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Fortification of yogurt with *Solanum melongena* phenolic extract: Physicochemical stability, bioaccessibility, bioavailability, and antioxidant effects

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ABSTRACT

This study investigated the impact of fortifying yogurt with *Solanum melongena* phenolic extract by assessing its physicochemical, biochemical, and antioxidant stability, including its behavior during *in vitro* simulated digestion. Fortification influenced the yogurt's pH, titratable acidity, and color, and improved its cohesiveness during shelf life. The fortified yogurt exhibited higher glucose levels and reduced lactic acid production compared to the control. During simulated digestion, the extract modulated alterations in the protein and fatty acid profiles of the yogurt. Among phenolic compounds, 3-*O*-caffeoylquinic acids exhibited good stability during the oral and gastric phases but decreased in the intestinal digest. Some antioxidant effects were enhanced during digestion, particularly against lipid peroxidation, but declined during storage. Furthermore, phenolic acids were absorbed in an *in vitro* Caco-2 cell model, supporting their bioavailability. Overall, yogurt fortification with *S. melongena* extract improved nutritional and functional properties, highlighting its potential as a sustainable functional food. Future studies should address sensory acceptance, safety aspects, and *in vivo* health outcomes.

1. Introduction

Natural extracts and bio-based ingredients have attracted growing interest in the food industry due to their functional and technological properties, as well as their potential health-promoting effects. These extracts are often rich in bioactive compounds such as phenolic acids, flavonoids, and vitamins, offering opportunities to enhance nutritional value, improve product stability, and replace certain artificial additives, meeting the rising consumer demand for functional and clean-label foods (Gómez-García, Campos, Aguilar, Madureira, & Pintado, 2021b; Shahidi & Ambigaipalan, 2015). This focus is particularly relevant considering the global rise in obesity and type 2 diabetes, which have reached alarming levels in recent decades and pose public health challenges.

Type 2 diabetes and obesity are closely linked metabolic conditions that significantly contribute to global morbidity and mortality (Chandrasekaran & Weiskirchen, 2024). The prevalence of diabetes has been steadily increasing for the past three decades, currently affecting about 422 million people worldwide, particularly in low- and middle-income countries (WHO, 2016). Similarly, it is estimated that overweight and obesity affect over 640 million adults and 110 million children and adolescents, being responsible for more than 1.2 million deaths across the WHO European Region annually, making it the fourth leading risk factor for mortality (WHO, 2022). Both conditions impose heavy burdens on healthcare systems and are associated with complications such as cardiovascular disease, kidney failure, and reduced quality of life (WHO, 2016). Addressing these epidemics requires comprehensive strategies, including healthier diets and sustainable lifestyle practices.

Crop by-products, such as plant materials or bioresidues remaining

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after fruit harvest, are promising candidates for upcycling into food-grade bio-based ingredients. Among these, eggplant (*Solanum melongena* L.) by-products are abundant and hold significant potential for value-added applications in food and related industries. They exhibit a phenolic-rich composition, including caffeoylquinic acids and *O*-glycosylated flavonoids, and display a wide range of bioactive properties, such as antioxidant and antimicrobial activities, notable antidiabetic effects *via* inhibition of α-glucosidase and advanced glycation end products (AGEs) formation, and moderate anti-obesity through pancreatic lipase inhibition capacity (Añibarro-Ortega et al., 2025). Despite their bioactive potential, these crop remains are often discarded or used as compost, providing minimal economic benefit to producers (Pantuzza Silva et al., 2021). Therefore, repurposing these underutilized raw materials into functional food ingredients supports circular production and enhances sustainability in the agri-food system.

Yogurt is a widely consumed fermented dairy product and serves as an ideal vehicle for incorporating natural ingredients with anti-diabetic and anti-obesity properties, owing to its versatility and broad consumer acceptance (Machado et al., 2022). Fortifying yogurt with bioactive compounds derived from plant by-products, such as *S. melongena* biomass, can enhance its stability, nutritional value, and shelf-life while delivering additional functional benefits. These functional dairy foods hold the potential to address the global rise in diabetes- and obesity-related conditions by offering a convenient and nutritious dietary option that supports improved health outcomes.

Research into plant extract-enriched yogurts has already demonstrated their potential as functional foods with antioxidant and antidiabetic properties. Extracts from medicinal plants, including Solanaceae species such as goji berry (*Lycium barbarum* L.), have been incorporated into yogurt, increasing total phenolic content and enhancing antioxidant activity and enzyme inhibition (Shori & Baba, 2023). These studies suggest that such enriched yogurts could help combat oxidative stress and regulate glucose metabolism. However, many studies have encountered limitations, such as significant declines in bioactivity during prolonged storage, inconsistency in sensory acceptance, and a lack of comprehensive analyses of the stability and bioavailability of bioactive compounds within the digestive tract (Ramírez, Valencia, Arbelaez, Herrera, & Rojano, 2020). These challenges underscore the need for further research to guarantee both functional and sensory outcomes.

This study investigated the effects of incorporating a phenolic-enriched functional extract from *S. melongena* into yogurt, focusing on key parameters such as physicochemical stability, protein and fatty acid profiles, phenolic compounds content, and antioxidant properties throughout its shelf-life. The phenolic content was evaluated to determine the stability of these bioactive compounds within the yogurt matrix, as well as their bioaccessibility and bioavailability following *in vitro* simulated gastrointestinal digestion.

2. Material and methods

2.1. Chemicals and plant material

Chemicals, standards, and biological materials used in this work, along with their suppliers, are listed in Table A.1. Aerial parts of *S. melongena*, namely leaves and branches, were harvested at the end of the fruit production cycle in October 2019 from a local farm in Bragança, Portugal. The collected plant material was immediately freeze-dried (FreeZone 4.5, Labconco, Kansas City, MO, USA), finely ground into powder, and stored under vacuum at −20 °C in the dark until further analysis.

2.2. Preparation of phenolic-enriched extract

The phenolic-enriched extract was obtained by stirring 10 g of powdered plant material with 300 mL of ethanol/water (80:20, *v/v*) for 1 h at room temperature. The mixture was filtered through Whatman no. 4 filter paper, and the solid residue was re-extracted under identical conditions. The combined extracts were concentrated by ethanol removal under reduced pressure in a rotary evaporator (Heidolph, Schwabach, Germany), and the remaining aqueous phase was lyophilized to yield a dried phenolic-enriched extract, subsequently employed for yogurt fortification. The phenolic composition and *in vitro* bioactive properties of this extract, including antioxidant, antimicrobial, antidiabetic, and anti-obesity potential, were previously described by the authors (Añibarro-Ortega et al., 2025), who also reported no toxicity to porcine liver primary cells at concentrations ≤400 μg/mL.

2.3. Preparation of fortified yogurt

The yogurts were prepared following the methods described by Chen et al. (2023) and Ferreira and Santos (2023), with some modifications. Commercial semi-skimmed milk was heated to 90 °C and subsequently cooled to 43 °C. A final concentration of 0.1% (*w/w*) extract in yogurt (Ferreira & Santos, 2023), previously dissolved in a minimum volume of ultrapure water, was then added to the milk. The mixture was inoculated with commercial plain yogurt at a 1:10 ratio. Alongside the fortified yogurt (Y + E), a control yogurt without extract (CY) was prepared using the same volume of ultrapure water. Both formulations were transferred into 30 mL glass flasks, incubated at 38 °C for 16 h, and then refrigerated at 4 °C to stabilize fermentation. Subsequently, the yogurts were stored at 4 °C for 21 days, with samples taken for analysis at four time points: day 0 (T0), day 7 (T1), day 14 (T2), and day 21 (T3).

2.4. Yogurt quality and stability assessment

2.4.1. pH, titratable acidity, color, texture, and syneresis

The pH was measured using a portable pH meter (Milwaukee PH55 PRO, Szeged, Hungary), calibrated with pH 4.01 and 7.01 buffers, by immersing the pH electrode in the yogurt and swirling until the reading stabilized (Stephen Ezeonu, 2016). Titratable acidity was determined by mixing 10 mL of yogurt with 10 mL of ultrapure water, adding 100 μL of 0.1% (*w/v*) phenolphthalein, and titrating with 0.1 N NaOH (Bullock & Gruen, 2023). Results were expressed as a percentage of lactic acid. Color measurements were taken using a Konica Minolta colorimeter CR-400 (Osaka, Japan) and expressed in the CIE L^* (lightness), a^* (redness–greenness), and b^* (yellowness–blueness) color parameters (Molina et al., 2019). Texture analysis was conducted with a Stable Micro Systems texture analyzer (Vienna Court, Godalming, UK) following Ueda et al. (2021). Firmness (g), consistency (g·*sec*), work of cohesion (g·sec), and cohesiveness (g) were recorded. Syneresis was determined by centrifuging a weighed amount of yogurt at 7870 ×*g* for 10 min at 4 °C, followed by weighing the supernatant (Machado et al., 2022). Results were expressed as the percentage of liquid (whey) separated from the yogurt matrix. All these physicochemical determinations were performed at last in triplicate.

2.4.2. Soluble sugars and organic acids

The yogurt samples were centrifuged at 4000 ×*g* for 10 min (Megafuge 16, Thermo Scientific, Waltham, MA, USA) and the resulting supernatants (whey) were filtered through 0.22-μm filter disks prior to analysis by high-performance liquid chromatography with a refractive index detector (HPLC-RI; equipment specifications in Table A.2). Separation was carried out under isocratic conditions using 70:30 (*v/v*) acetonitrile–deionized water at a flow rate of 1 mL/min. The analytical procedures were previously described by Barros et al. (2013). Results were expressed as g/100 g of yogurt.

For the analysis of organic acids, an ultra-fast liquid chromatography system equipped with a photodiode array detector (UFLC-PDA; equipment specifications in Table A.2) was employed, following the methodology described by Paschoalinotto et al. (2021). The identified compounds were quantified by comparing the chromatographic peak areas, recorded at 215 nm, with calibration curves constructed using

commercial standards (Table A.3). Results were expressed as mg/100 g of yogurt.

2.5. In vitro simulation of human gastrointestinal digestion

To evaluate the bioaccessibility of the phenolic compounds added to the yogurt, the samples were subjected to an *in vitro* gastrointestinal digestion following the INFOGEST protocol (Brodkorb et al., 2019). The yogurt underwent simulated salivary, gastric, and intestinal digestion using appropriate fluids, including α-amylase, rabbit gastric extract, lipase, pepsin, bile salts, and pancreatin.

Oral digestion was performed by diluting the food sample 1:1 (*w/w*) with simulated salivary fluid supplemented with human salivary α-amylase. Mastication was simulated using an orbital shaker (MaxQ 6000, Thermo Scientific, Waltham, MA, USA) at 200 rpm and 37 °C for 2 min. Following the INFOGEST protocol, the resulting oral bolus was further diluted 1:1 (*v/v*) with simulated gastric fluid containing gastric enzymes, specifically pepsin and gastric lipase from rabbit gastric extract. This mixture was shaken using the same orbital shaker at 130 rpm and maintained at pH 3.0 and 37 °C for 2 h. Subsequently, the gastric chyme obtained was diluted 1:1 (*v/v*) with simulated intestinal fluid containing bile salts and pancreatic enzymes (pancreatin from porcine pancreas). This intestinal phase was carried out at pH 7.0, incubated for 2 h at 45 rpm and 37 °C in the orbital shaker. Each digestion assay was conducted using 3 g of sample, with two replicates per sample. Additionally, a negative control consisting of distilled water instead of the sample was included.

From each digestion phase, an aliquot was collected for phenolic analysis by HPLC-DAD-ESI/MS^n, SDS-PAGE protein profiling, fatty acid profiling, and antioxidant activity assessment.

The bioaccessibility results were expressed for each phenolic compound as the percentage of the compound quantified in each gastrointestinal phase relative to the total amount quantified in the undigested yogurt. Additionally, an aliquot of the intestinal phase content was collected for fatty acid analysis and to assess the bioavailability of phenolic compounds with enterocytes.

2.6. Evaluation of the effects of simulated digestion on the chemical composition and antioxidant activity of the yogurt

2.6.1. Protein profile

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the methodology described by Gómez-García, Campos, Aguilar, Madureira, and Pintado (2021a), with minor adjustments. Yogurt samples (digested and undigested) were centrifuged at 4000 ×*g* for 10 min, and the supernatant was mixed in a 1:1 (*v/v*) ratio with a solution of sample buffer and β-mercaptoethanol (19:1, *v/v*). The resulting mixture was subjected to heat hydrolysis at 100 °C for 3 min. The electrophoresis was conducted using a 10 cm separating gel (8% acrylamide) and a 2 cm stacking gel (5.5% acrylamide). Following hydrolysis, 10 μL of each sample was loaded into the wells of the stacking gel. For band identification, 10 μL of polypeptide SDS-PAGE molecular weight standards from Bio-Rad Laboratories (Hercules, CA, USA) with weights of 225, 76, 52, 38, 31, 24, 17, and 12 kDa were employed. Electrophoresis was run at 75 V for 5 min and then at 150 V for 40 min (Bio-Rad Laboratories, Hercules, CA, USA). Following separation, the gels were fixed in a 10% trichloroacetic acid solution and subsequently stained overnight with 0.25% (*w/v*) Coomassie brilliant blue under gentle agitation. Any excess stain solution was removed by washing the gels with a destaining solution (25% methanol and 10% glacial acetic acid) until the gel became clear and the protein bands were visible. The resulting gels were scanned, and band identification was made by comparing them with the polypeptide standards.

2.6.2. Fatty acid profile

The fatty acid composition was evaluated using gas chromatography following transesterification, as described by Machado et al. (2022). The experimental conditions were as follows: split injector (25:1) with 1 μL injection volume); injector and detector temperatures set at 250 °C and 275 °C, respectively; hydrogen as the carrier gas at a flow rate of 1 mL/min. The oven temperature was initially set at 60 °C and gradually increased to 225 °C. A Supelco 37-component FAME mix certified reference material facilitated the identification and quantification of fatty acids. Each sample underwent triplicate analysis. Results were expressed in μg/g of yogurt.

2.6.3. Phenolic profile

The yogurt (undigested and digested) samples were centrifuged at 4000 ×*g* and the supernatants were filtered through 0.22-μm filter disks. Phenolic compounds were analyzed using a Thermo Scientific UltiMate 3000 HPLC system equipped with a diode array detector (DAD, at 280, 330, and 370 nm) coupled to an electrospray ionization mass detector (ESI-MS), following the protocol previously described by Añibarro-Ortega et al. (2020). The identification of phenolic compounds derived from the added *S. melongena* extract was achieved by comparing retention time and UV–Vis and mass spectra of the sample peaks with those of commercially available standards. Their quantification (expressed as μg/g of yogurt) was performed using the calibration curve in Table A.3.

2.6.4. Antioxidant activity

The antioxidant activity of both undigested and digested yogurt samples was evaluated through *in vitro* assays of oxidative hemolysis inhibition (OxHLIA) and thiobarbituric acid reactive substances (TBARS) formation inhibition, following protocols previously described (Babotă et al., 2022; Lockowandt et al., 2019). The yogurt samples were first centrifuged at 5000 ×*g* for 10 min, and the resulting supernatants were collected and diluted at concentrations ranging from 31.25 to 1000 mg/mL, using phosphate-buffered saline (PBS, pH 7.4) for the OxHLIA assay and Tris-HCl buffer (pH 7.4) for the TBARS assay. Trolox was used as the positive control.

OxHLIA assay. An erythrocyte solution (2.8%, *v/v*) was mixed with either yogurt solution, PBS (negative control), or water (baseline). The oxidant 2,2′-azobis(2-amidinopropane) dihydrochloride (160 mM) was added after a 10-min pre-incubation at 37 °C with shaking, and the optical density was kinetically monitored at 690 nm using a BioTek ELx800 microplate reader (BioTek Instruments, Winooski, VT, USA). Yogurt concentrations (mg/mL) required to maintain 50% erythrocyte integrity over a 60-min interval were expressed as IC_{50} values.

TBARS assay. A porcine brain homogenate (1:2, *w/v*) was incubated with yogurt solution, $FeSO_4$ (10 μM), and ascorbic acid (0.1 mM) at 37 °C for 1 h. Trichloroacetic acid (28% *w/v*) and thiobarbituric acid (TBA, 2% *w/v*) were then added, and the mixture was heated at 80 °C for 20 min. After centrifugation, the malondialdehyde (MDA)-TBA_2 complexes in the supernatant were quantified at 532 nm using a double-beam spectrophotometer (SPECTROstar Nano, BMG LABTECH GmbH, Ortenberg, Germany). Yogurt concentrations (mg/mL) required to inhibit 50% of TBARS formation were expressed as EC_{50} values.

2.7. Phenolics absorption assessment in a cellular model

2.7.1. Cell culture

Human colon carcinoma (Caco-2) cells (ATCC HTB-37, VA, USA) and HT29-MTX E12 (ECACC 12040401) were obtained from the American Type Culture Collection and the European Collection of Authenticated Cell Cultures, respectively, and cultured at 37 °C in a humidified atmosphere of 95% air and 5% CO_2 (Oliveira et al., 2023). Cells were maintained in high-glucose (4.5 g/L) Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% (*v/v*) heat-inactivated fetal bovine serum, 1% (*v/v*) penicillin-streptomycin-fungizone, and 1% (*v/v*)

of non-essential amino acids (NEAA).

2.7.2. Samples cytotoxicity

Cytotoxicity of gastrointestinal digest samples towards Caco-2 cells was evaluated according to ISO 10993–5:2009 standard (International Organization for Standardization, 2009). Briefly, cells were grown to 80–90% confluence, detached with TrypLE, and seeded at 1×10^4 cells/well in 96-well plates. After 24 h, the medium was replaced with culture medium supplemented with filtered samples (to ensure sterility and remove insoluble material) at concentrations ranging from 100 to 1 μg/mL. Dimethyl sulfoxide (10%, *v/v*) in culture medium served as a control for cell death, while plain culture medium served as a control for cell growth. After a 24-h incubation, PrestoBlue® reagent was added to each well and incubated for 2 h, after which fluorescence (Ex: 560 nm; Em: 590 nm) was measured using a microplate reader (Synergy H1, BioTek Instruments, Winooski, VT, USA). All assays were performed in quadruplicate.

2.7.3. Co-culture models

Co-culture models were performed through seeding of a Caco-2/HT29-MTX co-culture at a 90:10 proportion on the apical chamber of a 12-well Transwell (Corning, New York, NY, USA) plate as previously described by Machado et al. (2022). Co-culture was maintained for 21 days, with media changes every two days until assaying.

2.7.4. Transepithelial electrical resistance (TEER) measurements

Membrane integrity during permeability assays was evaluated by measuring transepithelial electrical resistance (TEER) using a Millicell ERS-2 Voltohmmeter (Merck, Darmstadt, Germany). Only the membranes with TEER values between 150 and 250 Ω/cm2 were selected for permeability experiments.

2.7.5. Phenolic compounds permeability assay

Permeability assay was conducted as previously described by Machado et al. (2022). Briefly, after 21 days, the medium in the apical chamber of the co-culture model was replaced with either plain medium (control), medium containing digested samples at non-cytotoxic concentrations (fortified and non-fortified yogurts), or 10% DMSO (stress control). Membrane integrity was monitored hourly for up to 6 h, after which the contents of both the apical and basal chambers were collected. Samples were then analyzed for phenolic compounds by HPLC-DAD-ESI/MS^n, as any phenolic compound taken up by enterocytes during the 3-h exposure period may be released into the medium by the end of the 6-h incubation. Additionally, a gastrointestinal negative control (digest without fortified yogurt) and a plain medium control (unexposed cells) were included in the assay.

2.8. Statistical analysis

Results from at least three independent experiments are presented as mean ± standard deviation (SD). The assumptions for the one-way analysis of variance (ANOVA), specifically the normal distribution of residuals and homogeneity of variance, were tested using Shapiro-Wilk's and Levene's tests, respectively. Depending on the homoscedasticity, the dependent variables were compared using Tukey's honestly significant difference (HSD) test (for homoscedastic data, $p > 0.05$) or Tamhane's T2 multiple comparison test (for heteroscedastic data, $p < 0.05$). Furthermore, linear discriminant analysis (LDA) was applied to compare samples using a stepwise procedure with Wilk's λ test, applying F-values of 3.84 for entering and 2.71 for removal of variables. All statistical tests were performed at a 5% significance level using SPSS Statistics (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.).

3. Results and discussion

3.1. Effects of fortification on the physicochemical properties of yogurt

3.1.1. pH, titratable acidity, color, texture, and syneresis

Yogurt is a fermented dairy product obtained through the action of specific bacterial cultures, primarily *Lactobacillus spp.* and *Streptococcus spp.*, which convert lactose into lactic acid. This process gives yogurt a characteristic gel-like texture, mildly acidic flavor, and creamy consistency. Acidity plays a crucial role in the fermentation process, significantly affecting the taste and texture of the final product. The pH of yogurt is primarily determined by the lactic acid produced during fermentation, which occurs mainly during the 16-h incubation period, though slower fermentative processes may continue during refrigerated storage (Hui, 1992). Ideally, yogurt should have a pH of around 3.9 and a titratable acidity of at least 0.9% lactic acid, attributes desirable for consumption (Hui, 1992). These values were consistent with the results of this study (Fig. 1 and Table A.4).

During storage, slight changes in the physicochemical properties of yogurt were observed, particularly between control and fortified samples. The pH decreased until day 7, with a more pronounced reduction in the control yogurt, corresponding to higher lactic acid levels as evidenced by titratable acidity measurements and HPLC analysis (Fig. 1). Subsequently, the pH of the fortified yogurt tended to increase, reaching values similar to day 0 by day 21, whereas the control yogurt's pH stabilized after an initial increase between days 7 and 14. Titratable acidity exhibited significant differences ($p < 0.05$) between the formulations at day 7, with the control showing higher acidity levels. In both formulations, titratable acidity increased throughout storage.

The addition of *S. melongena* bioactive extract likely delayed microbial fermentation and the conversion of lactose to lactic acid. This effect may be attributable to the extract's antimicrobial properties (Añibarro-Ortega et al., 2025), which could have inhibited or slowed down the metabolic activity of lactic acid bacteria (Amirdivani & Baba, 2011).

A color difference ($p < 0.05$) was observed between control and fortified yogurts (Fig. A.1). Fortified yogurt appeared slightly darker (lower L^* values), greener (lower a^* values), and yellower (higher a^* values) (Fig. 1), likely due to the natural pigments (*e.g.*, chlorophylls) in the plant extract. Lightness remained stable in both formulations until day 14 but increased significantly by day 21, eliminating differences between them ($p > 0.05$). Some variations in the a^* and b^* parameters during storage were statistically significant ($p < 0.05$) but likely imperceptible to the naked eye (see color in Table A.5).

Texture, a critical determinant of yogurt quality, was evaluated through four parameters: firmness, consistency, cohesiveness, and work of cohesion. Firmness refers to the resistance to deformation, consistency indicates the thickness or thinness of the product, cohesiveness describes how the yogurt holds together under force, and work of cohesion represents the energy required to disintegrate the yogurt's structure (Lewis, 2024). As shown in Fig. 1, the *S. melongena* extract did not affect firmness, which remained stable in the control yogurt but decreased in fortified yogurt over time. Consistency remained similar and stable in both formulations during storage, indicating minimal influence of the extract or storage on this attribute. However, cohesiveness was better maintained in the fortified yogurt by day 21, suggesting enhanced structural integrity. Work of cohesion decreased slightly but comparably in both formulations over time. Thus, the *S. melongena* extract improved yogurt cohesiveness over time without affecting firmness or consistency.

Syneresis, the separation of whey from the yogurt gel matrix, is a critical quality indicator reflecting gel stability. This parameter can also affect sensory attributes and consumer acceptability. As illustrated in Fig. 1, no significant differences ($p > 0.05$) in syneresis were observed within the same yogurt formulation during storage, nor between control and fortified yogurts. These results indicate that the bioactive extract did

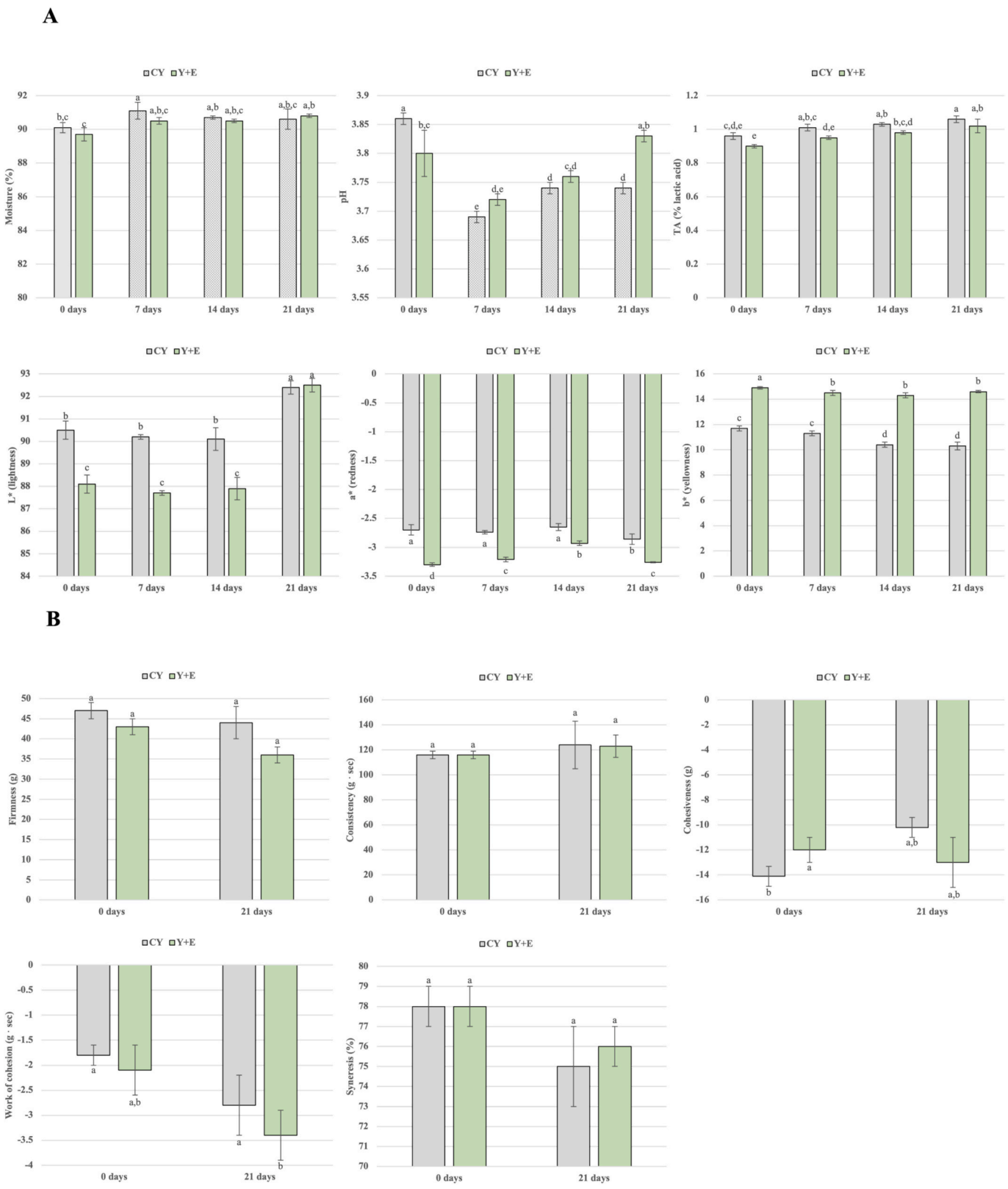

Fig. 1. Physicochemical properties (A), texture attributes (B), and content of soluble sugars and organic acids (C) of control (CY) and fortified (Y + E) yogurts during refrigerated storage. In each graph, different letters indicate statistically significant differences ($p \leq 0.05$) between samples.

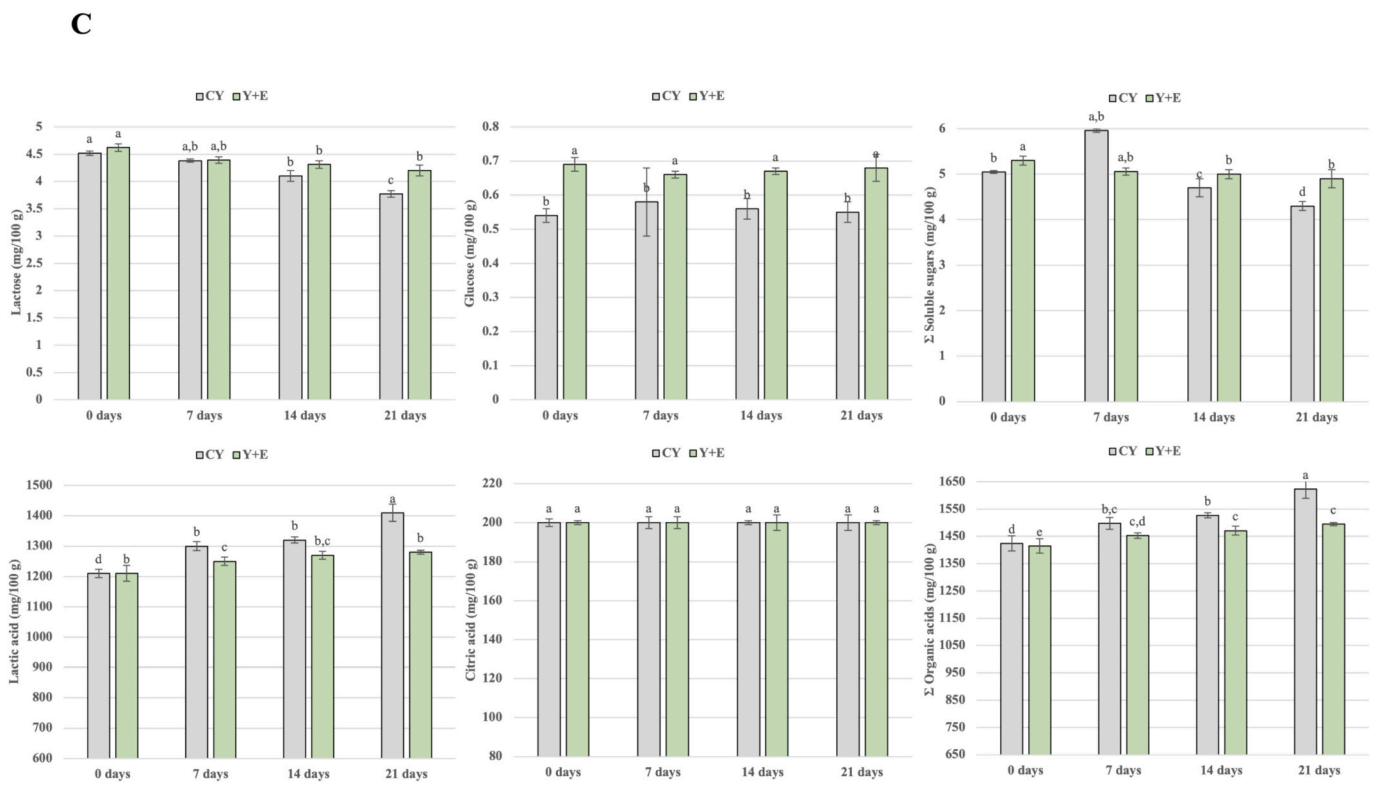

Fig. 1. (*continued*).

not adversely affect the yogurt gel matrix stability *via* whey separation throughout refrigerated storage.

3.1.2. *Soluble sugars and organic acids*

Soluble sugars play a key role in yogurt's sweetness and consumer acceptance. Glucose is the main contributor to perceived sweetness, whereas lactose, although less influential in this regard, affects digestibility, especially in lactose-intolerant individuals. During fermentation, yogurt bacteria metabolize glucose and lactose, lowering their concentrations and increasing lactic acid production, which enhances acidity and defines the yogurt's characteristic flavor profile (Savaiano, 2014).

In this study, glucose levels (although not nutritionally meaningful in the context of total dietary sugar intake) were significantly higher in fortified yogurt and remained stable in both formulations over 21 days (Fig. 1). This difference may be attributed both to the extract composition, which could contain free glucose, and to its antimicrobial effects (Añibarro-Ortega et al., 2025), which may have reduced microbial glucose metabolism, thereby preserving glucose and enhancing perceived sweetness. Lactose concentrations, initially similar between

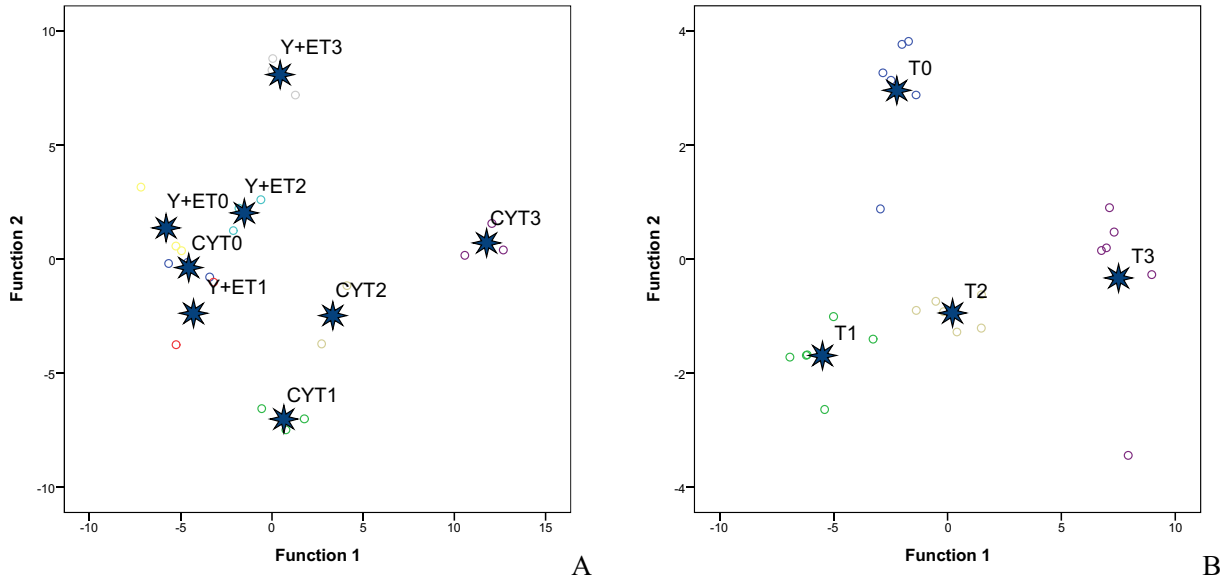

Fig. 2. Spatial distribution of markers set by the canonical discriminant function coefficients when considering (A) the eight yogurt samples and (B) the storage time regardless of the addition of extract.

formulations, progressively declined during storage, with a steeper reduction in the control yogurt by day 21. Control samples also showed higher lactic acid concentrations, consistent with greater titratable acidity. In contrast, fortified yogurt exhibited a less pronounced increase in lactic acid, indicating lower acidification and suggesting that the extract modulated microbial activity and fermentation dynamics. These findings align with those of Gul et al. (2022), who observed lower lactic acid production in yogurt enriched with antimicrobial compounds, reinforcing the role of the extract in influencing microbial activity.

Other detected organic acids included citric, fumaric, and succinic acids, though their concentrations were unaffected by refrigerated storage (Fig. 1) or, in the case of succinic acid, were below quantifiable limits. These acids are by-products of lactic acid bacteria metabolism and contribute to yogurt's flavor profile, but they may also originate from plant extracts.

3.1.3. Overall impact of the extract and storage time on stability attributes

Since pH, titratable acidity, glucose, lactose, and lactic acid are interdependent and critical to yogurt quality and stability, a linear discriminant analysis (LDA) was conducted to evaluate the changes in these parameters across the eight yogurt samples (Fig. 2A), the effect of the extract irrespective of storage time, and the influence of storage time independent of formulation (Fig. 2B). In the first scenario, the LDA model defined four discriminant functions. The first function, accounting for 54.7% of the total variance, was primarily associated with lactic acid content, while the second function, which explained 32.3% of the variance, was linked to lactose levels. Control samples stored for longer periods showed higher lactic acid levels, whereas fortified yogurt consistently maintained higher lactose concentrations throughout all storage periods. This suggests that the extract inhibited the conversion of lactose to lactic acid during storage.

When the effects of fortification were assessed independently of storage time, glucose was identified as the key differentiating parameter, with fortified samples showing higher concentrations. Regarding storage time, while the first function accounted for 87.8% of the variance, the second function explained 12.1% and demonstrated a strong positive correlation with pH and a negative correlation with titratable acidity and lactic acid (Fig. 2B). These findings highlight that storage time reduces pH while increasing titratable acidity and lactic acid content, underscoring the dynamic interplay between these parameters and their impact on yogurt stability.

3.2. Effects of fortification and simulated digestion on biochemical constituents and antioxidant properties of yogurt

3.2.1. Protein profile

The composition and relative abundance of proteins in yogurt, along with their interactions with other components such as polyphenols, can significantly influence texture and overall product quality (Akalin, Unal, Dinkci, & Hayaloglu, 2012). To investigate these interactions, the protein profile of yogurt was analyzed using SDS-PAGE. This technique allows the evaluation of possible interactions between dairy proteins and phenolic compounds from *S. melongena* extract, which may lead to the formation of protein–polyphenol aggregates visible as distinct bands on the electrophoresis gel. It also enables the monitoring of changes in the protein profile during simulated digestion, providing valuable insights into protein modifications throughout the process.

SDS-PAGE revealed no significant differences in protein band patterns between yogurts with and without the extract (Fig. 3), suggesting that the extract did not substantially alter the protein structure. This consistency was observed in freshly prepared yogurts, yogurt samples stored for 21 days, and yogurt samples analyzed after oral digestion. These findings suggest minimal interactions between the phenolic extract and dairy proteins, with negligible effects on the protein profile during refrigerated storage.

Four prominent bands were identified in the yogurt, ranging from *Ca.* 225 kDa to approximately 75 kDa (Fig. 3), likely corresponding to immunoglobulins and bovine serum albumin (Trentin et al., 2022). A broader band between 35 kDa and 50 kDa was attributed to caseins, the main structural proteins in yogurt, while bands below 24 kDa were associated with β-lactoglobulin (*Ca.* 18 kDa) and α-lactalbumin (*Ca.* 14 kDa), the primary whey proteins (Trentin et al., 2022). However, significant changes in the protein profile were observed during the gastric and intestinal digestion phases.

After gastric digestion, the yogurt samples exhibited prominent bands at approximately 52 kDa, 38 kDa, and below 17 kDa, along with a broad, diffuse band around 24 kDa. These changes indicate extensive protein breakdown and aggregation, primarily driven by pepsin activity

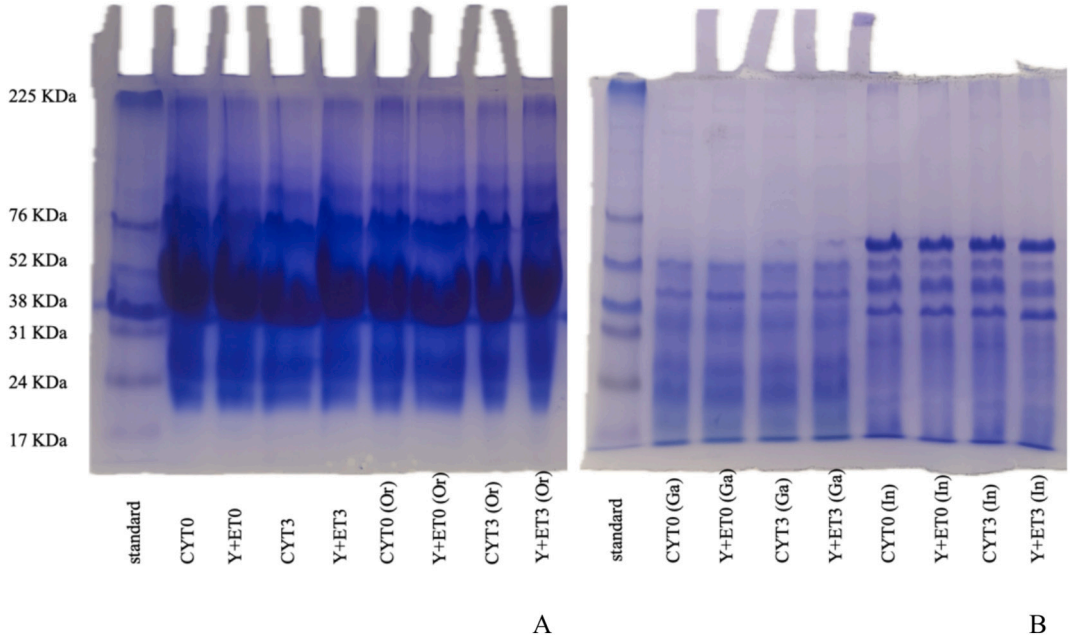

Fig. 3. SDS-PAGE electrophoresis gels of control (CY) and fortified (Y + E) yogurts at 0 (T0) and 21 days (T3) of refrigerated storage (4 °C): (A) undigested samples and samples after simulated oral (Or) digestion; and (B) samples after simulated gastric (Ga) and intestinal (In) digestion.

and the acidic stomach environment. In contrast, intestinal digestion produced new bands at approximately 65 kDa, 52 kDa, 45 kDa, and 38 kDa, alongside the persistent band below 17 kDa. These bands likely represent enzymatic hydrolysis products formed by digestive enzymes such as α-amylase, lipase, and pancreatin used in the *in vitro* simulated gastrointestinal digestion.

The marked alterations observed during gastric digestion highlight the strong proteolytic activity of pepsin, which cleaves proteins into smaller peptides. The broad band around 24 kDa reflected partially degraded caseins. Additional bands during intestinal digestion underscore the intricate enzymatic activity in the small intestine, which facilitates further protein hydrolysis.

These findings demonstrate the stability of yogurt proteins during storage and the initial digestion phases, as well as their significant transformations under the action of digestive enzymes in later stages. The stability of protein bands in both control and fortified yogurts during storage and oral digestion indicates that *S. melongena* phenolic extract does not induce long-term changes in yogurt protein composition. These results are important for evaluating potential interactions between phenolic compounds and yogurt proteins, their impact on texture and digestibility, and the development of functional dairy products fortified with natural extracts that may offer health-promoting benefits.

3.2.2. Fatty acid profile

The analysis of fatty acids in yogurt before and after simulated digestion is important for understanding whether the incorporation of the bioactive extract alters the lipid profile, which could, in turn, affect the bioaccessibility of phenolic compounds due to their potential entrapment within fat globules. To minimize this potential interference from milk fat, semi-skimmed milk was used in the formulation. Table 1 shows that the fatty acid profile of the control yogurt was predominantly composed of polyunsaturated (PUFA, 43%) and monounsaturated (MUFA, 43%) fatty acids, given the predominance of *cis*-7-hexadecenoic acid (29.0%), linoleic acid (18%), and γ-linolenic acid (19%). Upon incorporation of the *S. melongena* extract, a substantial shift in the fatty acid composition was observed, with the fortified yogurt being mainly composed of saturated fatty acids (SFA, 75%) and MUFA (21%), with palmitic acid (32%), oleic acid (19%), and arachidic acid (23%) emerging as the most prevalent. After simulated digestion, SFA dominated the lipid profile in both yogurt formulations, a pattern previously reported for dairy products such as yogurt (Machado et al., 2022).

The changes observed in the fatty acid composition of yogurt during simulated digestion suggest that the digestive system may modulate the bioaccessibility of these nutrients, equalizing the nutritional impact of yogurt regardless of its initial fatty acid composition. This highlights the complexity of assessing the health impacts of food, as nutrients can undergo significant transformations during digestion. The interaction between proteins and fats in yogurt plays an important role in this process. Milk proteins, primarily caseins, form micelles that organize into a network capable of entrapping fat droplets, resulting in a stable emulsion. During gastrointestinal digestion, this structure can temporarily reduce enzymatic access to proteins and lipids, slowing their breakdown, while simultaneously promoting a gradual release of nutrients, including fatty acids, which may favor more controlled absorption (Braun, Hanewald, & Vilgis, 2019).

3.2.3. Phenolic profile

The two phenolic acids tentatively identified in yogurt fortified with *S. melongena* extract are presented in Table 2 and a representative chromatogram is shown in Fig. A.2. Compounds 1 and 2 exhibited a pseudomolecular ion $[M-H]^-$ at *m/z* 353, with fragment ions at *m/z* 191 (quinic acid fragment), *m/z* 179, and *m/z* 135, and a maximum UV–Vis absorbance at 321 nm. Based on these features, the compounds were tentatively identified as *cis*- and *trans*-3-*O*-caffeoylquinic acids, respectively (Clifford, Johnston, Knight, & Kuhnert, 2003; Clifford, Zheng, & Kuhnert, 2006).

Table 1
Changes in fatty acid contents of control (CY) and fortified (Y + E) yogurts during simulated gastrointestinal digestion and refrigerated storage.

Fatty acid	Sample	Undigested yogurt		Digested yogurt	
(mg/100 g yogurt)		T0: 0 days	T3: 21 days	T0: 0 days	T3: 21 days
Myristic acid (C14:0)	CY	8.1 ± 0.3^{a}	8.1 ± 0.1^{a}	0.43 ± 0.1^{c}	–
	Y + E	7.3 ± 0.4^{b}	7.3 ± 0.1^{b}	0.343 ± 0.004^{c}	0.38 ± 0.02^{c}
Palmitic acid (C16:0)	CY	1.3 ± 0.1^{e}	1.3 ± 0.1^{e}	4.0 ± 0.1^{b,c}	2.7 ± 0.1^{d}
	Y + E	25.6 ± 1^{a}	25.6 ± 0.4^{a}	3.40 ± 0.02^{c,d}	4.4 ± 0.1^{b}
cis-7-Hexadecenoic acid (C16:1 c7)	CY	29.0 ± 0.1^{a}	27.6 ± 0.1^{a}	0.63 ± 0.05^{b}	0.48 ± 0.03^{b}
	Y + E	1.31 ± 0.02^{b}	1.32 ± 0.04^{b}	0.46 ± 0.01^{b}	0.5 ± 0.1^{b}
Stearic acid (C18:0)	CY	1.6 ± 0.1^{d,e}	1.6 ± 0.1^{d,e}	2.01 ± 0.04^{b,c}	1.39 ± 0.04^{e}
	Y + E	8.9 ± 0.3^{a}	8.8 ± 0.3^{a}	1.74 ± 0.02^{c,d}	2.22 ± 0.02^{b}
Oleic acid (C18:1 c9)	CY	9.5 ± 0.2^{b}	9.2 ± 0.1^{b}	6.7 ± 0.3^{c}	4.2 ± 0.2^{d}
	Y + E	15.3 ± 0.7^{a}	15.2 ± 0.2^{a}	5.10 ± 0.02^{d}	6.17 ± 0.01^{c}
Linolelaidic acid (C18:2 t9t12)	CY	16.1 ± 0.3^{a}	16.34 ± 0.02^{a}	0.470 ± 0.001^{c}	0.32 ± 0.01^{c}
	Y + E	0.87 ± 0.01^{b}	0.84 ± 0.03^{b}	0.304 ± 0.002^{c}	0.33 ± 0.02^{c}
Linoleic acid (C18:2 c9c12)	CY	5.2 ± 0.1^{a}	2.2 ± 0.1^{f}	4.8 ± 0.1^{b}	2.3 ± 0.1^{f}
	Y + E	3.3 ± 0.1^{e}	2.3 ± 0.1^{f}	3.58 ± 0.01^{d}	4.4 ± 0.1^{c}
Arachidic acid (C20:0)	CY	2.1 ± 0.2^{d}	2.01 ± 0.01^{d}	10 ± 1^{c}	10.2 ± 0.4^{c}
	Y + E	21.2 ± 0.2^{a}	18.8 ± 0.9^{b}	10.5 ± 0.1^{c}	9.9 ± 0.5^{c}
γ-Linolenic acid (C18:3 c6c9c12)	CY	16.7 ± 0.1a	16.0 ± 0.1^{b}	0.6 ± 0.1^{c}	5.00 ± 0.02^{c}
	Y + E	nd	nd	0.36 ± 0.03^{c}	0.52 ± 0.02^{c}
Fatty acid class					
SFA	CY	13.1 ± 0.6^{e}	13.04 ± 0.02^{e}	16.4 ± 0.9^{c}	15.2 ± 0.5^{d,e}
	Y + E	63 ± 2^{a}	60 ± 1^{b}	15.9 ± 0.1^{c,d}	17.2 ± 0.4^{c}
PUFA	CY	38.0 ± 0.3^{a}	34.0 ± 1.0^{b}	5.9 ± 0.2^{c}	3.1 ± 0.1^{e}
	Y + E	4.1 ± 0.1^{d}	3.2 ± 0.1^{e}	4.25 ± 0.02^{d}	5.2 ± 0.1^{c}
MUFA	CY	38.0 ± 0.2^{a}	36.76 ± 0.04^{b}	7.3 ± 0.3^{d}	4.7 ± 0.2^{f}
	Y + E	16.7 ± 0.7^{c}	16.6 ± 0.1^{c}	5.56 ± 0.02^{d,f}	6.7 ± 0.1^{d,e}

The mean ± SD of at least 3 replicates is reported for each fatty acid or fatty acid class, with different letters in every two lines indicating statistically significant differences ($p < 0.05$) between samples. SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; nd – not detected.

The fortified yogurt contained 21.1 ± 0.4 μg/g of phenolic acids, exhibiting the same major phenolic compounds previously reported by the authors in *S. melongena* crop by-product extract (Añibarro-Ortega et al., 2025). In the previously characterized extract, the *cis* isomer of chlorogenic acid predominated (6.8 ± 0.2 mg/g) compared to the *trans* isomer (1.18 ± 0.03 mg/g) (Añibarro-Ortega et al., 2025). However, in the fortified yogurt, the *trans* isomer was predominant (15.9 ± 0.2 μg/g) compared to the *cis* isomer (4.27 ± 0.07 μg/g). This difference in isomer predominance could be related to the temperature conditions employed during the yogurt incubation. Studies have shown that *cis* and *trans* stereoisomers of chlorogenic acid are sensitive to temperature changes in aqueous environments, which can promote isomerization between the

Table 2
Changes in caffeoylquinic acid (CQA) contents of the fortified (Y + E) yogurt during simulated gastrointestinal digestion and refrigerated storage.

Compound	Sample	Yogurt	Oral phase		Gastric phase		Intestinal phase	
		(μg/g yogurt)	(μg/g yogurt)	B (%)	(μg/g yogurt)	B (%)	(μg/g yogurt)	B (%)
cis-3-*O*-CQA	Y + ET0	4.27 ± 0.07^{a}	4.06 ± 0.05^{a}	95 ± 4	3.87 ± 0.02^{b}	91 ± 2	< LOQ	–
	Y + ET3	4.24 ± 0.05^{a}	4.07 ± 0.08^{a}	95 ± 1	3.82 ± 0.03^{b}	87 ± 2	< LOQ	–
trans-3-*O*-CQA	Y + ET0	15.9 ± 0.2^{a}	15.0 ± 0.4^{a}	95 ± 2	13.7 ± 0.1^{b}	87 ± 1	12.8 ± 0.1^{c}	78 ± 2
	Y + ET3	15.6 ± 0.2^{a}	15.2 ± 0.3^{a}	96 ± 1	13.9 ± 0.4^{b}	89 ± 2	13.2 ± 0.1^{c}	82 ± 4
Σ CQA	Y + ET0	20.2 ± 0.4^{a}	19.2 ± 0.4^{a}	95 ± 1	17.8 ± 0.6^{b}	89 ± 4	12.8 ± 0.1^{c}	63 ± 1
	Y + ET3	19.9 ± 0.3^{a}	19.3 ± 0.5^{a}	97 ± 1	17.8 ± 0.9^{b}	90 ± 3	13.2 ± 0.1^{c}	67 ± 1

The mean ± SD of at least 3 replicates is reported for each parameter, with different letters in every two lines indicating significant differences ($p \leq 0.05$) between samples. B – bioaccessibility; T0 – day 0; T3 – day 21.

two forms (Ianni et al., 2022). Therefore, the incubation temperature likely facilitated the conversion of the *cis* isomer into the thermodynamically more stable *trans* isomer, accounting for the observed isomer distribution in the final product. A similar explanation applies to the predominance of the 3-*O*- isomer in yogurt, despite the presence of both the 5-*O*- and 3-*O*- isomers in the *S. melongena* extract (Añibarro-Ortega et al., 2025). This sequential conversion may have occurred because the system favors the thermodynamically more stable isomer. Thus, the prevalence of the 3-*O*- isomer in the yogurt likely reflects this inherent stability.

During simulated *in vitro* digestion, the concentrations of chlorogenic acid isomers are presented in Table 2. For the *cis* isomer, 95 ± 4% of the initial concentration was detected in freshly produced yogurt after oral digestion and 91 ± 2% after the gastric phase. These values for yogurt refrigerated for 21 days were 95 ± 1% and 89 ± 2%, respectively. In both cases, the *cis* isomer was below the quantification limit (3.62 μg/mL) after intestinal digestion. The *trans* isomer showed slightly better stability, with 95 ± 2% of its initial concentration detected after oral digestion, 86 ± 1% after gastric digestion, and 78 ± 2% after intestinal digestion in freshly produced yogurt. For yogurt stored for 21 days, these values were 96 ± 1%, 87 ± 2%, and 82 ± 4% for the oral, gastric, and intestinal phases, respectively.

Overall, both isomers exhibited good stability in the early digestion phases (oral and gastric) regardless of storage duration. This suggested that the yogurt shelf-life does not considerably affect the initial digestion of these bioactive phytochemicals. The significant reduction in both isomers during the intestinal phase may result from hydrolysis by intestinal enzymes, solubility limitations, or conversion into other metabolites not assessed in this study (Pais, Coscueta, Pintado, Silvestre, & Santos, 2024). Nevertheless, the *trans* isomer exhibited greater stability during the intestinal phase compared to the *cis* isomer. This could be attributed to differences in their interactions with the digestive environment or, as noted earlier, the *trans* isomer's higher thermodynamic stability, which likely rendered it more resistant to degradation.

Table 3
Changes in antioxidant activity of control (CY) and fortified (Y + E) yogurts during simulated gastrointestinal digestion and refrigerated storage.

Antioxidant assay	Sample	Undigested yogurt		Digested yogurt	
		0 days (T0)	21 days (T3)	0 days (T0)	21 days (T3)
OxHLIA	CY	53.0 ± 0.5^{b}	64.4 ± 0.6^{c}	547 ± 11^{f}	571 ± 11^{g}
(IC_{50}, mg/mL)	Y + E	27.8 ± 0.3^{a}	53.9 ± 0.6^{b}	450 ± 11^{d}	483 ± 12^{e}
TBARS	CY	60 ± 2^{g}	72 ± 3^{h}	9.2 ± 0.2^{b}	17 ± 1^{d}
(EC_{50}, mg/mL)	Y + E	39 ± 1^{e}	45 ± 1^{f}	7.1 ± 0.4^{a}	10.4 ± 0.6^{c}

The mean ± SD of at least 3 replicates is reported for each activity, with different letters in every two lines indicating significant differences ($p \leq 0.05$) between samples. Trolox activity: IC_{50} for OxHLIA: 21.5 ± 0.2 μg/mL; EC_{50} for TBARS: 5.4 ± 0.3 μg/mL.

3.2.4. Antioxidant activity

The antioxidant activity of undigested and *in vitro* digested control and fortified yogurt samples was evaluated, and the results are presented in Table 3. Fermented foods often demonstrate enhanced antioxidant activity compared to their unfermented counterparts (Zhao et al., 2021). Notable changes in the antioxidant properties of the yogurt samples were observed following digestion. Specifically, the yogurt's ability to inhibit lipid peroxidation significantly increased after digestion, indicating an enhanced lipid-protective antioxidant potential. This supports the hypothesis that gastrointestinal digestion enzymes may boost the antioxidant activity of the digested yogurt (Oliveira & Pintado, 2015), thereby enhancing its effectiveness in mitigating lipid peroxidation. Conversely, the capacity of the yogurt samples to inhibit oxidative hemolysis decreased significantly post-digestion. These results suggest that the bioactive compounds responsible for protecting red blood cells against oxidative damage may undergo structural changes during digestion or may not remain at sufficient concentrations to exert a measurable effect. These differential trends may be partly explained by the bioaccessibility of caffeoylquinic acids (Table 2); while *cis*-3-*O*-caffeoylquinic acid degraded to levels below the limit of quantification in the intestinal phase, *trans*-3-*O*-caffeoylquinic acid was retained at ~78–82%. Other extract constituents may also have contributed to these effects.

During storage, all yogurt samples exhibited a general decline in antioxidant activity, even though caffeoylquinic acid levels were not significantly affected (Table 2). By the end of the yogurt's shelf life, the capacity to inhibit oxidative hemolysis in the fortified yogurt became statistically comparable to that of the control yogurt at the beginning of the storage period. This indicates that while digestion may enhance specific antioxidant properties, such as protection against lipid peroxidation, the overall stability of these activities decreases over time. This suggests that bioactive compounds other than caffeoylquinic acids may be involved (*e.g.*, *O*-glycosylated flavonoids, previously described in the extract (Añibarro-Ortega et al., 2025)).

Collectively, these results indicate that fortification provides a consistent antioxidant advantage throughout the yogurt's shelf life. Nonetheless, further research is warranted to better understand the mechanisms underlying these differential effects and to explore potential strategies to stabilize the antioxidant activity throughout refrigerated storage.

3.3. Phenolic acids uptake by enterocytes

After exposing the gastrointestinal yogurt digests to a Caco-2 cell monolayer, *trans*-3-*O*-caffeoylquinic acid was detected in the apical and basal chambers, as well as within the cells at the end of the assay. However, it was detected below the quantification limit (<3.62 μg/mL) in all cases. These results indicate that although the compound is not quantifiable after intestinal absorption, it was detected beyond the intestinal wall, suggesting that some absorption occurs in a quantity that could potentially exert biological activity. Additionally, it is well documented that phenolic acids are absorbed as early as the gastric

phase and continue through to the intestinal phase (Lafay & Gil-Izquierdo, 2008), which could explain the observed decrease in bioaccessibility. This implies that during the gastric phase, both isomers of these compounds are present in substantial quantities (*cis*: 3.82–3.87 μg/g yogurt; *trans*: 13.7–13.9 μg/g yogurt) and are highly susceptible to absorption. The concentrations of these phenolic acids absorbed during the gastric and intestinal phases could be sufficient to exert their health-promoting effects. Such concentrations could contribute to the potential antioxidant, anti-inflammatory, antidiabetic, anti-obesity, and other bioactive effects exhibited *in vitro* by the hydroethanolic extract of *S. melongena* crop by-product (Añibarro-Ortega et al., 2025), thereby offering various physiological and therapeutic benefits.

4. Conclusion

This study investigated the effects of fortifying yogurt with a bioactive *S. melongena* extract on its physicochemical, nutritional, and antioxidant properties during 21 days of storage at 4 °C, as well as the bioaccessibility and bioavailability of phenolic acids upon simulated digestion. The extract, particularly rich in 3-*O*-caffeoylquinic acids, significantly influenced pH, titratable acidity, and color, while delaying microbial fermentation and enhancing yogurt cohesiveness during storage. Fortification also resulted in higher glucose concentrations and reduced lactic acid production, whereas 3-*O*-caffeoylquinic acids remained stable throughout shelf life. Simulated digestion revealed extensive enzymatic modifications of proteins and notable shifts in the fatty acid profile in both control and fortified yogurts. Phenolic acids showed high stability during the oral and gastric phases but decreased in the intestinal phase, with absorption confirmed in the *in vitro* Caco-2 cell model, suggesting potential for exerting physiological effects. Fortification further enhanced the antioxidant activity of the yogurt against TBARS formation, although this effect declined over time.

Future research should investigate strategies to stabilize antioxidant activity during storage and assess the physiological effects of consuming fortified yogurt in animal models, with particular focus on its antioxidant and anti-diabetic potential. In addition to sensory evaluation to determine consumer acceptability, studies on interactions between phenolic acids and gut microbiota could provide further insights into the health-promoting properties of this novel dairy product. Finally, ensuring the extract safety, particularly the absence of toxic compounds such as solanine and other glycoalkaloids, will be critical before its application in foods for human consumption.

CRediT authorship contribution statement

Mikel Añibarro-Ortega: Writing – original draft, Validation, Investigation, Formal analysis, Conceptualization. **Ricardo Gómez-García:** Writing – review & editing, Investigation, Formal analysis. **Manuela Machado:** Investigation, Formal analysis. **Alexis Pereira:** Investigation, Formal analysis. **Eduardo M. Costa:** Writing – review & editing, Investigation, Formal analysis. **Manuela Pintado:** Writing – review & editing, Validation, Resources, Conceptualization. **Lillian Barros:** Writing – review & editing, Validation, Resources, Conceptualization. **José Pinela:** Writing – original draft, Validation, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2025.117725.

Data availability

No data was used for the research described in the article.

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