study, the thermal stability of the antioxidants at 200 μg mL^{-1} concentration was evaluated because it aligns with the recommended concentration for baked products.[74] The results revealed that the stability of all tested antioxidants was decreased after exposure to the high-temperature treatment. However, the retained thermal stability varied significantly among the samples. Notably, PSE exhibited the highest retained stability of 91% compared to all the synthetic antioxidants, including TBHQ (87%), BHT (62%), and BHA (63%). Mehta *et al.*[25] identified that PSE consists of tannins such as ellagic acid and punicalagin, which have the

ability to form stable intermolecular bonds to withstand high temperatures. Therefore, PSE may exhibit higher thermal stability. TBHQ demonstrates higher thermal stability than BHA and BHT due to its hydroquinone structure and low volatility, which provide stronger electron-donating properties and greater resistance to thermal degradation.[75] In contrast, BHA and BHT are more prone to oxidative cleavage at high temperatures due to their reactive hydroxyl groups, which break down under oxidative conditions.[75,76] They are also volatile compounds, making them prone to evaporation when exposed to heat and leading to a loss of antioxidant effectiveness.[76] This makes them less stable compared to natural antioxidants such as PSE. The thermal stability of the antioxidants can be ordered as PSE > TBHQ > BHA > BHT. This thermal resilience of PSE enhances its applicability as a natural alternative to synthetic antioxidants in baked products.

3.5 Antimicrobial activity

The investigated antioxidants at 200 μg mL^{-1} exhibited antibacterial activity against four foodborne pathogenic bacterial strains including two Gram-positive bacterial strains (*Staphylococcus aureus* and *Bacillus cereus*) and two Gram-negative bacterial strains (*Escherichia coli* and *Salmonella typhi*). The results of the antibacterial potential are presented in Table 2. As shown in Table 2, TBHQ showed significantly higher ($p < 0.05$) antibacterial inhibition among the tested antioxidants. In the present study, PSE showed zone inhibition against pathogenic bacteria as follows: *S. aureus* (8.83 ± 0.62 mm), *B. cereus* (14.17 ± 1.03 mm), *S. typhi* (7.50 ± 0.41 mm), and *E. coli* (10.83 ± 0.62 mm). The results revealed that the tested antioxidants have a more inhibitory effect on the growth of *S. aureus* due to their high sensitivity. Previous research by Anbuselvi and Jha[77] reported that the ethyl acetate fractions of crude PSE showed a 7.2 mm zone of inhibition against *S. aureus*, because bioactive compounds, including quercetin, phyllantine, phyllantidine, gallic acid, ascorbic acid, emblicanin A and emblicanin B in PSE, are responsible for targeting pathogenic bacteria that impede their growth.[26] Several studies have reported that Gram-positive bacterial strains are more sensitive to bioactive compounds present in extracts relative to Gram-negative bacterial strains,[37] owing to the cell walls of Gram-negative bacteria being constructed with lipopolysaccharides, which restrict the access of bioactive compounds in extracts.[78] Martin *et al.*[79] reported that ethanolic extracts of yerba mate showed antimicrobial activity against *S. aureus* and *L. monocytogenes* but not against *E. coli*. Among the tested strains, Gram-positive bacteria were much more sensitive to almost all the antioxidants, while BHA and BHT showed the lowest inhibition against Gram-negative bacteria strains (*E. coli* and *S. typhi*) (Table 2). An observational study by Walter *et al.*[80] presented that BHT was the least effective against Gram-negative bacteria such as *Salmonella* sp. and *E. coli* compared to the Gram-positive ones. Agada *et al.*[81] reported that BHA at 200 μg mL^{-1} was bactericidal to *S. aureus*, while *S. typhi* and *E. coli* were inhibited by 400 μg mL^{-1} of BHA. According to Shelef and Liang,[82] the effect of BHA was bacteriostatic at a concentration less than 200 μg mL^{-1}. By comparing the values, it can be stated that synthetic antioxidants BHT and BHA have significantly lower antibacterial activity than other tested antioxidants. Although the standard antibiotic (ampicillin) and TBHQ exhibited stronger inhibitory effects, PSE still demonstrated notable and broad antimicrobial activity in addition to its primary antioxidant function. This dual antioxidant and antibacterial activity highlights PSE as a promising multi-functional natural preservative for baked products.

3.6 *In vitro* cytotoxicity

The Caco-2 cell line is known for its ability to develop into cells resembling intestinal epithelium, which makes it a valuable model for testing the safety of antioxidants. It also helps in understanding how these compounds are absorbed and metabolized, and how they impact intestinal cells.[83] The higher IC_{50} suggest a higher cyto-compatibility effect over Caco-2 cells from the extracts, while lower IC_{50} represent higher cytotoxic activity. In the present study, cytotoxicity evaluation was focused on the concentration of 200 μg mL^{-1}, aligning with the recommended antioxidant concentration for incorporation into food products. After 72 h of incubation, TBHQ exhibited the lowest IC_{50} (53.48 ± 5.36 μg mL^{-1}) among the synthetic antioxidants (Table 3), followed by BHA (375.34 ± 0.14) and BHT (596.10 ± 2.35). A study by Esazadeh *et al.*[84] reported TBHQ-induced oxidative stress and apoptosis in intestinal epithelial cells with an IC_{50} value below 100 μg mL^{-1}, confirming high toxicity. Saito *et al.*[85] also confirmed that BHA and BHT induce ROS generation and disrupt cellular membranes, though with milder effects than TBHQ. In the present study, PSE exhibited the highest IC_{50} value (1025.37 ± 4.14), as displayed in Table 3. Martínez *et al.*[17] studied *Phyllanthus emblica* extract and

Table 2 Bacterial inhibition of phenolic extract and synthetic antioxidants[a]

	Diameter of the inhibition zone (mm)			
	S. aureus	*B. cereus*	*E. coli*	*S. typhi*
PSE	8.83 ± 0.62^{b}	14.17 ± 1.03^{a}	10.83 ± 0.62^{b}	7.50 ± 0.41^{c}
BHT	6.77 ± 0.21^{c}	6.00 ± 0.00^{d}	6.00 ± 0.00^{d}	6.00 ± 0.00^{d}
BHA	6.50 ± 0.00^{c}	6.00 ± 0.00^{d}	6.00 ± 0.11^{d}	6.00 ± 0.00^{d}
TBHQ	8.57 ± 0.33^{b}	6.57 ± 0.33^{c}	9.50 ± 0.41^{c}	8.00 ± 0.10^{b}
Ampicillin	11.50 ± 0.41^{a}	9.50 ± 0.40^{b}	19.50 ± 0.41^{a}	15.83 ± 0.24^{a}

[a] Each data point represented mean ± SD ($n = 3$). Different lowercase letters show significant differences in the same column ($p < 0.05$). PSE: *Phyllanthus emblica* seed extract, BHT: butylated hydroxytoluene, BHA: butylated hydroxyanisole, and TBHQ: *tert*-butylhydroquinone.

Table 3 Cytotoxicity of the phenolic extract and synthetic antioxidants toward Caco-2 cells[a]

Extract	IC_{50} value (μg mL^{-1}) 24 h	48 h	72 h
PSE	740.98 ± 1.25 ^{aC}	1167.11 ± 2.30 ^{aA}	1025.37 ± 4.14 ^{aB}
BHT	574.93 ± 2.41 ^{bC}	681.62 ± 4.21 ^{bA}	596.10 ± 2.35 ^{bB}
BHA	428.52 ± 3.64 ^{cB}	494.61 ± 5.27 ^{cA}	375.34 ± 0.14 ^{cC}
TBHQ	210.21 ± 1.21 ^{dA}	109.32 ± 0.92 ^{dB}	53.48 ± 5.36 ^{dC}

[a] Each data point represented mean ± SD ($n = 3$). Different lowercase letters show significant difference in the same column and different uppercase letters show significant difference in the same row ($p < 0.05$). PSE: *Phyllanthus emblica* seed extract, BHT: butylated hydroxytoluene, BHA: butylated hydroxyanisole, and TBHQ: *tert*-butylhydroquinone.

reported an IC_{50} > 1000 μg mL^{-1} in Caco-2 cells, similar to this study. Chowdhury *et al.*[86] found that *Phyllanthus niruri* extract showed mild cytotoxicity (IC_{50} – 900 μg mL^{-1}) in HepG2 cells, further confirming the low toxicity of *Phyllanthus* species. Harikumar and Kuttan[87] demonstrated that *Phyllanthus amarus* extract protects intestinal cells from oxidative stress, contrasting with the pro-oxidant nature of synthetic antioxidants. The results revealed that the PSE, BHT, and BHA cell viability increased up to 48 h and then gradually decreased after 72 h. However, the cell viability of TBHQ was decreased with time because TBHQ can get metabolized into *tert*-butyl-*p*-benzoquinone, which is more toxic and highly volatile resulting in the induction of apoptosis in affected cells.[88] Furthermore, Fig. 2 illustrates the effect of PSE and synthetic antioxidants on the morphology of Caco-2 cells over 24 h, 48 h, and 72 h of incubation at 200 μg mL^{-1}. The micrographs indicate that the untreated control cells maintained viable cells and consistent growth throughout the observation period, confirming the absence of any external cytotoxic influence. Similarly, PSE-treated cells exhibited active cell growth with no significant signs of cytotoxicity over time, suggesting high cell viability and low toxicity. In contrast, cells treated with synthetic antioxidants displayed varying degrees of morphological alterations indicative of cytotoxic effects. BHT-treated cells showed mild cytotoxic effects with slightly reduced cell density over time. BHA treatment resulted in noticeable cell damage at 72 h compared to 24 h and 48 h, suggesting that BHA induces cytotoxicity upon prolonged exposure. Importantly, TBHQ-treated Caco-2 cells exhibited increased cell shrinkage, apoptotic bodies and membrane blebbing alongside a yellowish-red discoloration of the media. Thus, TBHQ may cause severe oxidative stress and cytotoxicity. These effects are consistent with previous findings by Khezerlou *et al.*,[89] who reported similar cytotoxic and oxidative stress-related changes in cells exposed to TBHQ. The observed cytotoxicity trend was TBHQ > BHA > BHT > PSE. TBHQ and BHA are widely used synthetic antioxidants that have been previously reported to induce oxidative stress and trigger apoptosis in cells in the gastrointestinal tract.[84] Similarly, BHA and BHT are known to exert cytotoxic effects due to their ability to disrupt cellular membranes and induce ROS production.[85] In contrast, PSE is

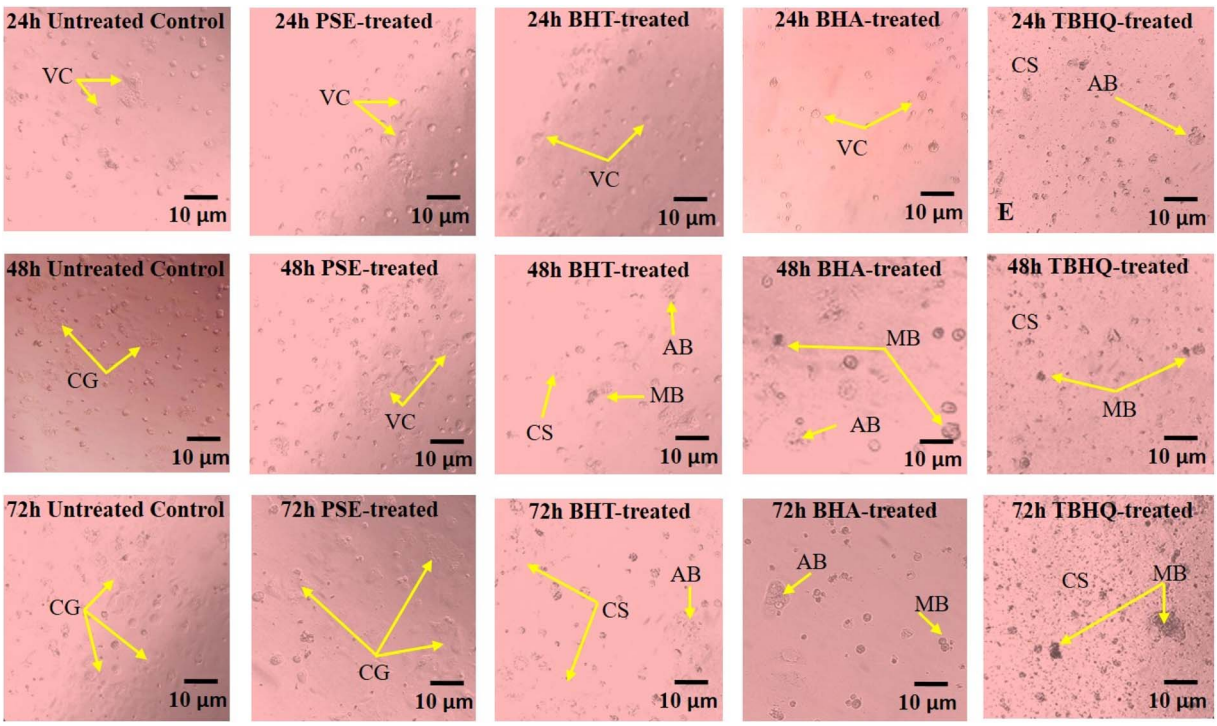

Fig. 2 Microscopic-view of morphological changes of Caco-2 cell line at 24 h, 48 h, and 72 h of incubation after treated with antioxidants at 200 μg mL^{-1} under 10× magnification power. PSE: *Phyllanthus emblica* seed extract, BHT: butylated hydroxytoluene, BHA: butylated hydroxyanisole, and TBHQ: *tert*-butylhydroquinone. VC = viable cells, CG = cell growth, AB = apoptotic body, MB = membrane blebbing, and CS = cell shrinkage. Scale bar:10 μm.

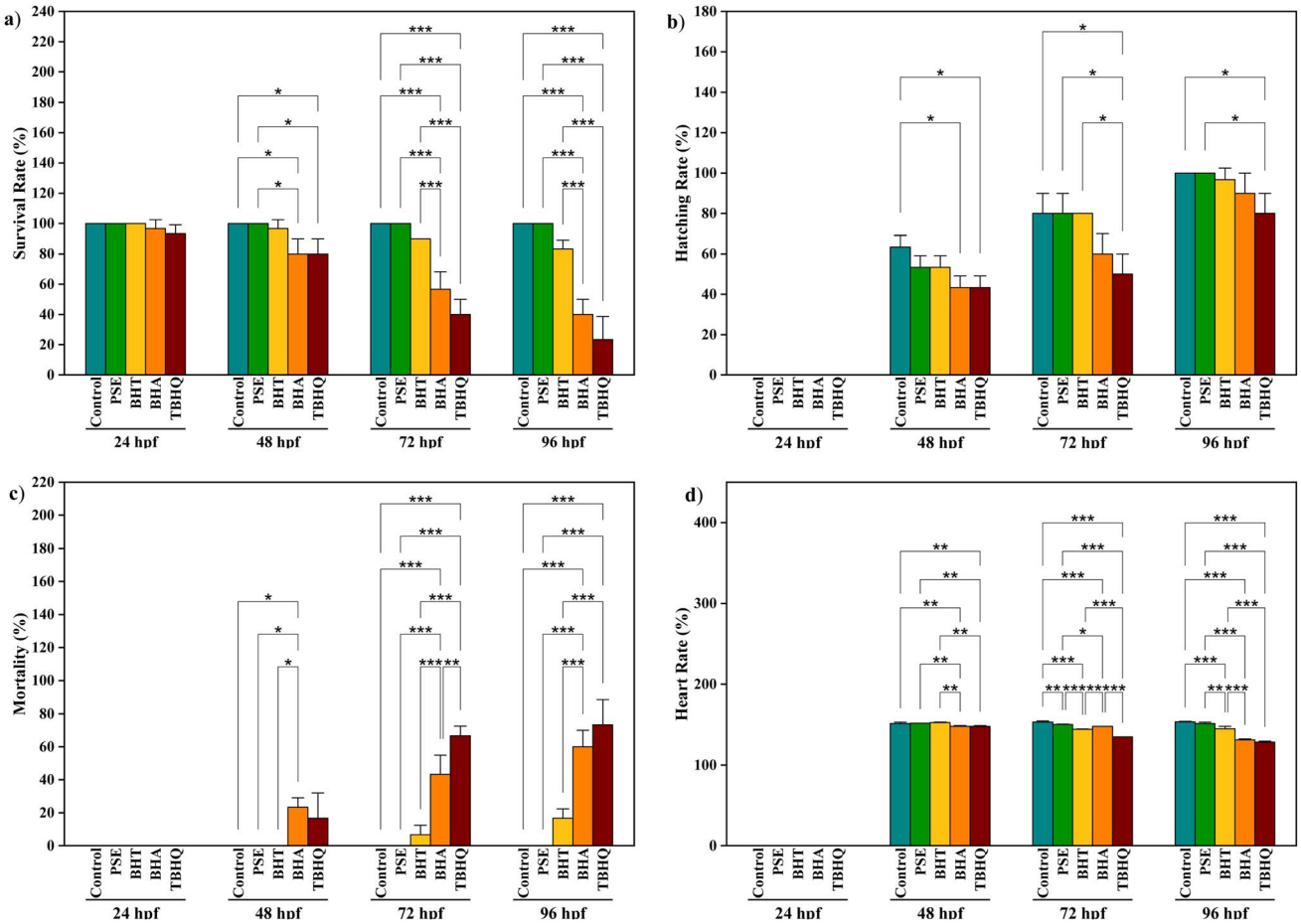

Fig. 3 Toxic effect of zebrafish embryos at 24, 48, 72, and 96 hpf exposed to antioxidants: (a) survival rate, (b) hatching rate (c) mortality, and (d) heartbeat rate. PSE: *Phyllanthus emblica* seed extract, BHT: butylated hydroxytoluene, BHA: butylated hydroxyanisole, and TBHQ: *tert*-butylhydroquinone. Data represent the mean ± SD of replicates ($n = 10$ embryos/group). Asterisk (*) indicates significant difference between groups ($p < 0.05$).

derived from a natural source that displays significantly lower cytotoxicity. The reduced cytotoxicity of PSE can be attributed to the presence of polyphenols such as tannins and flavonoids, which not only provide antioxidant activity but also exhibit protective effects against oxidative stress-induced cell damage.[90]

3.7 *In vivo* toxicity

Several studies have reported that high doses of polyphenolic-rich foods can potentially cause adverse effects through pro-oxidative effects.[91] The toxicological assessment of *Danio rerio* embryos exposed to natural and synthetic antioxidants at 24, 48, 72, and 96 hpf revealed distinct differences in their development. As shown in Fig. 2, embryos treated with PSE demonstrated consistently high survival rates (>90%), low mortality (<10%) and hatching rates exceeding 85% by 72 hpf. In contrast, embryos exposed to TBHQ exhibited significant developmental abnormalities. By 96 hpf, survival dropped below 50%, mortality exceeded 40% and hatching remained under 30%. Embryos treated with BHA and BHT showed intermediate levels of toxicity. BHT-exposed embryos had a survival rate of 70%, hatching rate of 60–65% and mortality of around 25%. In comparison, BHA exposure led to a slightly higher toxicity, with survival falling to 60%, hatching to 50%, and mortality nearing 35%. The normal heartbeat rate of zebrafish embryos ranges from 120 to 180 beats per minute (bpm).[92] The heartbeat rate of embryos exposed to antioxidants at 200 μg mL^{-1} was evaluated. The heartbeat evaluation started at 48 hpf because their heart begins to beat normally by 48 hpf.[93] As shown in Fig. 3, there was no significant ($p > 0.05$) difference in the mean heartbeat rate (bpm) of PSE-treated embryos (150.00 ± 2.05) compared to the control group (154.00 ± 0.47). In contrast, BHT, BHA, and TBHQ exhibited dose-dependent cardiotoxic effects, with TBHQ (128.00 ± 1.25) showing the most significant reduction in heart rate, followed by BHA (132.00 ± 1.25) and BHT (145.00 ± 2.49). These results suggest that PSE exhibits a high level of biocompatibility and minimal toxicity. This effect may be attributed to its richness in phenolic compounds, particularly gallic acid, tannic acid, myricetin, and syringic acid, which possess strong free radical scavenging properties and contribute to the enhancement of cellular defense mechanisms during early embryonic development.

LC_{50} refers to the concentration of a substance required to cause mortality in 50% of a test population, typically within a specified exposure period. A lower LC_{50} value indicates higher

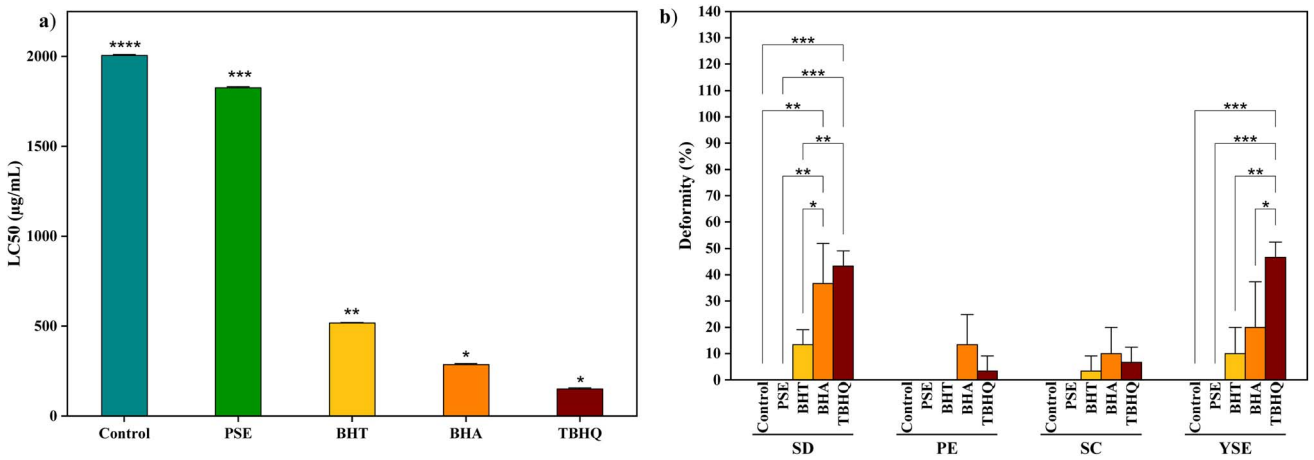

Fig. 4 Median lethal concentration (LC_{50}) and heartbeat rate of zebrafish embryos at 96 hpf exposed to antioxidants: (a) LC_{50} and (b) heartbeat rate. PSE: *Phyllanthus emblica* seed extract, BHT: butylated hydroxytoluene, BHA: butylated hydroxyanisole, and TBHQ: *tert*-butylhydroquinone. Data represent the mean ± SD of replicates ($n = 10$ embryos/group). Asterisk (*) indicates significant difference between groups ($p < 0.05$).

toxicity, while a higher LC_{50} value reflects lower toxicity. Fig. 4 represents a comparison of the LC_{50} (µg mL^{-1}) of PSE with synthetic antioxidants: BHT, BHA, and TBHQ over post-fertilization. PSE consistently shows the highest LC_{50} at 96 hpf, indicating the lowest toxicity among the tested antioxidants. Maintaining an LC_{50} threshold above 200 µg mL^{-1} is essential for our study as this level indicates low toxicity and aligns with the recommended concentration limits for safe food application.

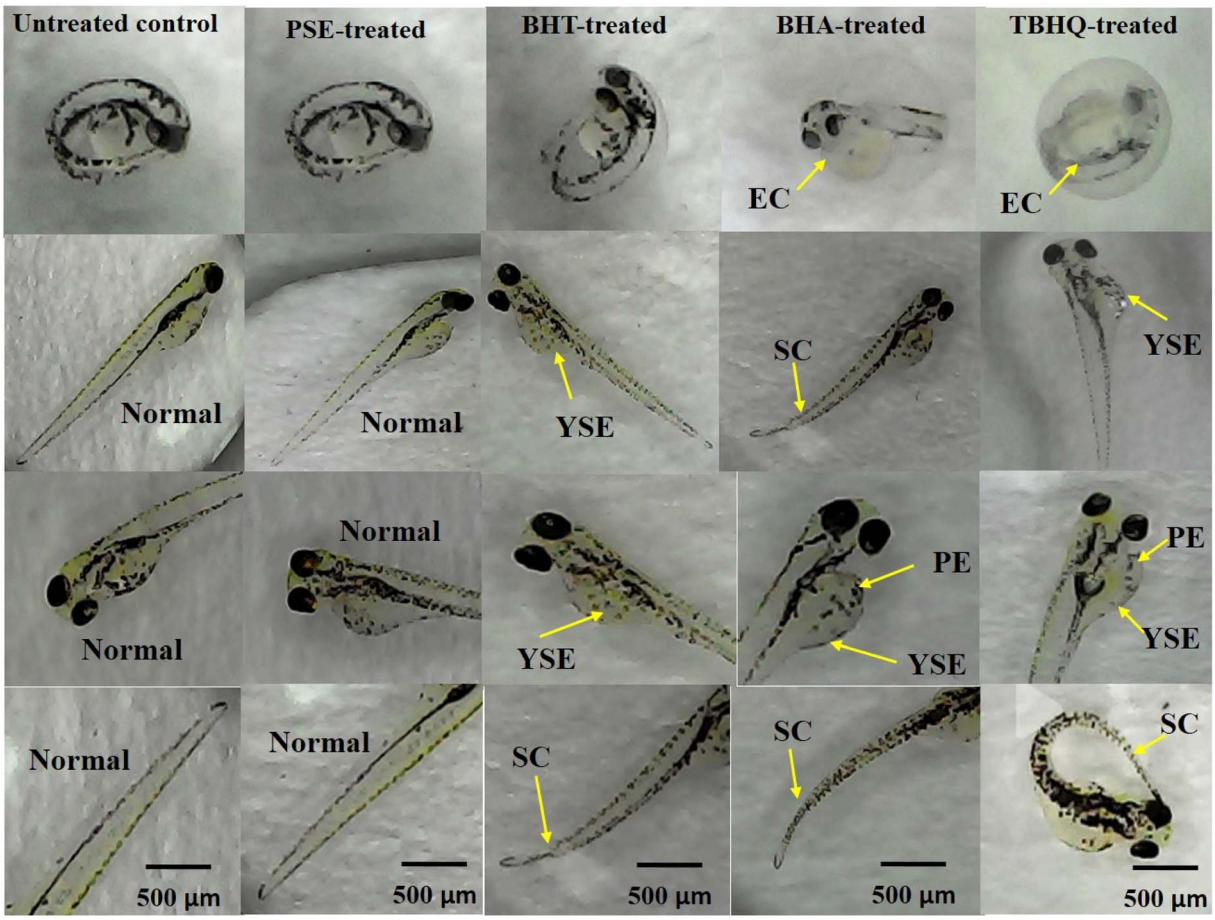

Fig. 5 Optical images of deformed zebrafish embryos and larvae at 96 hpf exposed to antioxidants. PSE: *Phyllanthus emblica* seed extract, BHT: butylated hydroxytoluene, BHA: butylated hydroxyanisole, and TBHQ: *tert*-butylhydroquinone. Malformations are indicated by arrows. EC = egg coagulation, SC = spinal curvature, YSE = yolk sac edema, and PE = pericardial edema. Scale bar: 500 µm.

Synthetic antioxidants show significantly ($p < 0.05$) lower LC_{50} due to higher toxicity over time compared to PSE. Among the synthetic antioxidants tested, TBHQ was found to be the most toxic with an LC_{50} of 189.25 ± 1.27 μg mL^{-1} at 96 hpf, which falls below the safety threshold of 200 μg mL^{-1}. BHT showed the lowest toxicity (400.21 ± 0.45 μg mL^{-1}), while BHA (204.28 ± 0.28 μg mL^{-1}) was more harmful than BHT but still less toxic than TBHQ. These results emphasize the importance of identifying safer alternatives with LC_{50} values above 200 μg mL^{-1}. Promisingly, the current study found that PSE had an LC_{50} of 1832.71 ± 1.22 μg mL^{-1} with a significantly safer profile compared to synthetic antioxidants. Furthermore, Yang *et al.*[94] conducted research by exposing zebrafish embryos to four synthetic phenolic antioxidants: BHA, BHT, TBHQ, and 2,2′-methylenebis (6-*tert*-butyl-4-methylphenol) (AO2246). The 96 hpf LC_{50} values indicated varying degrees of acute toxicity, with the toxicity ranking as AO2246 > TBHQ > BHA > BHT. Notably, exposure to BHA, TBHQ, and AO2246 resulted in decreased hatching rates because non-lethal concentrations of BHA (≤20 μM), TBHQ (≤20 μM), BHT (≤200 μM), and AO2246 (≤2 μM) resulted in reduced heart rates and body lengths in a dose-dependent manner.

Fig. 5 reveals distinct differences in developmental toxicity among the tested antioxidants. Embryos exposed to PSE exhibited predominantly normal morphology, similar to the untreated control, resulting in minimal toxic effects. In contrast, embryos exposed to synthetic antioxidants such as BHT, BHA, and TBHQ showed visible signs of developmental toxicity. Malformations such as spinal curvature, yolk sac edema, pericardial edema, and even egg coagulation were commonly observed, especially in the BHA- and TBHQ-treated groups (Fig. 5). These findings are in agreement with those of Leonard *et al.*[95] and Yang *et al.*,[94] who observed that TBHQ disrupts redox homeostasis, induces apoptosis, and causes morphological deformities in zebrafish embryos such as pericardial edema and spinal curvature. Also, Baran *et al.*[15] and Chen *et al.*[96] reported that both BHA and BHT can interfere with antioxidant enzyme systems, delay embryonic development, and increase the incidence of malformations such as yolk sac edema and axial deformities. These deformities suggest that synthetic antioxidants may interfere with normal embryonic development and raising concerns about their safety. In contrast, the natural extract appeared to be much safer for reinforcing its potential as a more biocompatible and less toxic alternative for food applications.

3.8 Effect of antioxidants on the shelf-life of baked products

3.8.1 Baked product properties. The proximate composition of both sponge cakes and biscuits prepared according to the previously described procedures including moisture, ash, fat, protein, fiber and carbohydrate contents aligned with reported standards,[42,43] as shown in Table 4. Importantly, the antioxidants had no significant effect ($p > 0.05$) on the nutritional properties of biscuits, as reported by Caleja *et al.*[97] The moisture content and the fat percentage are the major aspects of this study because they are primarily responsible for the stability of baked products. According to the results obtained, the moisture content of sponge cakes and biscuits was 14.47 ± 0.12% and 8.53 ± 0.94%, respectively. The higher moisture content of sponge cakes was more prone to spoilage by microbial growth and provided a soft texture compared to the biscuits.[98] The fat content of sponge cakes and biscuits was 29.69 ± 0.11% and 22.55 ± 0.20%, respectively, and significantly influenced their sensory and structural properties. However, the higher fat content increases the risk of lipid oxidation, which can lead to rancidity and off-flavors during storage of baked products.[99]

Table 5 shows that both sponge cakes and biscuits are not significantly ($p > 0.05$) different in resilience, cohesiveness, springiness, and color except hardness and chewiness. When considering the hardness, there is a significant ($p < 0.05$) difference across all sponge cakes and biscuits. In both sponge cakes and biscuits containing PSE, the hardness was lower than that of products containing synthetic antioxidants. The reduced hardness in PSE-added samples could be attributed to the inherent properties of the natural phenolic compounds, which may interact with the product matrix to retain moisture content and prevent excessive stiffness during storage.[100] For example, research by Sulaiman *et al.*[101] found that sponge cakes containing phenolic extracts from mango peel exhibited lower hardness than those containing synthetic antioxidants, likely due to the moisture-retaining capacity of phenolic compounds. Similarly, biscuits formulated with moringa leaf extract showed reduced hardness compared to BHT-containing biscuits.[102]

As depicted in Table 5, the cohesiveness and springiness values of both sponge cakes and biscuits were not significantly ($p > 0.05$) different from each other in samples containing antioxidants as well as the controls. A study by de la Rosa *et al.*[103] examined the addition of phenolic compounds to bread and found that these natural antioxidants did not significantly affect the cohesiveness and springiness of the bread, indicating that the internal structure remained stable. Similar results were reported by Zhang *et al.*,[10] where the addition of 1%, 2%, 5% of grape seed extract in wheat-based cakes did not significantly affect cohesiveness and springiness compared to control samples. The chewiness of PSE-added products was moderate. The results were lower than synthetic antioxidant-added samples but higher than the TBHQ-added sponge cakes and

Table 4 Proximate composition of control baked products g/100 g[a]

	Moisture	Ash	Fat	Protein	Fiber	Carbohydrate
Sponge cake	14.47 ± 0.12	1.03 ± 0.03	29.69 ± 0.11	10.19 ± 0.46	1.38 ± 0.07	43.23 ± 0.23
Biscuits	8.53 ± 0.94	0.92 ± 0.23	22.55 ± 0.20	0.92 ± 0.47	2.97 ± 0.33	60.49 ± 0.18

[a] Each data point represented mean ± SD ($n = 3$).

Table 5 Physical parameters of control and antioxidant-added baked products[a]

	Control	BHT-added	BHA-added	TBHQ-added	PSE-added
Sponge cake					
Hardness (g)	2564.50 ± 0.15^{b}	2648.00 ± 0.24^{a}	2640 21 ± 0.27^{a}	2456.33 ± 0.11^{c}	2325.33 ± 1.35^{d}
Resilience	0.29 ± 0.45^{a}	0.30 ± 0.70^{a}	0.25 ± 0.20^{a}	0.27 ± 1.25^{a}	0.28 ± 0.64^{a}
Cohesiveness	0.82 ± 0.05^{a}	0.79 ± 0.15^{a}	0.80 ± 0.00^{a}	0.81 ± 0.07^{a}	0.79 ± 1.62^{a}
Springiness (mm)	0.72 ± 0.64^{a}	0.69 ± 0.06^{a}	0.65 ± 1.32^{a}	0.70 ± 0.08^{a}	0.73 ± 0.64^{a}
Chewiness (mJ)	356.22 ± 0.17^{b}	400.33 ± 0.71^{a}	435.66 ± 0.10^{a}	236.33 ± 0.91^{d}	296.35 ± 0.01^{c}
Color L^*	76.21 ± 0.78^{a}	71.56 ± 0.61^{a}	75.50 ± 0.27^{a}	74.47 ± 0.42^{a}	75.28 ± 0.15^{a}
a^*	3.93 ± 0.06^{a}	3.90 ± 0.31^{a}	3.90 ± 0.03^{a}	4.03 ± 0.15^{a}	3.93 ± 0.06^{a}
b^*	24.37 ± 0.50^{a}	25.60 ± 0.58^{a}	23.85 ± 0.29^{a}	24.04 ± 0.25^{a}	24.37 ± 0.50^{a}
Biscuits					
Hardness (g)	5075.00 ± 1.11^{c}	5167.00 ± 0.16^{b}	5644.00 ± 0.60^{a}	4967.0 ± 0.10^{d}	4658.26 ± 1.15^{e}
Adhesiveness (mJ)	92.00 ± 0.33^{a}	88.00 ± 0.27^{a}	85.67 ± 0.19^{a}	89.37 ± 0.11^{a}	90.84 ± 0.61^{a}
Resilience	0.04 ± 0.10^{a}	0.03 ± 0.09^{a}	0.04 ± 0.34^{a}	0.02 ± 0.05^{a}	0.02 ± 0.55^{a}
Cohesiveness	1.11 ± 0.13^{a}	1.24 ± 0.87^{a}	1.04 ± 0.88^{a}	1.13 ± 0.45^{a}	1.05 ± 0.05^{a}
Springiness (mm)	29.22 ± 0.55^{a}	30.45 ± 0.50^{a}	28.64 ± 0.15^{a}	32.88 ± 0.41^{a}	31.68 ± 0.44^{a}
Chewiness (mJ)	1618.80 ± 0.05^{b}	1645.99 ± 0.14^{a}	1538.36 ± 0.60^{d}	1534.72 ± 0.10^{d}	1572.20 ± 1.22^{c}
Color L^*	67.40 ± 1.05^{a}	65.12 ± 0.45^{a}	65.64 ± 0.22^{a}	66.28 ± 0.16^{a}	67.34 ± 0.15^{a}
a^*	1.64 ± 0.04^{a}	0.98 ± 0.44^{a}	1.22 ± 0.08^{a}	0.88 ± 0.17^{a}	0.97 ± 0.34^{a}
b^*	25.23 ± 0.47^{a}	24.00 ± 0.50^{a}	25.33 ± 1.25^{a}	24.28 ± 0.33^{a}	24.89 ± 0.12^{a}

[a] Each data point represented mean ± SD ($n = 3$). Different lowercase letters show significant differences in the same row ($p < 0.05$), L^*, lightness; a^*, redness/greenness; b^*, yellowness/blueness. PSE: *Phyllanthus emblica* seed extract, BHT: butylated hydroxytoluene, BHA: butylated hydroxyanisole, and TBHQ: *tert*-butylhydroquinone.

biscuits (Table 5). A similar study by de la Rosa *et al.*[103] also found that the inclusion of phenolic compounds in bread formulations resulted in moderate chewiness due to interactions between phenolic compounds and gluten proteins, which altered the dough's rheological properties.

Importantly, the addition of antioxidants did not impact the color parameters (L^*, a^*, b^*) of sponge cakes and biscuits. This indicates that it doesn't cause significant discoloration or browning. This finding is crucial for consumer acceptability because color serves as a primary indicator of freshness and quality.[104] De la Rosa *et al.*[103] also reported that the addition of phenolic compounds did not significantly alter the color parameters of the bread, indicating no noticeable discoloration or browning.

3.8.2 Oxidative stability of baked products. The formation of primary and secondary oxidation products is a key indicator of lipid oxidation and serves as a critical measure of the oxidative stability and shelf-life of baked products, directly influencing their quality and consumer acceptance. In the present study, both sponge cakes and biscuits without antioxidants (control samples) showed a significant ($p < 0.05$) increase in PV and TBARS over the storage period. Fig. 6a shows that the peroxide value (PV) of PSE-added sponge cakes (0.71 meq per kg) was not significantly different ($p > 0.05$) from that of TBHQ-added sponge cakes (0.78 meq per kg). But, PSE-added cakes had significantly ($p < 0.05$) lower PVs than those with BHT (0.88 meq per kg), BHA (1.19 meq per kg), and the control (2.26 meq per kg). Similarly, as depicted in Fig. 6c, the control cakes displayed a marked increase in TBARS during storage. After 28 days, PSE-added sponge cakes maintained the lowest TBARS (1.72 ± 0.05 mg kg^{-1}), which was significantly lower than those containing BHT (2.41 ± 0.04 mg kg^{-1}), BHA (2.79 ± 0.04 mg kg^{-1}), and TBHQ (2.00 ± 0.02 mg kg^{-1}). In biscuits (Fig. 6b), the peroxide values of PSE-added (2.04 meq per kg), BHT-added (2.30 meq per kg), and TBHQ-added (2.21 meq per kg) samples showed no significant ($p > 0.05$) differences after 84 days of storage. As shown in Fig. 6d, TBARS values increased during storage, with the control showing the highest level (2.46 ± 0.04 mg kg^{-1}). However, biscuits containing PSE had the lowest TBARS value (1.31 ± 0.01 mg kg^{-1}) after 84 days, which was significantly ($p < 0.05$) lower than those with BHT (2.46 ± 0.04 mg kg^{-1}), BHA (2.10 ± 0.02 mg kg^{-1}), and TBHQ (1.48 ± 0.01 mg kg^{-1}).

The findings of this study align with those of Kozłowska *et al.*,[105] who reported that bakery fats with peroxide values (PVs) exceeding 3 meq per kg can still maintain acceptable quality, as moderate PV levels do not necessarily correlate with sensory deterioration. In their study, the addition of 1.0% green tea extract (GTE) significantly reduced lipid oxidation, resulting in a PV of 3.2 meq per kg after 28 days, compared to 4.2 meq per kg in cakes containing 0.02% BHT and 6.0 meq per kg in control cakes. Similarly, Zaky *et al.*[106] observed a significant ($p < 0.05$) increase in PV in biscuits during storage, with control samples showing the highest PV of 21.84 meq per kg by the end of the storage period. Biscuits containing 0.5%, 1%, 2%, and 3% grape pomace extract (GPE) showed PVs ranging from 1.38 ± 0.03 to 12.11 ± 0.04 meq per kg, whereas those with BHA ranged from 1.45 ± 0.03 to 15.13 ± 0.05 meq per kg. After six months, PV values for the GPE-added biscuits were 12.11 ± 0.07, 11.08 ± 0.08, 7.54 ± 0.02, and 6.31 ± 0.06 meq per kg, respectively, compared to 15.13 ± 0.05 meq per kg for the BHA-added biscuits. In the same study, Zaky *et al.*[106] also compared the effects of BHA and GPE on TBARS levels in biscuits over six months. Initially, GPE caused a slight reduction in TBARS

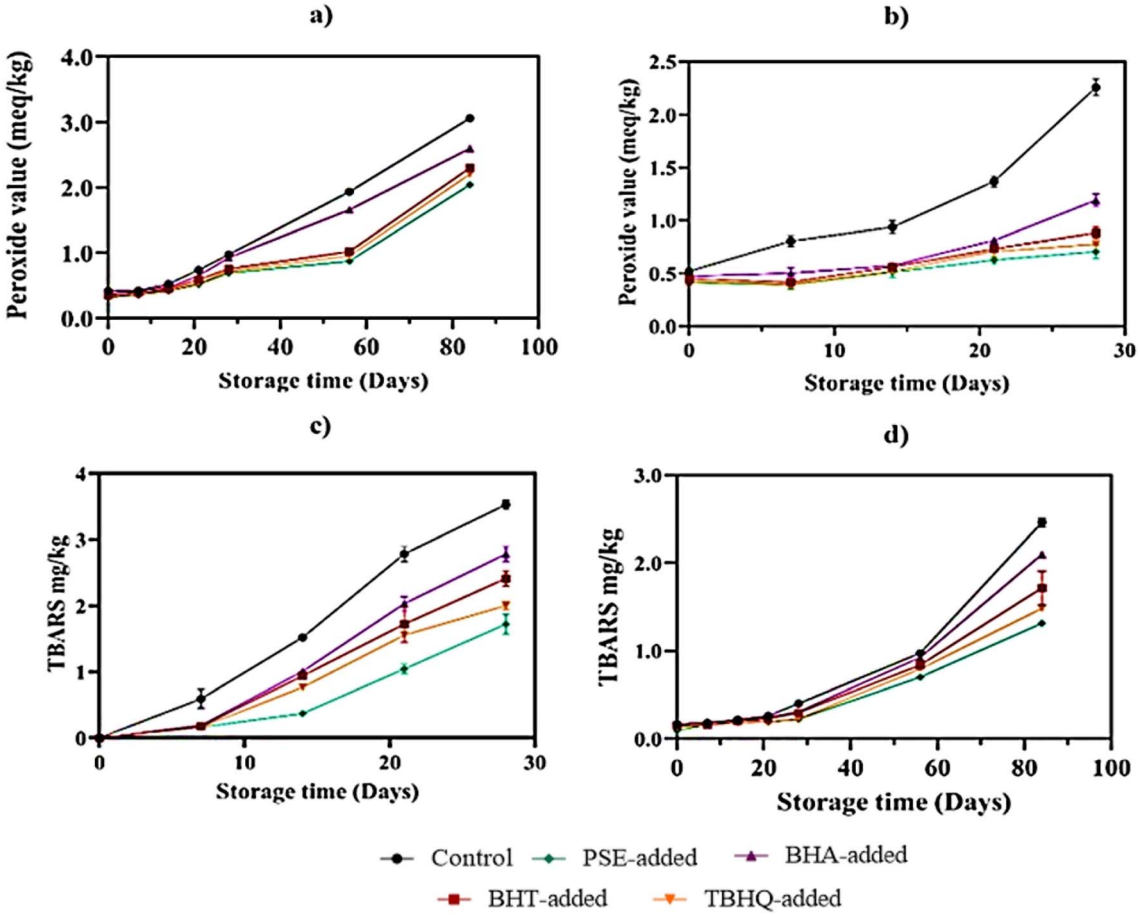

Fig. 6 Effect of antioxidants on primary and secondary oxidation in baked products during storage at room temperature. (a) Formation of peroxides in sponge cakes. (b) Formation of peroxides in biscuits. (c) Formation of TBARS in sponge cakes. (d) Formation of TBARS in biscuits. PSE: *Phyllanthus emblica* seed extract, BHT: butylated hydroxytoluene, BHA: butylated hydroxyanisole, and TBHQ: *tert*-butylhydroquinone.

values (0.381–0.377 mg kg^{-1}) compared to the control (0.385 mg kg^{-1}) and BHA (0.383 mg kg^{-1}). Over time, GPE demonstrated the greatest protective effect, retaining TBARS values below 0.563 mg kg^{-1}, while the control and BHA samples reached 0.805 mg kg^{-1} and 0.669 mg kg^{-1}, respectively.

According to the current study, PSE-added baked products were more effective than those containing synthetic antioxidants such as BHT and BHA in reducing lipid oxidation. The lower PV and TBARS values indicate that the phenolic acids and flavonoids present in PSE can readily donate hydrogen atoms to free radicals, thereby interrupting the oxidative chain reaction and enhancing oxidative stability.[107]

3.8.3 Microbial quality. Baked products are usually safe from harmful microbes because baking at high temperatures kills most pathogens. However, contamination can still happen during packaging and storage, which may affect the quality of the products.[108] According to FDA 2013, the microbial shelf-life of baked products is determined based on the time taken for the APC to exceed 1.0×10^6 CFU g^{-1} and the YMC to exceed 1.0×10^4 CFU g^{-1}.[109] In Table S2, the control sponge cakes without antioxidants had the shortest shelf-life because of exceeding the acceptable APC (9.9×10^4) and YMC (1.0×10^3) within 6 days. In contrast, sponge cakes containing synthetic antioxidants exhibited APC and YMC of 1.0×10^6 and 5.1×10^3 for BHT, and 9.7×10^3 and 1.0×10^4 for BHA, extending the microbial shelf-life to 9 days. Remarkably, sponge cakes containing PSE (9.2×10^5, 1.2×10^4) and TBHQ (1.0×10^6, 1.0×10^4) showed the longest shelf-life by remaining the APC and YMC below thresholds and shelf-life increased up to 12 days. Similarly, as depicted in Table S3, the microbial shelf-life of biscuits demonstrated a clear benefit from antioxidant addition. Control biscuits without antioxidants were safe for 28 days with APC and YMC as 8.9×10^5 and 4.8×10^3, respectively. The biscuits with BHA extended the shelf-life to 42 days based on APC (6.0×10^5), while YMC (9.1×10^3) allowed for up to 56 days. BHT (1.0×10^6, 1.0×10^4) addition further prolonged the microbial shelf-life to 70 days. Notably, biscuits with PSE (8.0×10^5, 9.0×10^3) or TBHQ (9.0×10^5, 1.0×10^4) exhibited the highest microbial stability by maintaining safety for up to 84 days with respect to APC and YMC levels.

A study by Senanayake *et al.*[53] evaluated the effects of coconut oil meal (CME) and sesame oil meal (SME) phenolic extracts (20 mg/100 g of margarine) on the shelf life of vanilla cake. The findings revealed that both CME and SME extended the

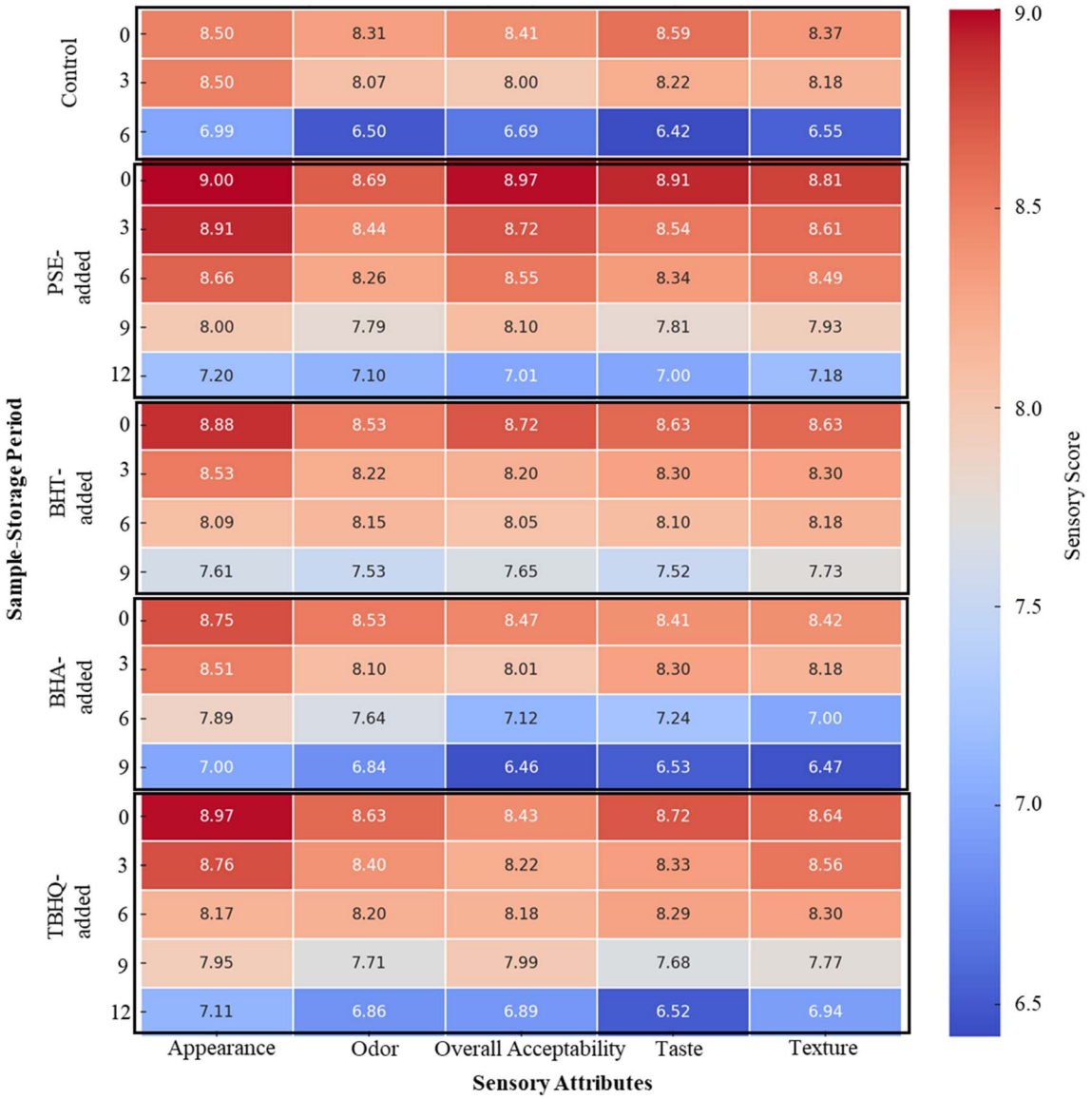

Fig. 7 Heat map of the sensory preference of the panelists for control and antioxidant-added cakes during the storage period according to sensory scores based on an 11-point hedonic scale (1 = greatest imaginable dislike and 11 = greatest imaginable like). PSE: *Phyllanthus emblica* seed extract, BHT: butylated hydroxytoluene, BHA: butylated hydroxyanisole, and TBHQ: *tert*-butylhydroquinone.

microbial shelf life of the cakes up to 13 days. In contrast, cakes with no added antioxidants (control) and those with the synthetic antioxidant butylated hydroxytoluene (BHT) exceeded the maximum allowed microbial counts by day 7 and day 11, respectively. This suggests that CME and SME are effective natural alternatives to synthetic antioxidants in enhancing both microbiological and chemical stability of cakes during storage.

Current research findings suggest that, baked products enriched with antioxidants demonstrated notable antimicrobial activity compared to control products without added antioxidants. Among the tested samples, those containing PSE and TBHQ exhibited significantly ($p < 0.05$) higher antimicrobial effects suggesting their potential in enhancing the microbial safety of baked goods during storage and reducing the risk of spoilage caused by microbial contamination.

3.8.4 Sensory quality. The sensory properties of baked products containing phenolic and synthetic antioxidants were evaluated using a 30 semi-trained panel. The data of the sensory perceptions are presented in Fig. 7 and 8. The sensory evaluation was conducted when the maximum allowable microbial activity was not exceeded. The mean sensory scores indicated that sponge cakes containing PSE showed no significant differences ($p > 0.05$) in color, odor, taste, texture, and overall acceptability compared to the control, and those containing BHT, BHA, and TBHQ throughout the storage period. Although all samples exhibited some decline in sensory attributes such as color, odor, taste, texture, and overall acceptability over time, control samples deteriorated the fastest, showing significantly ($p < 0.05$) lower scores by day 6 (Fig. 7). Among the synthetic antioxidants, TBHQ showed better performance in preserving

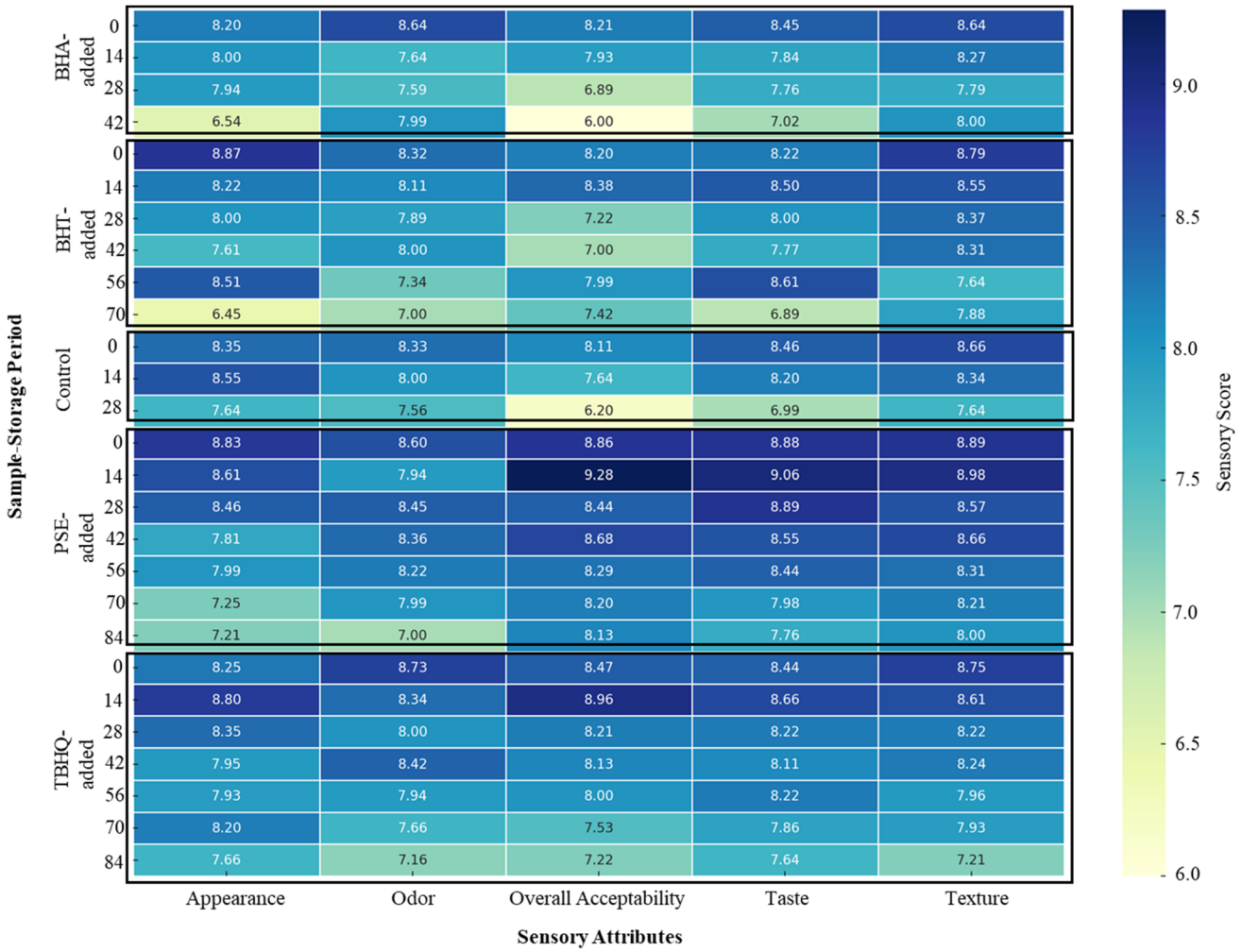

Fig. 8 Heat map of the sensory preference of the panelists for control and antioxidant-added biscuits during the storage period according to sensory scores based on an 11-point hedonic scale (1 = greatest imaginable dislike and 11 = greatest imaginable like). PSE: *Phyllanthus emblica* seed extract, BHT: butylated hydroxytoluene, BHA: butylated hydroxyanisole, and TBHQ: *tert*-butylhydroquinone.

sensory properties compared to BHT and BHA, though a noticeable decline was observed by day 12. In contrast, PSE-added samples displayed the least reduction in sensory quality, with higher scores across all attributes, particularly in taste and overall acceptability, even at day 12 (Fig. 7). The research outcome was similar to the study by Caleja *et al.*,[97] which examined the use of fennel and chamomile extracts as natural antioxidants in biscuits and compared their performance with BHT. The incorporation of both phenolic extracts and BHT did not cause significant changes in the sensory properties of biscuits over 60 days compared to control samples. This suggests that natural additives can serve as convenient alternatives for consumers seeking products free from synthetic additives.

In the sensory evaluation of biscuits, as shown in Fig. 8, the sensory properties of control biscuits were significantly ($p < 0.05$) decreased by day 28. This degradation of color, odor, taste, texture, and overall acceptability is due to the microbial contamination, lipid oxidation and extreme storage conditions. Among all the synthetic antioxidants, the sensory attributes of the BHA-added biscuits significantly ($p < 0.05$) decreased within the storage. However, the overall acceptability remained above acceptable levels until day 42 (Fig. 8). TBHQ-added biscuits demonstrated better sensory stability compared to BHA and BHT-added biscuits. Color, odor, and taste showed minor changes up to day 42, with significant reductions becoming apparent after day 56. In contrast, PSE-added biscuits maintained higher acceptability throughout storage with minimal reductions even after 84 days. The overall acceptability remained high with only a slight decline from day 0 to day 84 (Fig. 8).

These results are consistent with the findings of Hefnawy and El-Shourbagy,[110] who evaluated the sensory properties of biscuits prepared with water extract of grape leaf (GLE), ethanolic (70%) extract of carrot (CE), and water extract of turmeric (TPE) over a two-month storage period at 25 ± 2 °C. The biscuits were formulated with either 200 ppm of synthetic antioxidants (BHA and TBHQ) or 1% (w/w) of the plant extracts. The study found that biscuits containing phenolic extracts were well accepted in terms of color, crumb color, texture, and mouthfeel,

showing sensory characteristics comparable to both the control samples and those containing BHA and TBHQ. This research study indicates that addition of natural antioxidants does not affect the sensory parameters of baked products. For instance, a study by Kozłowska *et al.*[105] on sponge cakes fortified with 1.0% green tea extract (GTE) showed that while some consumers detected a slight difference in flavor, the overall acceptability remained high. Moreover, Zaky *et al.*[106] evaluated the effect of 0.5%, 1%, 2%, and 3% grape pomace extract (GPE) on the sensory characteristics of biscuits and found no significant differences in sensory scores between GPE-enriched and control biscuits. These findings suggest that the addition of natural antioxidants do not adversely affect sensory quality. The moderate reduction in hardness and chewiness in PSE-added sponge cakes and biscuits may be perceived as a positive attribute, indicating a softer and more pleasant mouthfeel. This contributes to higher consumer acceptance, as evidenced by the high sensory scores.

4 Conclusion

Phyllanthus emblica seed extract (PSE) emerges as a powerful natural antioxidant with strong potential to replace synthetic preservatives in food systems. Beyond its antioxidant potential, PSE exhibits notable antimicrobial properties. Compared to synthetic antioxidants such as BHT, BHA, and TBHQ, PSE demonstrates superior thermal stability making it highly suitable for baked products. Toxicological assessments, including cytotoxicity assays and the zebrafish (*Danio rerio*) embryonic toxicity test, indicate that PSE is significantly safer than conventional synthetic antioxidants, presenting minimal toxic effects. These findings support its potential application as a safer ingredient for food preservation. The incorporation of PSE into baked products enhances their oxidative stability by mitigating the formation of primary and secondary oxidation products, thus preventing rancidity and off-flavors. Furthermore, the antimicrobial properties of PSE contribute to the extended microbial shelf life due to inhibiting microbial growth, maintaining product freshness. Sensory evaluations further confirm that PSE maintains the sensory attributes (color, taste, aroma, texture, and overall acceptability) of these products compared to those enriched with synthetic antioxidants.

While the present study demonstrates the strong potential of PSE as a natural antioxidant and antimicrobial agent in baked products, several areas want further investigation. Future research should explore its application across diverse food matrices, including meat, dairy, and oil/fat-based products, to assess its broad-spectrum effectiveness. Long-term *in vivo* safety evaluations and chronic exposure studies are needed to confirm its safety for regular consumption. Additionally, the development of encapsulation or other delivery strategies could enhance the stability, bioavailability, and controlled release of PSE in complex food systems. Mechanistic studies to elucidate the interactions and synergistic effects of its phenolic compounds would provide deeper insights into its antioxidant and antimicrobial actions. Finally, examining the performance of PSE under varying storage conditions and processing methods, along with evaluating its commercial feasibility and consumer acceptability, will facilitate its practical application as a safe and effective alternative to synthetic preservatives in the food industry.

Ethical approval

The animal study was approved by the Ethics Review Committee (ERC), Faculty of Graduate Studies, USJ, SL (FGS/ERCAS/2024/11/03), and the sensory evaluation procedure was approved by the ERC, Faculty of Medical Sciences, USJ, Sri Lanka (ERC 11/24).

Author contributions

Ahinsa Lankanayaka (0009-0009-7356-5938): conceptualization, investigation, visualization, and writing—original draft. K. G. L. R. Jayathunge: supervision, review, and editing. Pasan C. Bandara: supervision, review and editing. Danushika C. Manatunga: supervision, review, and editing. Sameera Samarakoon: supervision, review, and editing. Nimal Punyasiri: supervision, review, and editing. Chathuri M. Senanayake (0000-0002-7116-6396): conceptualization, funding acquisition, supervision, review and editing. All authors read and approved the final version of the manuscript.

Conflicts of interest

The authors declare no conflict of interest.

Data availability

The datasets used and/or analyzed during the present study are included in this article and its supplementary information (SI). Supplementary information: Fig. S1: antioxidant activities of phenolic extract and synthetic antioxidants: (a) DPPH radical scavenging activity, (b) reducing power, and (c) ABTS radical scavenging activity; Table S1: phenolic compounds and flavonoids identified in ethyl acetate extract of *Phyllanthus emblica* seed; Table S2: effect of phenolic and synthetic antioxidants on aerobic plate count and yeast and mold count of sponge cakes; Table S3: effect of phenolic and synthetic antioxidants on aerobic plate count and yeast and mould count of biscuits. See DOI: https://doi.org/10.1039/d5fb00609k.

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