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Physicochemical, nutritional, and antioxidant properties of yogurt fortified with *Carpobrotus edulis* (L.) N. E. Br. fruit peel extracts

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ABSTRACT

Biotechnological valorization of invasive species supports sustainable management by transforming ecological threats into valuable resources. While *Carpobrotus edulis* fruits are rich in bioactive compounds, their use as functional ingredients remains unexplored. This study assessed the feasibility of incorporating *C. edulis* fruit extracts into yogurt to enhance its functional properties. To achieve this, water, hydroethanolic, and ethanol extracts were prepared from fruit peels and pulps and characterized for their total phenolic and flavonoid content and *in vitro* radical scavenging (RSA) properties against DPPH and ABTS•+. The peel extracts had the highest phenolic and flavonoid content, and the strongest RSA, and were further analyzed for *in vitro* cytotoxicity on mammalian cells, chemically profiled by UHPLC-ESI-MS/MS, and incorporated into yogurts. Fortified yogurts were analyzed for proximate composition, mineral content, physicochemical properties, and RSA immediately after preparation ($t = 0$) and after seven days of storage at 4 °C ($t = 7$). The peel extracts had low cytotoxicity and were rich in bioactive compounds, notably catechin and caffeic acid glucoside. The incorporation of water and hydroethanolic extracts improved yogurt's water-holding capacity (WHC) and reduced syneresis at $t = 0$, although a decline in WHC and an increase in syneresis were observed at $t = 7$. The antioxidant properties of the yogurt were enhanced at both time points, and fortification resulted in increased Na, K, and Mg levels. These findings underscore the potential of *C. edulis* fruit peel extracts as a functional yogurt additive, promoting invasive species valorization while enhancing food quality and nutrition.

1. Introduction

Food additives have revolutionized the food industry by extending shelf life, enabling mass production, and facilitating global distribution (Bimpizas-Pinis et al., 2022). However, artificial additives are increasingly linked to health concerns, including gut disorders, obesity, and immune system problems (Pearlman et al., 2017; Laudisi et al., 2019; Wang et al., 2024). This growing awareness drives consumer demand for safer, natural alternatives and fosters the "clean label" movement in the food industry (Román et al., 2017). Moreover, functional foods, *i.e.*, foods promoting positive effects on health in addition to their basic nutritional purposes (Granato et al., 2017) are already integrating the diet of a high number of consumers and are gaining prominence worldwide. The potential global market for functional foods in 2027, is expected to generate revenues of over 268 billion U.S. dollars worldwide (Statista, 2020).

Yogurt's popularity is attributed to its affordability, general consumer appeal, and intrinsic health benefits, and has become an effective vehicle for delivering nutraceuticals (Pereira et al., 2016; Rashwan et al., 2023). Yogurt's high-water content makes it particularly suitable for enrichment with hydrophilic extracts, enhancing its health benefits and aligning with modern dietary trends (Cristhian et al., 2018). This versatility has established yogurt as a prime candidate for functional food innovation. It can be fortified with various plant-based ingredients, including fruits, hazelnuts, and cereals, to introduce new flavors while improving its nutritional and biofunctional properties (Cristhian et al.,

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2018; Szołtysik et al., 2021; Pérez et al., 2021; Albayati et al., 2024). Halophytes, known for their ability to synthesize bioactive compounds like phenolics with antioxidant and antimicrobial effects, also hold potential as natural functional ingredients (Hulkko et al., 2023; Lopes et al., 2023), including for yogurt enhancement. These resilient plants thrive in saline soils (0.5 - 1.0 M NaCl) and withstand harsh environmental conditions such as drought, extreme temperatures, and intense light, making them valuable for promoting biodiversity, sustainability, and climate change adaptation. Their capacity to grow in non-arable saline lands using seawater or brackish water positions them as an eco-friendly resource for food innovation without competing for traditional agricultural areas. However, despite their high biotechnological potential, the application of halophytes in yogurt development remains underexplored. One study demonstrated that incorporating *Portulaca oleracea* L. (purslane) water extract into yogurt improved its antioxidant, antimicrobial, sensory, and nutritional attributes while enhancing its texture and stability (Al-Quwaie et al., 2023). Similarly, *Hippophae rhamnoides* L. (sea buckthorn) extract was used to fortify probiotic yogurt, resulting in enhanced nutritional value and sensory acceptance by a consumer evaluation panel (Brodziak et al., 2021).

Carpobrotus edulis L. (Hottentot fig, syn. *Mesembryanthemum edule* L.) is a highly invasive halophyte native to South Africa but widely naturalized in coastal regions around the world, where it often outcompetes native flora due to its rapid growth and adaptability to harsh environmental conditions (Castañeda-Loaiza et al., 2020). Despite being an ecological threat, Hottentot fig presents significant potential as a valuable source of bioactive compounds, offering opportunities for the sustainable utilization of invasive species. Hottentot fig fruits are rich in carbohydrates, essential minerals such as calcium (*Ca*) and magnesium (Mg), and bioactive compounds, including phenolic acids and flavonoids like quercetin, kaempferol, and gallic acid. These compounds possess highly relevant antioxidant and antimicrobial properties, enhancing their potential for food preservation and functional food applications (Rúa et al., 2017; Castañeda-Loaiza et al., 2020; Periferakis et al., 2022; Shabir et al., 2022). This study aimed to fortify cow milk yogurt with Hottentot fig fruit extracts (peels, pulps) and evaluate the resulting product's nutritional, physicochemical, and antioxidant properties. Previous research has primarily focused on the chemical composition and bioactivities of C. edulis fruits, with limited insights into their integration into food matrices, particularly dairy products. This study presents a novel approach by using C. edulis fruit peel extracts as functional additives in yogurt, an application not previously explored for this invasive species, to our best kowledge. This approach aligns with contemporary circular economy and ecosystem management practices, offering a sustainable strategy to valorize invasive species while potentially reducing the economic costs associated with their control and eradication (Máximo et al., 2020).

2. Materials and methods

2.1. Materials and reagents

Sigma-Aldrich (Germany) provided the 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and butylated hydroxytoluene (BHT). Further chemicals and solvents were supplied by VWR International (Belgium). Methanol, acetonitrile, water LC-MS optima grade, and formic acid LC-MS grade were supplied by Fisher Scientific (Hampton, USA).

2.2. Collection of Hottentot-fig fruits and sample processing

About 10 kg of ripe fruits from Hottentot fig plants were randomly collected in "Praia do Garrão", South of Portugal (coordinates: 43°38′19.39″N 116°14′28.86″W) in July 2018. Species identification was made by Dr. Maria João Rodrigues (CCMAR). Fruits were manually peeled, and peels and pulps were separated and stored at −20 °C. Frozen samples were then freeze-dried to a powder and kept at −20 °C.

2.3. Preparation of the extracts

Extracts from the peels and pulps of Hottentot fig fruits were prepared using three different solvents: 96 % ethanol, 100 % water, and a 50:50 hydroethanolic mixture. The plant biomass and solvents were combined in a 1:40 (w/v) ratio and allowed to extract overnight (16 h), at room temperature (RT, approximately 20 °C) with continuous stirring. The extracts were then filtered (Whatman no 4) and dried under reduced pressure at 40 °C. The dry extracts were weighed, and stock solutions were prepared at a concentration of 50 mg/mL, using the same solvent employed during the extraction process.

2.4. Antioxidant properties

2.4.1. Total phenolics (TPC) and total flavonoids content (TFC)

TPC and TFC were determined by the Folin-Ciocalteu (F-C), and aluminium chloride methods adapted to 96-well microplates (Rodrigues et al., 2014). Results were calculated using a calibration curve of the respective standard (at concentrations between 0.002 and 2 mg/mL), and were expressed as gallic acid equivalents (GAE), for TPC, and rutin equivalents (RE) in milligrams per gram of dried extract (dry weight, DW).

2.4.2. Radical scavenging activity (RSA) on DPPH and $ABTS^{\bullet+}$ radicals

The extracts, at concentrations ranging from 10 to 0.04 mg/mL, were evaluated for RSA on DPPH and $ABTS^{\bullet+}$ radicals according to fully described protocols, on 96 well plates (Rodrigues et al., 2014). The absorbances were measured at 517 nm (DPPH) and 734 nm ($ABTS^{\bullet+}$) using a Biotek Synergy 4 microplate reader. Gallic acid in water (2 mg/mL) was used as a positive control. Results were expressed as antioxidant activity in percentage (%), compared to the negative control, containing the corresponding solvent and as half-maximal inhibitory concentration (IC_{50}, mg/mL).

2.5. In vitro cytotoxic properties

Three mammalian cell lines were used in this study, two from the tumoral origin (HepG2: human hepatocarcinoma cells, and RAW 264.7: murine leukemic macrophage cells), and one from the non-tumoral origin (S17: murine bone marrow stromal cells). HepG2 and S17 cells were kindly provided by the Centre for Biomedical Research (CBMR, University of Algarve, Portugal), while RAW cells were provided by the Faculty of Pharmacy and Centre for Neurosciences and Cell Biology (University of Coimbra, Portugal). Cell lines were cultured in DMEM (HepG2 and S17 cells) or RPMI media (RAW cells) supplemented with heat-inactivated fetal bovine serum, 1 % L-glutamine (2 mM), and 1 % penicillin (50 U/mL)/streptomycin (50 μg/mL). Cells were maintained at 37 °C in a humidified atmosphere with 5 % CO_2, for 24 h. Once the cells reached the exponential phase, they were placed in 96-well tissue plates at a concentration of 5×10^3 cells/well for HepG2 and S17 and at 10×10^3 cells/well for RAW and incubated again for 24 h at 37 °C to adhere. The extracts were dissolved in dimethyl sulfoxide (DMSO), at 100 μg/mL and filtered through a 0.22 μm filter. The extracts were then applied to each well and cells were again incubated for 72 h. The control cells were treated with DMSO at the highest concentration used in the test wells (0.5 %). Cell viability (%) was determined by the MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) colorimetric assay, as described previously (Castañeda-Loaiza et al., 2020). Results were expressed as a percentage of cellular viability relative to the control cells.

2.6. Phytochemical profiling of the extracts by ultrahigh-Performance Liquid Chromatography Coupled with Electrospray Ionization Mass/Mass Spectrometry (UHPLC-ESI-MS/MS)

The extracts were analyzed by *UHPLC-ESI-MS/MS* using a Dionex Ultimate 3000RS UHPLC system coupled to a mass spectrometer (Q Exactive Orbitrap, Thermo, Waltham, USA). To get chromatographic separation, 2 µL of each sample was injected into the HPLC system equipped with reverse phase C-18 column (Phenomenex Kinetex XB-C18 (100 mm x 2.1, mm i. d., 2.6 µm). The column was thermostated at 25 °C (± 1 °C). The elution was carried out at a flow rate of 200 µL/min using water (A) and methanol (B) as eluents, both were acidified with 0.1 % formic acid. Elutions were performed using the following gradient: isocratic 5 % B (0 - 3 min), linear gradient increasing from 5 % B to 100 % (3 - 43 min), 100 % B (43 - 61 min), a linear gradient decreasing from 100 % B to 5 % (61 - 62 min) than isocratic, 5 % B (62 - 70 min).

The Thermo Q Exactive Orbitrap mass spectrometer equipped with electrospray ionization source was used in positive or negative polarity at the resolving power of 70 000 (full MS, range: *m/z* 100 - 1500) and 35,000 (ddMS2). Spray voltages were 4.0 kV in positive and 3.8 kV in negative ion modes. The capillary temperature was 320 °C. The acquired data were processed using TraceFinder 3.1 software (Thermo Fisher Scientific). The secondary metabolites were identified based on our previous published works and our MS2 databases. In every case, the exact molecular mass, isotopic pattern, characteristic fragment ions, and retention time were used to identify the compounds. The difference between the measured and calculated monoisotopic molecular masses was always <5 ppm.

2.7. Fortification of yogurts

Yogurts were made by mixing 250 mL of semi-skimmed cow milk (Auchan brand), with 30 g of natural yogurt (Auchan brand) purchased at the local market and 15 g of full fat milk powder (Nido, Nestlé). The nutritional composition of used milk, milk powder, and? yogurt, according to the reference label, is depicted in Table S1 (Supplementary material). The peel extracts were dissolved in a small amount of milk (taken from the 250 mL volume), added to the mixture of milk, yogurt and milk powder, and yogurt preparation took place in a Thermomix (TM5, Vorwerk), for 5 min, 50 °C, velocity 3. Yogurts were then divided into portions (80 mL each) and fermented for 8 h, in a yogurt maker, at 45 °C. Afterwards, yogurts were stored at 4 °C, in the dark, for 16 h ($t = 0$), and 7 days ($t = 7$), respectively. Four groups of samples were prepared, each comprising three yogurts, 80 mL each: 1) control (without extract); 2) yogurts fortified with the water extract; 3) yogurts fortified with the ethanol extract, and 4) yogurts fortified with the hydroethanolic extract. The extracts were incorporated into the yogurts at amounts providing two times the highest IC_{50} value obtained for the antioxidant activity of each extract to ensure a sufficient and effective concentration of bioactive compounds in the final product.

2.8. Physicochemical properties of yogurts

Yogurts were evaluated for color, pH, syneresis (S), and water holding capacity (WHC). Color evaluation was performed using the CIELAB which refers to the color space defined by the Commission Internationale de l'Éclairage (CIE) in 1976. A PSE-CSM 10 colorimeter (Instruments LDA, UK) was used, using the D65 illuminant. First, the device was calibrated using standard white and black tiles. After the calorimeter calibration, samples were put into the apparatus cell and the color was measured, through the color parameters L*, a*, and b*, in triplicates. The three-color parameters were used to describe lightness (L* = 0 (black) and L* = 100 (white)), a* representing red to green color coordinates (-a* = greenness and +*a** = redness), while b* representing yellow to blue color coordinates (-b* = blueness and +*b** = yellowness). Finally, the total color difference (ΔE) after 7 days of storage was calculated according to the following formula:

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$$

The pH was measured by the direct method using a potentiometer (Crison, Instruments, S.A., Spain). Nine measurements were taken per sampling time, using 3 different yogurts.

Syneresis and WHC were conducted as determined according to established protocols (Bakry et al., 2019) with some alterations. Yogurt samples (5 g) were centrifuged at 4000 x g for 15 min at 4 °C. After centrifugation, the supernatant (whey) and precipitate (strained yogurt) were both weighted separately. Syneresis was determined as the percentage of whey weight compared to the original weight of the sample (Bakry et al., 2019). The WHC was determined as the percentage of the precipitate compared to the original weight of the sample (Bakry et al., 2019).

2.9. Nutritional properties of yogurts

Yogurts were analyzed for moisture, ash, total protein, and minerals. Moisture was measured by drying fresh yogurt samples at 102 °C for 3 h, following the AOAC method 925.23. Ash was determined by incineration of the dried biomass at 550 °C for 3 h in a 48,000 Furnace, Thermolyne (Thermo Scientific™). Crude protein was estimated by measuring nitrogen (N) content in freeze-dried samples using the analyser Elementar Vario EL III (Hanau - Germany). Total protein was obtained by multiplying the N content by the general conversion factor of 6.38 (Codex Alimentarius Commission, 2010).

For the mineral analysis, 25 mg of each sample was digested using a mixture of 6 mL nitric acid (HNO_3) and 2 mL of hydrogen peroxide (H_2O_2) in a CEM Discover microwave synthesizer (USA). The resulting digests were then diluted with ultrapure water. Mineral concentrations in the diluted samples were measured using Agilent 4200 Microwave Plasma-Atomic Emission Spectroscopy (MP-AES; USA). Calibration curves were established using a multielement standard solution, ensuring accurate quantification. Agilent MP Expert software was employed to process the data, subtracting background signals from the analytical signals to ensure precise mineral content determination.

2.10. In vitro antioxidant properties of yogurts

Extracts were prepared from control and fortified yogurts, according to Kennas et al. (2020) with some modifications. Freeze-dried samples were mixed with ethanol at a ratio of 1:10 and extracted overnight (16 h) while stirring at RT (approx. 20 °C). Extracts were then filtered (Whatman No.1) and centrifuged (4000 rpm, 10 min). The supernatant was collected and evaporated in a rotary evaporator at 40 °C under vacuum until complete removal of the solvent. The obtained dry extract was then dissolved in ethanol and evaluated for RSA towards DPPH and ABTS, as described in Section 2.4.2.

2.11. Statistical analyses

All experiments were analyzed at least in triplicate and expressed as the mean ± standard error of the mean (SEM). Whether the data was parametric or nonparametric, ANOVA test was applied for parametric data, and Tukey HSD test or the Kruskal-Wallis test were used for nonparametric data. Sample differences were considered significant if the P values were equal or inferior to 0.05. Statistical tests were made using XLSTAT, Statistical Software for Excel, Version 2021.2.2. IC_{50} were calculated with GraphPad Prism v. 5.0 software.

3. Results and discussion

3.1. Antioxidant properties

In this work, extracts from Hottentog fig fruit peels and pulps were first analyzed for total contents in phenolics and flavonoids, and results are depicted in Table 1. The total levels of phenolics and flavonoids in plant extracts are crucial indicators of their antioxidant potential, as these compounds are primarily responsible for neutralizing free radicals and reducing oxidative stress in biological systems (Shi et al., 2022). Generally, the higher the concentration of these bioactive compounds, the greater the extract's capacity to act as an effective antioxidant *in vitro* and *in vivo* models (Nwozo et al., 2023).

The extraction yields varied significantly based on the specific fruit part and the solvent employed. The highest (50 %) and the lowest yields (14 %) were obtained in the hydroethanolic extraction of the peel and pulp, respectively. Water extraction produced moderate yields of 24.6 % for the peel and 26.6 % for the pulp. Pure ethanol extraction resulted in yields of 34.0 % for the peel and 30.0 % for the pulp. Overall, the hydroethanolic extraction was the most effective for extracting compounds from the peel, whereas the pulp generally yielded less across all solvents tested. The higher extraction yield achieved with hydroethanolic extracts is attributed to the solvent mixture's ability to solubilize a broader spectrum of compounds, including polar and moderately non-polar substances. The presence of water enhances the extraction of hydrophilic compounds such as sugars and proteins, contributing to the overall higher yield.

Generally, the fruit peel extracts had higher levels of total phenolics and flavonoids, similar to the results observed previously in the same species (Castañeda-Loaiza et al., 2020). Significant differences were also observed between the extracts (Table 1). Ethanol was the most efficient solvent for extracting phenolics from the peel (TPC: 98.4 mg GAE/g, DW, TFC: 5.74 mg QE/g, DW), followed by hydroethanolic extracts (TPC: 96.0 mg GAE/g, DW, TFC: 4.21 mg QE/g, DW). Water was the least efficient solvent for extracting such compounds (TPC: 60.1 mg GAE/g, DW, TFC: 2.49 mg QE/g, DW). Pure ethanol demonstrates greater selectivity towards less polar compounds, such as phenolics and flavonoids. As a result, the ethanolic extract exhibited higher TPC and TFC, despite yielding a lower overall extract amount compared to the hydroethanolic extraction. Our results are similar to those previously described by Neves et al. (2021) for hydroethanolic extracts of Hottentog figs fruits (TPC: 102 mg GAE/g DW). The TPC of the fruit peel extracts detected in this work (Table 1) was lower than those described previously Castañeda-Loaiza et al. (2020), while the TFC for both parts of the fruit was higher. These differences could be explained by the different extraction techniques used since the ultrasound extraction technique, used previously, is considered more efficient for extracting phenolics, as observed in the halophyte sea knotgrass (*Polygonum maritimum* L.) (Rodrigues et al., 2019). In summary, the hydroethanolic extraction proved most effective for overall yield, while pure ethanol was superior for extracting phenolics and flavonoids, particularly from fruit peels.

Since the peel extracts had the highest levels of total phenolics and flavonoids, they were further appraised for their capacity to scavenge DPPH and $ABTS^{\bullet+}$ free radicals (Table 2). The ethanol extract exhibited the most effective RSA towards both radicals, with IC_{50} values of 0.414 and 0.202 mg/mL on DPPH and $ABTS^{\bullet+}$, respectively. The hydroethanolic extract was the second most effective, with IC_{50} values of 0.69 mg/mL (DPPH) and 0.310 mg/mL ($ABTS^{\bullet+}$). The water extract had the lowest RSA on both radicals (DPPH, IC_{50} = 1.555 mg/mL; $ABTS^{\bullet+}$, IC_{50} = 1.089 mg/mL). In general, all the extracts were more effective in scavenging $ABTS^{\bullet+}$ radicals. The acetone and ethanol extracts of Hottentot fig fruits, particularly from the peels, have been shown to exhibit high antioxidant activity (Castañeda-Loaiza et al., 2020). This antioxidant capacity is closely linked to the high levels of total phenolics, and flavonoids present in the fruit peels. Positive linear correlations between total phenolic content, measured via the F-C assay, and antioxidant assays such as TEAC (Trolox Equivalent Antioxidant Capacity) and DPPH have been consistently reported by other researchers, supporting the strong antioxidant potential of these bioactive compounds (Gulcin, 2020). Overall, ethanol extracts exhibited the strongest RSA, aligning with their higher phenolic and flavonoid content, while all extracts were generally more effective against ABTS•+ radicals.

3.2. In vitro cytotoxic properties

In this work, the extracts from peels exhibited the highest antioxidant properties and were further appraised for cytotoxic activity towards cell lines from tumoral and non-tumoral origin (Fig. 1). While natural products are often regarded as safer and less prone to adverse side effects than synthetic alternatives, some plants may contain toxic compounds, emphasizing the importance of assessing the safety of their extracts before incorporating them into food products (Ahmed et al., 2023). *In vitro* cytotoxicity assays using mammalian cell lines represent a crucial initial step in evaluating the potential toxicity of natural extracts providing a reliable and cost-effective method for assessing cellular responses to bioactive compounds and offering valuable insights into their safety profiles (Femina et al., 2023). No significant reduction in cell viability was observed following treatment with the extracts, with most values remaining above the reference threshold of 80 % (Fig. 1). The

Table 1
Extraction yields (%), and total contents of phenolics (TPC) and flavonoids (TFC) of water, hydroethanolic and ethanol extracts from Carpobrotus edulis L. (Hottentog fig) fruit peel and pulp.

Extract	Fruit part	Extraction yields (%)	Total contents of phenolics	
			TPC (mg GAE/g DW)	TFC (mg QE/g DW)
Water	Peel	24.6	60.1 ± 1.32^{b}	2.49 ± 0.24^{c}
	Pulp	26.6	18.8 ± 0.604^{e}	n.d
Hydroethanolic	Peel	50.0	96.0 ± 6.16^{a}	4.21 ± 0.37^{b}
	Pulp	14.0	29.8 ± 2.92^{d}	4.38 ± 0.79^{b}
Ethanol	Peel	34.0	98.4 ± 6.98^{a}	5.74 ± 0.30^{a}
	Pulp	30.0	43.3 ± 1.61^{c}	1.88 ± 0.09^{c}

Results are presented as means ± standard deviation (SD) of at least three repetitions. The comparison was made between extracts, for each group of compounds. In each column, different letters represent significant differences ($P < 0.05$) according to Tukey's HSD test for parametric data.
GAE: gallic acid equivalents; QE: quercetin equivalents; n.d: not detected.

Table 2
Radical scavenging activity (RSA) on 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) free radicals, of water, hydroethanolic and ethanol extracts from Carpobrotus edulis L. (Hottentog fig) fruit peels and pulp. Results are shown as half-maximal inhibitory concentration (IC_{50}) values in mg/mL.

Treatment/Extract	DPPH	$ABTS^{\bullet+}$
Water	1.555 ± 0.099^{d}	1.089 ± 0.080^{c}
Hydroethanolic	0.690 ± 0.085^{c}	0.310 ± 0.044^{b}
Ethanol	0.414 ± 0.099^{b}	0.202 ± 0.038^{b}
Gallic acid	0.007 ± 0.001^{a}	0.025 ± 0.004^{a}

Results are presented as means ± standard deviation (SD) of at least three independent experiments done in triplicates ($n = 9$). Comparison was made between extracts, for the same assay. In each column, different letters represent significant differences ($P < 0.05$) according to Tukey's HSD test for parametric data. Gallic acid: positive control.

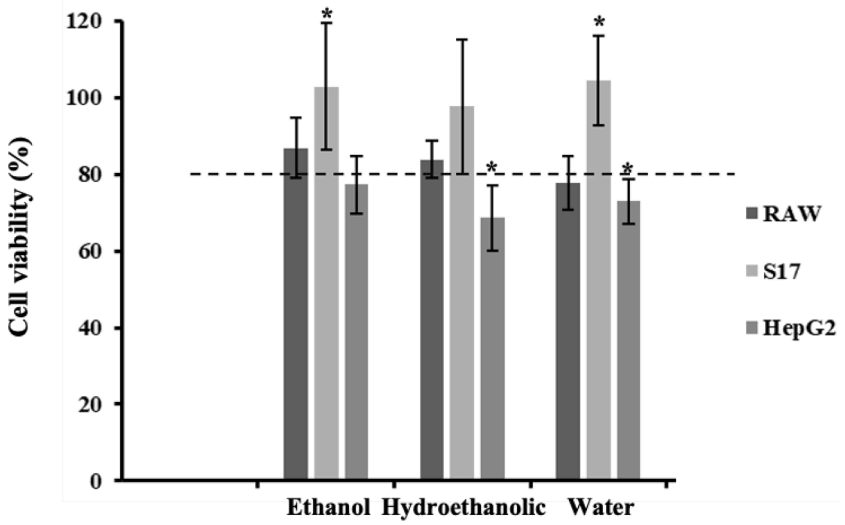

Fig. 1. *In vitro* cytotoxic properties of water, hydroethanolic and ethanol extracts from Carpobrotus edulis (Hottentog fig) fruit peel on HepG2 (human hepatocarcinoma), RAW 264.7 (murine leukemic macrophage), and S17 (murine bone marrow stromal) cells. Values represent the mean ± standard deviation (SD) of at least three experiments performed in triplicate ($n = 9$). Comparison was made between the extracts, and the reference viability level (80 % cell viability, dashed line). The asterisk represents significant differences ($P < 0.05$) according to Tukey's HSD test for parametric data and Kruskal Wallis for non-parametric data.

only exception was observed in HepG2 cells treated with the hydroethanolic extract, where cell viability dropped to near 68 %. This indicates that, while the extracts are generally non-toxic, the hydroethanolic extract may exhibit mild cytotoxicity in certain cancer cell lines. These findings align with previous studies on the same species. For example, ethanol, acetone, and water extracts from the fruit pulps and peels of the same species were non-toxic to human keratinocyte cells (HaCaT) (Castañeda-Loaiza et al., 2020). Overall, the extracts demonstrated minimal cytotoxicity supporting their potential safety for food applications.

3.3. Phytochemical composition of the peel extracts

To gain more knowledge on the phytochemical composition of the peel extracts, an analysis was made by UHPLC-ESI–MS/MS, and results are summarized on Tables S2 - S4 (Supplementary Material). A total of 31, 30 and 29 secondary metabolites were detected in the ethanol, hydroethanolic and water extract, respectively. The same metabolites were detected in the three samples, except for oleanolic acid, which was not detected in the hydroethanolic and oleanolic acid, and quercetin, which was not detected in the water extract.

Results indicate that malic acid, citric acid and quinic acid are the main metabolites in the extracts, followed by dimethoxy-tetrahydroxyflavone-*O*-(rhamnosyl)hexoside, dimethoxy-tetrahydroxyflavone-*O*-(pentosyl) hexosylhexoside ferulate, dimethoxy-tetrahydroxyflavone and methoxy-pentahydroxyflavone-*O*-(rhamnosyl)hexoside. Dimethoxy-tetrahydroxy flavone and its derivatives are the main flavonoids in the examined extract. No data was found on the structure of this flavonoid in the literature and analyzing the fragmentation patterns we could not determine unambiguously the positions of the hydroxy and methoxy substituents on the flavonoid rings. Examining the peak areas (Tables S2 - S4, Supplementary Material), it was observed that in all cases the areas of the metabolites found in the ethanolic extract were the largest followed by the hydroethanolic and water extracts. Therefore, our results suggest that in terms of the main metabolites, ethanol could be the most preferable solvent for preparing Hottentog fig fruit peel extracts.

The secondary metabolite profiles of the fruit peel were largely consistent with those reported by Castañeda-Loaiza et al. (2020). However, our analysis revealed the presence of additional polar compounds, such as quinic acid, malic acid, citric acid, protocatechuic acid, and its hexoside derivative, which were not detected in the acetone, water, and ethanol extracts previously prepared by ultrasound-assisted extraction. On the other hand, certain compounds identified by Castañeda-Loaiza et al. (2020), including several Procyanidin B isomers, quercetin glycosides, and azelaic acid, were not detected in our analysis. While most secondary metabolites in the fruit peel align with previous findings, the differences in compound detection highlighted the impact of extraction methods on metabolite profiles. In summary, ethanol extraction yielded the highest concentration of key metabolites, suggesting its higher efficiency for extracting bioactive compounds from Hottentot fig peels, although differences in metabolite profiles compared to previous studies highlight the influence of extraction methods.

3.4. Physicochemical properties of fortified yogurts

Color is an essential characteristic that affects consumer perceptions regarding the quality of food products (Spence, 2023) and their expected shelf-life. Usually, changes in color during storage are not desired, but different colored products can be attractive to the market (Spence, 2023).

Lightness (L*) in yogurt indicates the brightness of the product, with higher L* values representing a lighter, whiter appearance. This measurement is crucial for evaluating the visual quality and consumer appeal of yogurt as it can be affected by the ingredients and additives used (Spence, 2023). In this work, L* was significantly lower in yogurts fortified with the hydroethanolic extract, at $t = 0$, while no differences were observed at $t = 7$ (Table 3). When looking at the influence of the storage period, a decrease in L* was only observed in samples fortified with the ethanol extract, from $t = 0$ (83.1) to $t = 7$ (79.6). Redness (a*) (Table 3) significantly increased in samples fortified with the extracts, when compared with the control group, for $t = 0$, while at $t = 7$ an increase was observed in yogurts fortified with the water and ethanol extracts. No differences were noted for the control yogurts, and in those fortified with the water extract from $t = 0$ to $t = 7$, while a significant decrease in a* was noticed between both fortified yogurts with ethanol and hydroethanolic extract along the storage period. A similar trend was observed in yellowness (b*) (Table 3). The color parameters L* and b* were enhanced by adding a water extract of purslane (*P. oleraceae*) to yogurts compared to control yogurt (Al-Quwaie et al., 2023). The changes of a* and b* values can be due to the pigments present in Hottentog peel extracts, betalains which are water-soluble pigments producing

Table 3
Color changes of yogurts fortified with water, hydroethanolic and ethanol extracts from *Carpobrotus edulis* L. (Hottentog fig) fruit peel, after storage at 4 °C, in the dark, for 16 h ($t = 0$), and 7 days ($t = 7$).

		Treatment/extract			
Parameter	Storage	Control	Water	Hydroethanolic	Ethanol
L*	$t = 0$	81.36 ± 0.770[a]	80.42 ± 1.843[a]	78.25 ± 1.145[b]	83.12 ± 0.603[a*]
	$t = 7$	80.93 ± 0.227[a]	78.93 ± 1.151[a]	79.38 ± 0.477[a]	79.62 ± 0.532[a]
a*	$t = 0$	−0.840 ± 0.039[b]	−0.109 ± 0.236[a]	−0.153 ± 0.092[a*]	0.062 ± 0.053[a*]
	$t = 7$	−0.651 ± 0.090[b]	−0.508 ± 0.048[a]	−0.721 ± 0.277[b]	−0.484 ± 0.096[a]
b*	$t = 0$	3.791 ± 0.249[b]	4.732 ± 0.150[a]	4.799 ± 0.080[a*]	5.552 ± 0.094[a*]
	$t = 7$	4.478 ± 0.410[a]	4.712 ± 0.116[a]	4.146 ± 0.206[a]	4.165 ± 0.383[a]
ΔE		1.844 ± 0.536[b]	1.843 ± 0.714[b]	2.776 ± 1.051[b]	4.316 ± 0.767[a]

Results are presented as means ± standard deviation (SD) of at least three independent experiments, done in triplicate ($n = 9$). Different letters in each row represent significant differences between samples, for the same time of storage, while differences between times of storage, for each group of samples, in the same column, are marked with an asterisk (*) ($P < 0.05$) (Tukey's HSD test for parametric data and Kruskal Wallis for non-parametric data).
L*: represents lightness. L* = 0 (black), L* = 100 (white).
a*: represents red and green color coordinates. -a* = greenness and +*a** =redness.
b*: represents yellow and blue color coordinates. -b* = blueness and +*b** = yellowness.
ΔE: represents the total color difference after 7 days of storage.

red-violet coloration of fruits (Kimler et al., 1970; Azeredo et al., 2009). On the other hand, L* is related to different factors including the concentration and type of pigments present, the water content, and surface water availability (Viuda-Martos et al., 2012). Regarding the overall color change (ΔE), only yogurts fortified with the ethanol extract had a statistically different and noticeable ΔE, maybe related to a higher concentration of colored compounds in this extract. However, such changes were not noticeable to the naked eye but still, they are a good control, for predicting the behavior of a new food product, and its changes during storage. In brief, the incorporation of extracts influenced the color parameters of yogurts, with the hydroethanolic extract reducing L*at initial storage, and the ethanol extract leading to noticeable ΔE over time, highlighting the role of pigment composition and extract type on yogurt appearance during storage.

The pH of yogurt typically ranges between 4.1 and 4.6, which ensures the right balance of tartness and smoothness while inhibiting the growth of harmful bacteria and is a result of lactic acid bacteria transforming lactose into lactic acid (Walstra & Wouters, 2006). This parameter is critical in yogurt as it directly influences the product's texture, flavor, microbial stability, and shelf-life (Walstra & Wouters, 2006). The pH also affects the protein structure, contributing to the yogurt's thickness and creaminess (Walstra & Wouters, 2006). The initial pH of control yoghurts was 4.349, in agreement with other previously reported values of plain cow and sheep milk yogurts (Serafeimidou et al., 2013). Fortification of yogurts with the Hottentog fig peel extracts did not significantly modify the pH at day 0, with values ranging from 4.219 to 4.463 in yogurts fortified with water and hydroethanolic extract, respectively (Table 4). After seven days of storage, the pH values increased slightly in the yogurts fortified with the hydroethanolic extract (4.582) and increased significantly in the case of ethanol (4.799) and water (4.701) extracts (Table 4). Fortification of yogurts with sea buckthorn fruit mousse lowered the pH in relation to the control, while pH value also increased at $t = 7$ (Brodziak et al., 2021). Opposite results were observed with fortification of yogurts with purslane, where the yogurt's pH reduced during storage (Al-Quwaie et al., 2023). In summary, fortification with Hottentot fig peel extracts did not significantly alter the initial pH of yogurts, though slight increases were observed during storage, particularly with ethanol and water extracts, suggesting that the extract type can influence pH stability over time.

Table 4
Values of pH, syneresis, and water holding capacity (WHC) of yogurts fortified with water, hydroethanolic and ethanol extracts from *Carpobrotus edulis* L. (Hottentog fig) fruit peel, after storage at 4 °C, in the dark, for 16 h ($t = 0$), and 7 days ($t = 7$).

		Storage	
Parameter	Treatment/extract	$t = 0$	$t = 7$
pH	Control	4.349 ± 0.032	4.587 ± 0.126
	Water	4.219 ± 0.078	4.701 ± 0.045*
	Hydroethanolic	4.463 ± 0.108	4.582 ± 0.135
	Ethanol	4.360 ± 0.043	4.799 ± 0.022*
Syneresis (%)	Control	60.89 ± 3.947[a]	64.09 ± 3.223
	Water	45.46 ± 1.783[c]	59.50 ± 3.107*
	Hydroethanolic	52.86 ± 2.754[b]	58.29 ± 3.582
	Ethanol	54.70 ± 4.493[ab]	58.40 ± 2.459
WHC (%)	Control	39.10 ± 3.947[c]	35.90 ± 3.223
	Water	54.53 ± 1.783[a*]	40.49 ± 3.107
	Hydroethanolic	47.13 ± 2.754[bc]	41.70 ± 3.582
	Ethanol	45.29 ± 4.493[bc]	41.59 ± 2.459

Results are presented as means ± standard deviation (SD) of three independent experiments, done in triplicate ($n = 9$). The comparison was between different extracts and the control group (row) and between storage time at $t = 0$ and $t = 7$ (column). Different letters in each column and asterisk (*) in the same row represent significant differences ($P < 0.05$) according to Tukey's HSD test for parametric data and Kruskal Wallis for non-parametric data.

Syneresis (S) and water holding capacity (WHC) are critical parameters regarding yogurt quality. Syneresis is an undesirable characteristic that can be seen as expelling liquid, caused by the shrinkage and weakening of the gel network/yogurt coagulum. It is largely influenced by the temperature and pH of the yogurt, where higher temperatures and lower pH (< 4) cause a more pronounced syneresis (Walstra & Wouters, 2006). WHC refers to the ability of the yogurt matrix to retain water within its structure, preventing the separation (syneresis) of liquid (whey) from the solid parts/coagulum of the yogurt. In this work, syneresis significantly decreased at $t = 0$, as a result from increased WHC, in yogurts fortified with hydroethanolic and water extracts, compared with control samples, and increased from $t = 0$ to $t = 7$, from decreased WHC in yogurts supplemented with the water extract (Table 4). No differences were observed between samples, at $t = 7$. Similar results were obtained by Al-Quwaie et al. (2023), who reported similar improvements in WHC for yogurts fortified with water extracts of purslane. The observed decline in WHC and the increase in syneresis during storage suggest a gradual deterioration of the yogurt matrix's structural integrity, potentially driven by modifications in protein-polyphenol interactions or reorganization within the gel network. The rearrangement of proteins in yogurt, typically leading to increased syneresis and decreased WHC during storage, is mainly due to the aggregation of casein micelles caused by isoelectric precipitation from lactic acid bacteria activity (Everett & McLeod, 2005; Lucey, 2002). However, fortification with polyphenol-rich extracts appears to slow this natural process. Polyphenols have a strong affinity for proteins, leading to the formation of soluble protein-polyphenol complexes through multiple weak interactions, primarily hydrophobic, between amino acid side chains and polyphenol aromatic rings (Oliveira et al., 2015; Charlton et al., 2002). This interaction may modify protein structures, enhancing protein affinity and cohesion, resulting in reduced syneresis and improved WHC (Kwon et al., 2019). Adding polyphenol-rich extracts could play a crucial role in stabilizing the gel matrix, forming stable complexes and stronger internal bonds, which reduces protein rearrangement during storage. This stabilization maintains a more robust casein network, retains water more effectively, and reduces syneresis, ultimately

improving the yogurt's quality and consumer acceptability. However, the formation of protein-polyphenol complexes is influenced by factors, such as the nature of the polyphenols and proteins, temperature, and the presence of other components that might interfere with these interactions (Pringent et al., 2003). In sum, fortification with hydroethanolic and water extracts improved yogurt quality by enhancing WHC and reducing syneresis at initial storage, likely due to polyphenol-protein interactions that stabilized the gel matrix. However, a decline in WHC and increased syneresis during storage indicate the need for further research to optimize these interactions for prolonged yogurt stability.

3.5. Nutritional properties of fortified yogurts

The moisture content of the yogurts ranged between 81.7 % and 85.6 % (Table 5), The water content in dairy products can vary widely, ranging from approximately 2.5 % to 94 % (w/w), with yogurts typically containing between 82 % and 85 %, depending on their fat and protein content (Fox et al., 2015). The moisture content of food products is closely linked to water activity, a critical factor in food technology that, along with pH and temperature, significantly influences the stability and shelf life of food by affecting chemical and microbiological changes (Fox et al., 2015). No significant differences were observed between different storage times, except in the control group, where moisture decreased from 85.58 % at $t = 0$ to 82.5 % at $t = 7$ (Table 5). When comparing the control group with the fortified yogurts, significant differences were observed in moisture content depending on the type of extract and storage time (Table 5). At $t = 0$, a significant decrease in moisture content was recorded in yogurts fortified with the ethanol extract, likely due to the interactions between the extract components and the yogurt matrix, potentially affecting water retention. No differences were observed for $t = 7$. To conclude, moisture content in fortified yogurts generally remained stable, except for ethanol-fortified samples at initial storage, where decreased moisture likely resulted from extract-matrix interactions affecting water retention. This suggests that the type of extract can influence yogurt's moisture dynamics, potentially impacting texture and shelf life.

Table 5
Moisture, ash, and total protein of yogurts fortified with water, hydroethanolic and ethanol extracts from Carpobrotus edulis L. (Hottentog-fig) fruit peel, after storage at 4 °C, in the dark, for 16 h ($t = 0$), and 7 days ($t = 7$).

Parameter	Treatment/ extract	Storage	
		$t = 0$	$t = 7$
Moisture (%)	Control	85.58 ± 0.094^{a*}	82.51 ± 0.266^{a}
	Water	84.63 ± 0.032^{a}	83.68 ± 0.576^{a}
	Hydroethanolic	83.43 ± 0.149^{a}	84.61 ± 0.405^{a}
	Ethanol	81.71 ± 0.236^{b}	83.79 ± 0.161^{a}
Ash (%)	Control	0.882 ± 0.019^{b}	0.791 ± 0.117^{b}
	Water	1.111 ± 0.101^{a}	1.036 ± 0.088^{a}
	Hydroethanolic	1.161 ± 0.082^{a*}	1.010 ± 0.064^{a}
	Ethanol	0.969 ± 0.055^{a}	0.914 ± 0.012^{a}
Crude protein (g/100 g)	Control	29.67 ± 1.312^{a}	30.64 ± 0.448^{a}
	Water	29.42 ± 1.243^{a}	27.69 ± 0.354^{a}
	Hydroethanolic	30.08 ± 0.729^{a}	27.85 ± 0.785^{a}
	Ethanol	28.05 ± 1.584^{a}	28.43 ± 2.490^{a}

Results are presented as means ± standard deviation (SD) of three independent experiments, done in triplicate ($n = 9$). The comparison was made between yogurts fortified with different extracts, and the control group (column) and between storage time at $t = 0$ and $t = 7$ (row). Different letters in each column and an asterisk (*) in the same row represent significant differences ($P < 0.05$) according to Tukey's HSD test for parametric data and Kruskal Wallis for non-parametric data.

The ash content of the yogurts varied between 0.791 % (control group, $t = 7$) and 1.161 % (yogurt fortified with the hydroethanolic extract, $t = 0$) (Table 5). A significant increase in ash content was observed between the control group and yogurts fortified with all the extracts, at both storage periods (Table 5). A similar trend was observed by Jung et al. (2016), where higher concentrations of red ginseng extracts led to increased ash levels in functionalized yogurts. However, no significant differences were found between storage times, except in yogurts fortified with hydroethanolic extracts, where ash content significantly decreased over time. This is likely due to the high ash content of the fruits of C. edulis, which accumulate minerals effectively (Broomhead et al., 2019). This may explain the higher ash and the mineral content, detailed in Table 6, and explained in the next paragraph. The increase in ash content following fortification suggests that Hottentot fig extracts contribute to the overall mineral enrichment of yogurt, aligning with the species' known capacity for mineral accumulation. However, storage may influence ash stability, particularly in hydroethanolic extracts, warranting further investigation.

The levels of the macro minerals found in the yogurts followed this descending order: potassium (K), calcium (*Ca*), sodium (Na), and magnesium (Mg). The content of K ranged from 14.2 mg/g (yogurt fortified with the ethanol extract, $t = 0$) to 15.5 mg/g (yogurt fortified with the water extract, $t = 7$). Notably, the addition of ethanol and hydroethanolic extracts led to a significant decrease in K content at $t = 0$ ($P < 0.05$), though this effect was not sustained at $t = 7$. The *Ca* content in the yogurts ranged from 9.58 mg/g (yogurt fortified with the ethanol extract, $t = 0$) to 10.3 mg/g (control yogurt and fortified with the water extract, $t = 7$), with fortification having no significant impact on its levels. The Na and Mg levels were measured between 2.65 mg/g (yogurt fortified with the ethanol and hydroethanolic extracts $t = 0$) and 3.28 mg/g (yogurt fortified with the water extract, $t = 0$); 0.88 mg/g (yogurt fortified with the ethanol extract, $t = 0$) and 1.12 mg/g (yogurt fortified with the water extract, $t = 0$), respectively. A significant increase in Na and Mg content was observed with the addition of water extracts at $t = 0$ ($P < 0.05$), though this effect was not sustained at $t = 7$ (Table 6). Regarding trace elements like manganese (Mn), iron (Fe), zinc (Zn), and copper (Cu), fortification with water and hydroethanolic extracts resulted in a significant increase in Mn at both storage times ($P < 0.05$). Fortification with the hydroethanolic extract resulted in lower Fe levels in yogurts at $t = 7$, while the levels of Zn, and Cu remained unaffected (Table 6). Given the relatively small amount of extract used, the contribution of these minerals was minor.

Regarding toxic elements, only nickel (Ni) was detected across all samples, including the control, at levels ranging from 0.83 μg/g (yogurt fortified with the ethanol extract, $t = 7$) to 1.27 μg/g (control yogurts at $t = 0$). In previous studies, Cr was the only toxic element in C. edulis fruits, with other metals like cobalt (Co), nickel (Ni), and selenium (Se) were undetectable (Broomhead et al., 2019). Although halophytes are known to accumulate toxic elements when grown in contaminated sites, these are typically stored in underground organs (Caetano et al., 2008). The detection of Ni in the control sample suggests that the source of contamination may be the dairy product itself, likely due to environmental pollutants from industrial, urban, and agricultural activities (Capcarova et al., 2017). Despite the presence of Ni, the levels found in yogurt are low, with the estimated exposure requiring consumption of approximately 12 yogurts daily to reach the tolerable daily intake (TDI) for nickel established by EFSA, which is 13 μg/kg body weight (Schrenk et al., 2020). In summary, fortification of yogurts with Hottentot fig peel extracts introduced slight variations in mineral content, enhancing

Table 6
Mineral content of yogurts fortified with water, hydroethanolic and ethanol extracts from Carpobrotus edulis L. (Hottentog fig) fruit peel after storage at 4 °C, in the dark, for 16 h ($t = 0$), and 7 days ($t = 7$).

	Storage and treatment/extract							
	$t = 0$				$t = 7$			
Mineral	Control	Water	Hydroethanolic	Ethanol	Control	Water	Hydroethanolic	Ethanol
Macro-elements (mg/g)								
Na	2.70 ± 0.12^{b}	3.28 ± 0.00^{a}	2.65 ± 0.04^{b}	2.65 ± 0.05^{b}	2.75 ± 0.36^{a}	3.08 ± 0.09^{a}	2.72 ± 0.12^{a}	2.74 ± 0.34^{a}
Ca	10.1 ± 0.56^{a}	10.1 ± 0.05^{a}	9.82 ± 0.06^{a}	9.58 ± 0.19^{a}	10.3 ± 0.29^{a}	10.3 ± 0.4^{a}	10.0 ± 0.45^{a}	10.2 ± 0.15^{a*}
K	15.3 ± 0.94^{a}	14.9 ± 0.06^{a}	14.3 ± 0.14^{b}	14.2 ± 0.39^{b}	15.2 ± 0.49^{a}	15.5 ± 0.63^{a}	14.8 ± 0.78^{a}	14.7 ± 0.26^{a}
Mg	0.90 ± 0.04^{b}	1.12 ± 0.01^{a}	0.93 ± 0.02^{b}	0.88 ± 0.02^{b}	0.94 ± 0.13^{a}	1.06 ± 0.02^{a}	0.95 ± 0.04^{a}	0.95 ± 0.10^{a}
Micro and trace-elements (µg/g)								
Fe	1.99 ± 0.16^{a}	3.13 ± 0.25^{a}	2.25 ± 0.35^{a}	2.56 ± 0.29^{a}	1.86 ± 0.29^{a}	2.19 ± 0.30^{a}	1.20 ± 0.12^{b}	2.18 ± 0.13^{a}
Mn	0.30 ± 0.02^{b}	4.92 ± 0.02^{a}	1.68 ± 0.03^{a}	0.78 ± 0.04^{b}	0.25 ± 0.00^{d}	4.30 ± 0.02^{a}	1.50 ± 0.07^{b}	0.81 ± 0.04^{c}
Zn	32.9 ± 2.04^{a}	32.8 ± 0.43^{a}	31.8 ± 1.32^{a}	31.3 ± 0.99^{a}	34.0 ± 1.98^{a}	31.8 ± 0.73^{a}	32.7 ± 1.54^{a}	33.8 ± 0.75^{a}
Cu	0.79 ± 0.05^{a}	0.73 ± 0.06^{a}	0.76 ± 0.02^{a}	0.81 ± 0.04^{a}	0.62 ± 0.03^{a}	0.52 ± 0.03^{a}	0.59 ± 0.03^{a}	0.59 ± 0.03^{a}
Cr	< LOQ	< LOQ	0.12 ± 0.00	< LOQ	0.03 ± 0.00^{a}	0.06 ± 0.00^{a}	< LOQ	< LOQ
Ni	1.27 ± 0.06^{a}	1.06 ± 0.11^{a}	1.04 ± 0.15^{a}	1.14 ± 0.03^{a}	1.06 ± 0.09^{a}	0.97 ± 0.21^{a}	1.00 ± 0.08^{a}	0.83 ± 0.06^{a}
Pb	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Cd	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ

Data represent the mean ± standard deviation (SD) of three experiments ($n = 3$). The comparison was made for each mineral, between different treatments for each storage period (represented by small letters) and between storage periods, for the same treatment and mineral. Statistical differences ($P < 0,05$) according to Tukey's HSD test for parametric data and Kruskal Wallis for non-parametric data. Limits of quantification (LOQ): Cr 0.01 µg/g, Pb 0.53 µg/g, Cd 0.17 µg/g.

levels of K, Na, Mg, and Mn, depending on the extract type and storage duration. Despite detecting nickel across samples, its concentration remained within safe limits, posing negligible health risks.

The crude protein content of the yogurts ranged from 27.69 g/100 g (yogurt fortified with water extract, $t = 7$) to 30.64 g/100 g (control, $t = 7$) (Table 5), which is considerably higher than the 3.95 - 4.86 g/100 g range reported for plain yogurts by Serafeimidou et al. (2013). This increase is attributed to the addition of plain milk powder with a high protein content (26.4 g/100 g) to the yogurts, as shown in Table S1 (Supplementary Material). There were no significant differences in crude protein levels between the different samples and storage times, indicating that the added extracts did not alter the protein content of the yogurts. This was expected, given that Hottentot fig fruits are primarily composed of carbohydrates and have a low protein content (Broomhead et al., 2019). In brief, protein levels in the fortified yogurts remained consistent across samples and storage times, with no significant influence from the addition of Hottentot peel extracts, likely due to the naturally low protein content of the fruit.

3.6. Antioxidant properties of the yogurts

Yogurts fortified with the water and ethanol extracts exhibited the highest RSA towards DPPH compared to the control at $t = 0$ and $t = 7$, with EC_{50} values of 199.2 mg/mL and 481.5 mg/mL, respectively (Table 7). While the RSA of water extract fortified yogurts decreased over time, ethanol extracts fortified yogurts showed an increase in antioxidant activity. Similarly, in the $ABTS^{\bullet+}$ assay, water and ethanol extract-fortified yogurts maintained the highest RSA at both $t = 0$ and $t = 7$, with EC_{50} values of 176.5 mg/mL and 437.2 mg/mL, respectively. The antioxidant activity of yogurts fortified with water extracts decreased over the storage period, while those enriched with ethanol extracts exhibited an improvement in antioxidant capacity over time. The antioxidant activity observed in control samples may be attributed to sulfur-containing amino acids in milk, such as cysteine, along with vitamins A, E, carotenoids, zinc, selenium, and enzyme systems like superoxide dismutase, catalase, and glutathione peroxidase (Khan et al., 2019). Previous studies have also demonstrated that incorporating plant extracts rich in phenolic compounds can significantly enhance the antioxidant potential of yogurts. For instance, adding a water extract from purslane to yogurts resulted in a significant decrease in malondialdehyde (MDA) levels, attributed to the antioxidant properties of the purslane (Al-Quwaie et al., 2023). Adding extracts to yogurt rich in antioxidants like polyphenols and flavonoids, can significantly prevent

Table 7
Radical scavenging activity (RSA, IC_{50} values in mg/mL) on 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) radicals, of ethanol extracts made from fortified yogurts with water, hydroethanolic and ethanol extracts from Carpobrotus edulis (Hottentog fig) fruit peel after storage at 4 °C, in the dark, for 16 h ($t = 0$), and 7 days ($t = 7$).

		Storage	
RSA	Treatment/extract	$t = 0$	$t = 7$
DPPH	Control	589.6 ± 57.62^{d}	594.3 ± 25.94^{d}
	Water	199.2 ± 9.521^{b*}	325.3 ± 10.68^{b}
	Hydroethanolic	536.8 ± 20.74^{d}	538.5 ± 17.50^{d}
	Ethanol	481.5 ± 42.91^{c}	354.2 ± 39.03^{c*}
	Gallic acid	0.015 ± 0.000^{a}	0.015 ± 0.001^{a}
$ABTS^{\bullet+}$	Control	515.0 ± 45.74^{d}	490.0 ± 21.66^{d}
	Water	176.5 ± 15.69^{b*}	287.6 ± 3.551^{b}
	Hydroethanolic	582.4 ± 42.91^{d}	463.7 ± 4.541^{d*}
	Ethanol	437.3 ± 22.70^{c}	388.7 ± 11.52^{c}
	Gallic acid	0.025 ± 0.004^{a}	0.025 ± 0.004^{a}

Results are presented as means ± standard deviation (SD) of three independent experiments, done in triplicate ($n = 9$). The comparison was made between different samples (column) and storage periods (row). Different letters in each column and an asterisk (*) in the same row represent significant differences ($P < 0.05$) according to Tukey's HSD test for parametric data and Kruskal Wallis for non-parametric data. Gallic acid: positive control.

food spoilage due to lipid oxidation, a common issue in dairy products that leads to off-flavors, discoloration, and reduced nutritional value (Ahmad et al., 2022). These products can also help to reduce oxidative stress in the body, thereby lowering the risk of diseases such as cancer and neurodegeneration (Ahmad et al., 2022). Although the fortified yogurts exhibited significantly higher antioxidant activity compared to the control, a noticeable reduction in RSA was observed when compared to the original extracts (Table 2). This reduction is likely due to polyphenols binding to milk proteins, leading to their precipitation. The acidic environment of yogurt may further assist this interaction, as noted by Helal and Tagliazucchi (2018), who reported a similar phenomenon in cinnamon-fortified yogurt, where a large portion of phenolic compounds became bound to milk proteins, reducing their bioavailability. In sum, incorporation of water and ethanol extracts significantly enhanced the antioxidant activity of yogurts, although storage affected this activity differently depending on the extract.

4. Conclusion

This study suggests that Hottentot fig fruit peel extracts could be further explored as natural additives to fortify yogurt, enhancing its nutritional and functional properties. The hydroethanolic and water extracts exhibited higher extraction yields and improved the antioxidant potential of yogurts. The ethanolic extracts, though lower in yield, were richer in phenolic and flavonoid compounds, which contributed to stronger radical scavenging activities. Yogurt fortification with the extracts improved yogurt's WHC and reduced syneresis at the initial storage stage, though these benefits decresased after 7 days, indicating the need for further optimization to maintain long-term stability. The inclusion of extracts did not significantly affect the yogurt's pH, moisture, protein content, or mineral composition over the storage period, ensuring the product's physicochemical stability. Additionally, cytotoxicity assays confirmed the extracts' safety for food applications. Our results highlight the dual benefits of valorizing an invasive species for sustainable food innovation while enhancing yogurt's nutritional profile. The integration of Hottentot fig fruit peel extracts into dairy products presents a promising strategy for promoting natural, functional ingredients in food systems. Future research should focus on optimizing extract concentrations, assessing consumer acceptance, and exploring scalability for commercial applications.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the author(s) used Grammarly and ChatGPT 4 to improve language and readability. After using this tool, the author(s) reviewed and edited the content as needed and take (s) full responsibility for the content of the publication.

Ethical statement – studies in humans and animals

This study did not involve any experiments on humans or animals. All research conducted for this manuscript complies with ethical standards and focused solely on plant-based materials and *in vitro* methodologies. No human participants or animal subjects were used in this work.

CRediT authorship contribution statement

Kim Bratkič: Writing – original draft, Investigation. **Maria João Rodrigues:** Investigation. **Viana Castañeda-Loaiza:** Investigation. **Catarina Pereira:** Investigation. **Isabel Ratão:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Formal analysis, Conceptualization. **Célia Quintas:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Formal analysis, Conceptualization. **Andreja Čanžek Majhenič:** Writing – review & editing. **József Jeko:** Investigation. **Zoltán Cziáky:** Writing – review & editing, Writing – original draft, Investigation. **Luísa Custódio:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors whose names are listed immediately below certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.afres.2025.100962.

Data availability

Data will be made available on request.

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