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Nutriosome-based delivery of tomato by-product polyphenols: formulation, characterization, and integration into functional gummies

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ABSTRACT

Tomato processing by-products represent a sustainable source of antioxidant bioactives, but their use as functional ingredients requires formulation strategies able to improve their stability and oral delivery. In this study, a hydroethanolic tomato by-product extract, rich in carotenoids (β-carotene, phytoene and lycopene) and phenolic compounds (mainly quercetin-3-galactoside), was incorporated into phospholipid-based vesicles prepared by an organic solvent-free direct sonication method. Both liposomes and nutriosomes exhibited nanometric size (<100 nm), low polydispersity index (~0.213), a highly negative surface charge (~−43 mV). Both systems retained more than 75% of their initial antioxidant activity after dialysis and showed good physicochemical stability during 180 days of storage at 4 °C. Under simulated salivary, gastric and intestinal pH and ionic conditions, both formulations maintained their colloidal stability. However, nutriosomes showed superior technological performance following freeze-drying and rehydration, displaying only a limited increase in particle size while preserving a narrow size distribution. In Caco-2 cells, all formulations were biocompatible, maintaining cell viability above 90% after 48 h. Under oxidative stress conditions, free extract and liposomes failed to significantly counteract hydrogen peroxide-induced cytotoxicity, whereas nutriosomes restored cell viability to approximately 93%. Owing to their superior technological and biological performance, nutriosomes were successfully incorporated into gummy formulations, which completely dissolved under simulated gastric conditions while releasing vesicles that retained their nanoscale characteristics, with a mean diameter of ~249 nm and a polydispersity index of ~0.211. Overall, these findings demonstrate that nutriosomes effectively enhance both the processing stability and biological efficacy of tomato by-product phytocomplexes, supporting their development as sustainable functional ingredients for gummy-based oral nutraceuticals.

1. Introduction

The human intestinal tract harbours a highly diverse microbial ecosystem, the gut microbiota, which plays a central role in host physiology [1]. The balance between the host and its gut microbiota is essential for maintaining intestinal homeostasis, whereas its disruption leads to dysbiosis, a condition increasingly associated with a wide range of pathological conditions, including inflammatory bowel diseases (IBD), irritable bowel syndrome (IBS), obesity, and diabetes [2]. Alterations in gut microbiota composition have been closely linked to the overproduction of reactive oxygen species (ROS). This oxidative stress contributes to intestinal inflammation by impairing epithelial barrier integrity, activating immune responses, and disrupting normal metabolic functions. Furthermore, dysbiosis alters the profile of microbial metabolites, which can themselves promote inflammation, enhance ROS generation, and induce epigenetic modifications. Collectively, these mechanisms may establish a self-perpetuating cycle in which inflammation-driven oxidative stress exacerbates microbial imbalance, thereby sustaining and amplifying the dysbiotic state [3]. The relevance of this dysbiosis-oxidative stress axis is particularly evident in paediatric gastrointestinal disorders, where alterations in gut microbiota composition may contribute to intestinal inflammation and impaired barrier function. In this context, the development of safe, well-tolerated, and age-appropriate oral formulations represents a major challenge, as

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paediatric administration requires not only biological efficacy but also palatability, ease of intake, and high patient acceptability [4,5]. Natural antioxidant compounds, including polyphenols and carotenoids, have emerged as promising therapeutic candidates due to their well-documented antioxidant properties and their ability to modulate gut microbiota composition. By scavenging ROS and influencing microbial diversity and metabolic activity, polyphenols may help restore intestinal homeostasis and interrupt the vicious cycle linking oxidative stress and dysbiosis [6,7]. Their capacity to promote beneficial bacteria while inhibiting pathogenic strains further supports their potential prebiotic-like activity [8].

Recently, increasing attention has been directed towards agri-food by-products as sustainable and cost-effective sources of polyphenols. These materials, once considered waste, have been re-evaluated as valuable raw materials for the production of high-value compounds, with potential applications in the food, pharmaceutical, and cosmetic industries [9,10]. Among these, tomato processing by-products represent a particularly promising source of bioactive compounds, being rich in carotenoids, mainly lycopene, as well as polyphenols such as flavonoids and phenolic acids. Owing to this composition, they are considered attractive ingredients for the development of functional foods and nutraceutical formulations [11].

However, the application of natural bioactives derived from agri-food side streams, particularly polyphenols and carotenoids, is often limited by their poor aqueous solubility, low stability under gastrointestinal conditions, and limited bioavailability. To overcome these limitations, nanotechnology-based delivery systems have been extensively investigated [12]. Among them, liposomes, phospholipid-based vesicles capable of encapsulating both hydrophilic and lipophilic compounds, offer several advantages, including high biocompatibility and safety, low toxicity, and the ability to improve the pharmacokinetic profile and biodistribution of bioactive molecules [13,14]. Despite these promising properties, conventional liposomes have several limitations, especially for oral administration as they may not adequately protect polyphenols from degradation in the upper gastrointestinal tract or ensure controlled release in the intestine. To overcome these limitations, Nutriose® FM06, a soluble dextrin resistant to digestion in the upper gastrointestinal tract and fermentable in the colon, has been included as a key component for the development of nutriosomes [15,16]. These advanced vesicular systems combine the protective properties of phospholipid carriers with the prebiotic potential of Nutriose, thereby improving the delivery and intestinal residence of bioactive compounds in the intestinal environment, promoting their interaction with the gut microbiota, and potentially enhancing their biological effects [17]. As a result, nutriosomes can enhance the stability, bioavailability, and overall efficacy of encapsulated polyphenols [18].

The development of oral formulations intended for paediatric use requires particular attention to acceptability and compliance. Although liquid formulations are commonly used in children, solid or semi-solid dosage forms with pleasant sensory properties may offer advantages in terms of handling, dosing convenience, and patient adherence [19]. In this regard, gummy-based formulations represent a promising platform for paediatric administration, as they are palatable, easy to administer, non-invasive, and suitable for incorporating functional ingredients or bioactive-loaded delivery systems [20].

In light of these considerations, the present study focused on the development of a functional gummy formulation enriched with antioxidant molecules extracted from tomato processing by-products and encapsulated in liposomes and nutriosomes. The resulting vesicular systems were characterized in terms of physicochemical properties, evaluated for their biocompatibility and protective effect against oxidative stress in Caco-2 cells, and finally incorporated into gummy formulations, which were subsequently assessed for their structural and functional properties.

2. Materials and methods

2.1. Materials

All chemicals used in the experiments were of analytical grade. The Folin & Ciocalteu phenolic reagent, sodium carbonate, gallic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH), and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), along with other analytical-grade reagents, were purchased from Sigma-Aldrich (Milan, Italy). Lipoid S75 (phosphatidylcholine content: approximately 70%) was purchased from AVG srl (Milan, Italy). Food-grade gelatin powder derived from the partial hydrolysis of animal connective tissues (porcine skin), with a Bloom strength of 280, was purchased from Galeno Srl (Italy). Nutriose® FM06, a soluble dextrin from maize, was kindly provided by Roquette (Lestrem, France). Water was purified using a Milli-Q system from Millipore (Milford, MA, United States). Carrageenan was purchased from Farmalabor Srl (Canosa di Puglia, Italy). Ethanol and methanol were obtained from Carlo Erba (Milan, Italy). Cell culture medium, foetal bovine serum, penicillin, streptomycin, fungizone, and all other reagents for cell studies were purchased from Thermo-Fisher Scientific Inc. (Waltham, MA, United States).

2.2. Extract preparation

Tomato processing by-products, mainly consisting of peels, seeds, and residual pulp, were obtained from a local agro-industrial company (Casar, Sardinia, Italy). The material was dried at 40 °C for 48 h in a forced-air oven and subsequently ground to obtain a fine powder. An aliquot of 5 g of powder was dispersed in 25 mL of an ethanol-water mixture (50:50, v/v). The dispersion was subjected to sonication two sets of 50 cycles (5 s on and 2 s off, 13 μm amplitude) using a high-intensity ultrasonic disintegrator (Soniprep 150, MSE Crowley, London, UK). During sonication, the sample was kept in an ice bath to limit heat accumulation, and a 5-min pause was introduced between the two sets of 50 cycles. After extraction, the dispersion was centrifuged (4000 rpm, 15 min) to separate the supernatant from the pellet (Eppendorf 5804, Hamburg, Germany). Ethanol was then removed from the supernatant using a rotary evaporator (Rotavapor RII, Büchi, Italy). The remaining aqueous phase was freeze-dried for 48 h at −56 °C and 0.5 mbar to obtain the solid extract as a porous powder (Lyovapor™ L-200, Büchi, Italy).

2.3. Extract characterization

2.3.1. Analysis of carotenoids using UHPLC-MS-DAD

Tomato by-product extract was analyzed for carotenoid content using an indirect method adapted from Multari et al. [21]. Briefly, 100 mg of extract was spiked with 10 μL of an internal standard (ISTD) mixture (5 μg/mL), reaching a final concentration of 0.1 ppm [22,23]. Samples were extracted with 5 mL of a mixture of methanol/acetone/hexane (25:25:50, v/v), vortexed, and mixed on an orbital shaker for 10 min. The mixture was then sonicated in a bath sonicator for 5 min at 10 °C and 59 kHz, followed by centrifugation (10 min; 1800 g; 4 °C). The organic layer was collected, and the extraction step was repeated twice. The combined organic extracts were dried under nitrogen at 35 °C for 30 min. The residues were subsequently saponified overnight at room temperature under shaking conditions, using 4 mL of 15% KOH in methanol (w/v). After saponification, 3.5 mL of 9% NaCl (w/v) and 4 mL of a mixture of hexane/diethyl ether (3:1, v/v) were added. The mixture was shaken for 10 min at room temperature, vortexed, and centrifuged (5 min; 1800 g; 4 °C). The organic phase was collected, and the extraction step was repeated twice more, adding only the organic solvent while retaining the aqueous phase in the tube. The combined organic extracts were washed three times with 5 mL of water, dried under nitrogen at 35 °C for 15 min, and reconstituted in 0.4 mL of methyl tert-butyl ether/ethanol (1:2, v/v) containing rosmarinic acid

(0.3 μg/mL; stock solution 0.54 mg/mL in ethanol). Samples were filtered through 0.22 μm regenerated cellulose (RC) membranes and stored at −20 °C until analysis.

Carotenoid profiling was performed using an UHPLC–MS system consisting of an Acquity UPLC instrument coupled to a diode array detector (DAD) and a single quadrupole mass spectrometer (QDa Acquity, Waters, Milford, MA, USA) [24]. The system was controlled by MassLynx version 4.2 (Waters, Milford, MA, USA).

Chromatographic separation was achieved on a BEH C18 column (1.7 μm, 2.1 × 100 mm) maintained at 55 °C. The mobile phases consisted of acetonitrile/water (1:1, v/v) (eluent A) and isopropanol (eluent B), both containing 0.1% (v/v) formic acid and 10 mM ammonium formate.

The elution gradient was as follows: initial conditions at 65% A (0.25 mL/min), decreased to 45.7% A over 4 min, then to 36.1% A within 0.5 min, followed by isocratic elution for 3.5 min, and finally reduced to 0% A over 6 min and maintained for 1.5 min. A cleaning step was performed before re-equilibration using three injections (10 μL each) of a blank solution (methyl tert-butyl ether/ethanol, 1:2, v/v) under the following conditions: 100% B for 0.6 min, followed by re-equilibration to initial conditions (65% A) over 3 min. The total run time, including cleaning, was 20.3 min.

The autosampler temperature was maintained at 20 °C. DAD spectra were acquired in the range of 270–600 nm with a resolution of 1.2 nm. The mass spectrometer operated with an electrospray ionization (ESI) source in positive ion mode at 150 °C, with a capillary voltage of 2.5 kV, a desolvation temperature of 450 °C, a desolvation gas flow of 800 L/h, and a cone gas flow of 150 L/h. Data were acquired in both full-scan (100–1200 *m/z*) and selected-ion recording (SIR) modes.

For quantification, 1 μL of concentrated extract was injected in triplicate. Carotenoids were identified by comparing ultraviolet–visible (UV–Vis) spectra and MS data with those of external standards. Calibration curves were constructed using standard solutions of (all-E)-violaxanthin, lutein epoxide, antheraxanthin, (all-E)-lutein, (all-E)-zeaxanthin, β-cryptoxanthin, and (all-E)-β-carotene in the concentration range of 5–100 ppm.

2.3.2. Polyphenol detection and quantification

5 mg of tomato by-product extract was dissolved in a water-ethanol mixture (50:50, v/v) to obtain a final concentration of 1600 ppm. Ultra-performance liquid chromatography (UPLC) analysis was carried out using an Acquity UPLC system (Waters, Milford, MA, USA) equipped with a binary pump, an online vacuum degasser, an autosampler, and a column compartment. Phenolic compounds were separated on a Waters Acquity HSS T3 column (1.8 μm, 100 × 2.1 mm, Milford, MA, USA) maintained at 40 °C. The mobile phases consisted of water with 0.1% formic acid (eluent A) and acetonitrile with 0.1% formic acid (eluent B). The flow rate was set at 0.4 mL/min.

The gradient program was as follows: 0 min, 5% B; 0–3 min, linear increase to 20% B; 3–4.3 min, isocratic at 20% B; 4.3–9 min, linear increase to 45% B; 9–11 min, linear increase to 100% B; 11–13 min, held at 100% B; 13.01–15 min, return to initial conditions (5% B).

The injection volume for both samples and standard solutions was 2 μL. After each injection, the needle was washed with 600 μL of weak wash solution (water/methanol, 90:10, v/v) followed by 200 μL of strong wash solution (methanol/water, 90:10, v/v). Samples were maintained at 6 °C during the analysis.

Mass spectrometric detection was performed using a Waters Xevo TQMS system (Waters, Milford, MA, USA) equipped with an electrospray ionization (ESI) source. The capillary voltage was set at 3.5 kV in positive mode and −2.5 kV in negative mode. The source temperature was maintained at 150 °C, while the desolvation temperature was set at 500 °C. Cone gas and desolvation gas flow rates were 50 L/h and 800 L/h, respectively. Unit mass resolution was applied to each quadrupole. Flow injection analysis of individual metabolites was performed to optimise Multiple Reaction Monitoring (MRM) conditions, following the method described by Vrhovsek et al. [25]. For most metabolites, optimisation was carried out automatically using Waters IntelliStart software, while for selected compounds, optimal cone voltages and collision energies were determined manually through collision-induced dissociation (CID) experiments. A dwell time of at least 25 ms was applied to each MRM transition.

2.3.3. Total phenolic content

The total phenolic content (TPC) of the tomato by-product extract was determined using the Folin–Ciocalteu assay. Briefly, an aliquot (100 μL) of the extract solution (10 mg/mL in ethanol) was mixed with 500 μL of Folin-Ciocalteu reagent and 3 mL of 20% (w/v) sodium carbonate. The mixture was diluted to a final volume of 10 mL with distilled water, stirred for 1 min, and incubated in the dark at room temperature for 90 min. Absorbance was measured at 765 nm against a blank using a microplate reader (Epoch Reader, BioTek Instruments, AHSI S.p.A., Bernareggio, Italy). A five-point calibration curve was constructed using gallic acid (0.01-2 mg/mL), showing good linearity over the investigated concentration range ($R^2 = 0.9972$). Results were expressed as mg gallic acid equivalents per g of extract (mg GAE/g).

2.3.4. Antioxidant activity (DPPH and FRAP assays)

The reducing capacity of the tomato by-product extract was evaluated using the ferric reducing antioxidant power (FRAP) assay, a colorimetric method that measures the reduction of ferric ions to ferrous ions. The reduction of ferric ions was quantified by comparing the results with a ferrous sulphate standard. The FRAP reagent was prepared by mixing the following three solutions (10a:1b:1c v/v): a) 10 mM 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) in 40 mM hydrochloric acid (HCl); b) 300 mM sodium acetate; c) 20 mM ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$). A calibration curve was constructed using ferrous sulphate heptahydrate ($FeSO_4 \cdot 7H_2O$) at different concentrations (0-2000 μM). Each sample was incubated for 15 min with the FRAP reagent, and absorbance was measured at 593 nm against the blank. The antioxidant activity was expressed as μmol of Fe^{2+} equivalents/g of extract (μmol Fe^{2+} eq./g).

The radical-scavenging activity of the tomato by-product extract was also evaluated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. This test is commonly employed to assess the scavenging ability of a compound. Briefly, the extract (40 μL) was mixed with a methanolic DPPH solution (960 μL, 40 μg/mL) and incubated for 30 min in the dark at room temperature. The absorbance was measured at 517 nm against the blank, and the antioxidant activity (AA%) was calculated according to a previously reported method [16] using Equation (1):

$$\textit{Antioxidant activity}\ (\%) = \frac{ABS_{blank} - ABS_{sample}}{ABS_{blank}} \times 100 \quad (1)$$

A calibration curve was constructed using 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) at various concentrations (0-5 mg/mL), and the results were expressed as mg of Trolox equivalents/g of extract (mg TE/g).

2.4. Preparation and characterization of tomato by-product extract-loaded vesicles

All formulations were prepared by direct sonication, avoiding the use of organic solvents [26]. A pre-formulation study was carried out by keeping the concentration of the tomato by-product extract constant (20 mg/mL) while exploring different phospholipid concentrations (60, 80, 100, and 120 mg/mL of S75). Final liposomes were obtained by weighing phospholipid S75 (120 mg/mL) and tomato by-product extract (20 mg/mL) into a glass vial, followed by hydration with water. Nutriosomes were obtained by adding Nutriose® FM06 (120 mg/mL) to the other components prior to hydration with water. All dispersions were sonicated using a high-intensity ultrasonic disintegrator Soniprep 150 sonicator (MSE Crowley, London, UK) for a total of 20 cycles (5 s on

and 2 s off per cycle), divided into four sets of five cycles, at a probe amplitude of 13 μm. The samples were allowed to cool between each set to limit heat accumulation during sonication. Reference formulations without the extract (empty liposomes and empty nutriosomes) were also prepared to evaluate the effect phytocomplex incorporation on vesicle assembly.

The physicochemical properties of the vesicles, namely mean diameter, polydispersity index (PDI), and zeta potential, were measured using a Zetasizer Ultra (Malvern Instruments, Worcestershire, UK).

Samples were stored at 4 °C until use. Stability under storage conditions was evaluated by monitoring the physicochemical properties of extract-loaded vesicles every 30 days for 180 days [27–29].

To improve shelf-life, vesicular systems were frozen at −80 °C and freeze-dried for 48 h at −56 °C and 0.5 mbar using a laboratory-scale freeze dryer (Lyovapor™ L-200, Büchi, Italy). The lyophilized samples were rehydrated with water to the initial volume (2 mL) and gently shaken prior to analysis. Their physicochemical properties (mean diameter, PDI, and zeta potential) were then evaluated and compared with freshly prepared formulations.

Vesicular formulations were purified from non-encapsulated compounds by dialysis. Briefly, 1 mL of dispersion was loaded into polycarbonate dialysis tubing (Spectra/Por®, molecular weight cut-off (MWCO) of 12-14 kDa, pore size ~3 nm; Spectrum Laboratories Inc., USA) and dialyzed against 1 L of distilled water at 25 °C for 2 h under constant stirring, with water replacement after 1 h.

Retention of antioxidant activity after dialysis (RAA%) was determined as an indirect measure of the antioxidant phytocomplex retained in the vesicular dispersion following purification. Vesicular formulations were purified from non-incorporated compounds by dialysis as described above, and antioxidant activity was measured before and after dialysis using the DPPH assay (Section 2.3.4). RAA% was calculated as the percentage of antioxidant activity measured after dialysis relative to that measured before dialysis. Since the non-associated fraction was removed during purification, RAA% was considered an indirect estimate of the fraction of the antioxidant phytocomplex retained within or associated with the vesicular system, although it does not provide compound-specific encapsulation efficiency [30].

2.5. Stability study of tomato by-product extract-loaded vesicles under simulated gastrointestinal pH and ionic conditions

Simulated fluids with pH values mimicking salivary, gastric, and intestinal conditions were prepared according to a previously established protocol [18,31].

The salivary fluid (pH 6.75) was obtained by dissolving 0.238 g of sodium bicarbonate, 0.019 g of potassium bicarbonate, and 0.8 g of sodium chloride in 100 mL of water. The pH was adjusted to 6.75 using a diluted 1 M phosphoric acid solution. The gastric fluid (pH 1.20, high ionic strength) was prepared by dissolving 1.75 g of sodium chloride in 94 mL of bi-distilled water, followed by volume adjustment to 100 mL with 0.1 M hydrochloric acid. The intestinal fluid (pH 7.00, high ionic strength) was prepared by dissolving 0.726 g of disodium hydrogen phosphate, 0.356 g of sodium dihydrogen phosphate, and 1.754 g of sodium chloride in water, and adjusting the final volume to 100 mL. The pH was subsequently adjusted to 7 using diluted hydrochloric acid.

Vesicle stability was evaluated in the three simulated fluids. Samples were diluted (1:100) and incubated at 37 °C in salivary fluid (pH 6.75) for 10 min, in gastric fluid (pH 1.20) for 2 h, or in intestinal fluid (pH 7.00) for 6 h.

At the end of each incubation period, the mean diameter and PDI were measured at 37 °C.

2.6. Biocompatibility assay

In vitro biocompatibility of the samples was evaluated using the human colorectal adenocarcinoma cell line Caco-2, according to a previously reported protocol [16]. Complete culture medium consisted of high-glucose Dulbecco's Modified Eagle Medium (DMEM) supplemented with L-glutamine, 10% foetal bovine serum, and 1% penicillin–streptomycin solution (10,000 units/mL penicillin and 10,000 μg/mL streptomycin). Caco-2 cells were cultured in 75 cm^2 flasks using this complete medium.

For the experiment, cells were seeded in 96-well plates (1×10^4 cells/well) and incubated for 24 h. Subsequently, cells were treated with the aqueous dispersion of the extract (20 mg/mL), liposomes, or nutriosomes, appropriately diluted in complete DMEM to obtain final extract concentrations of 20, 2, 0.2, and 0.02 μg/mL.

After 48 h of incubation, the culture medium was removed and 100 μL of MTT solution ([3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide], final concentration 0.5 mg/mL) was added to each well. Following 3 h of incubation, the MTT solution was removed, and the resulting formazan crystals were dissolved in 100 μL of dimethyl sulfoxide (DMSO). Absorbance was measured at 570 nm using a microplate reader (Synergy LX Reader, BioTek Instruments, AHSI S.p.A., Bernareggio, Italy).

Experiments were performed at least three times, each in triplicate. Results were expressed as percentage cell viability relative to untreated control cells (100% viability).

2.7. Protective effect against oxidative stress

Caco-2 cells were seeded into 96-well plates (1×10^4 cells/well). After 24 h of incubation, the cells were exposed to hydrogen peroxide (0.5 mM) and simultaneously treated with the extract in aqueous dispersion (20 mg/mL), or loaded either in liposomes or nutriosomes, properly diluted with complete DMEM to achieve the desired concentration (20 μg/mL of extract). After 4 h, MTT assay was performed as described in Section 2.6. Cells exposed to hydrogen peroxide alone (0.5 mM) were used as the positive control. The results were expressed as the percentage of the control (100% viability) [28,32].

2.8. Preparation and characterization of gummy candies containing nutriosomes

As nutriosomes were identified as the most promising formulations, they were incorporated into gummy formulations. Briefly, 1 g of food-grade gelatin powder with a Bloom strength of 280, was dispersed in 2.5 g of water and heated until complete dissolution. In parallel, 2.27 g of sucrose, 0.02 g of citric acid, and 0.67 g of glycerol were dissolved in a separate beaker under gentle heating. The two solutions were then combined and thoroughly mixed to obtain a homogeneous matrix. Freeze-dried nutriosomes, previously rehydrated with 2 mL of water, were subsequently added and uniformly dispersed within the formulation. The final mixture was poured into three wells of a 24-well plate and allowed to solidify at room temperature for 1 h.

The uniformity of mass was assessed according to the European Pharmacopoeia, following the monograph “Uniformity of mass of single-dose preparations” [33]. In the absence of a specific method for chewable formulations, the procedure applicable to tablets was adopted. Preparations were considered non-compliant if more than two individual units deviated by more than 5% from the average mass, or if any single unit deviated by more than 10%.

Water loss during storage was evaluated by storing nutriosome-loaded gummies (n = 10) either in sealed plastic bags or under open-air conditions (temperature: 25 ± 2 °C; relative humidity: 45 ± 5%). Samples were weighed after preparation (t0) and every two days for 10 days. Weight changes were monitored and recorded, and water loss was calculated as the percentage decrease in sample mass relative to the initial mass, according to Equation (2):

$$\text{Water loss}\ (\%) = \frac{w_{t0} - w_t}{w_{t0}} \cdot 100 \quad (2)$$

where w_{t0} is the initial weight, and w_t is the weight recorded at a specific time point (i.e., day 2, day 4, day 6, day 8, or day 10).

2.9. Release of nutriosomes from gummy candies

The release of nutriosomes from the gummy formulation was evaluated following a dissolution test under acidic conditions to assess gummy dissolution and vesicle recovery under simulated gastric conditions. Briefly, a single gummy was immersed in 250 mL of the acidic medium (pH 1.20) described in Section 2.5, simulating gastric conditions, and maintained at 37 °C under constant agitation (50 rpm). The experiment was conducted for 2 h. At the end of the test, the dispersion containing the released nutriosomes was analyzed for mean diameter and PDI. Experiments were performed in triplicate.

2.10. Statistical analysis of data

Results were expressed as mean ± standard deviation of at least three independent experiments. Analysis of variance (ANOVA) was used for multiple comparisons, while Student's t-test was used for comparisons between two groups, as appropriate. Statistical significance was set at $p < 0.05$. Data analysis was performed using XL Statistics for Excel.

3. Results

3.1. Extract characterization

The carotenoid content was determined in a liquid sample prepared by dispersing 100 mg of tomato by-product extract in 0.4 mL of solvent using the indirect method described above, and the results are reported in Table 1. The concentrations reported in Table 1 are expressed in ppm relative to the analyzed sample. Among the carotenoids, β-carotene (39.93 ppm), phytoene (23.92 ppm), and lycopene (11.29 ppm) were the most abundant compounds in the extract, while lutein and zeaxanthin were present at considerably lower levels (0.23 and 1.41 ppm, respectively).

The polyphenol profile of the tomato by-product extract was also analyzed, with concentrations expressed in ppm. Among the compounds identified, quercetin-3-galactoside was by far the most abundant (10.150 ppm), followed by neochlorogenic acid (0.227 ppm) and chlorogenic acid (0.201 ppm), while the remaining polyphenols were present at relatively low levels (<0.3 ppm). These results indicate that quercetin-3-galactoside was the predominant identified phenolic compound and may contribute to the bioactive properties of the extract.

The total phenolic content of the tomato by-product extract was also determined using the Folin-Ciocalteu colorimetric assay and expressed as gallic acid equivalents. A total phenolic content of ~27.23 mg GAE/g of extract was obtained (Table 2). It should be noted that, due to the non-specific redox nature of the Folin–Ciocalteu assay, the value expressed as gallic acid equivalents may also include contributions from non-phenolic reducing compounds potentially present in the extract and should therefore be interpreted as an estimate rather than a selective quantification of phenolic compounds.

The antioxidant activity of the fresh tomato by-product extract was ~3.20 mg TE/g according to the DPPH assay and ~129 μmol Fe^{2+} eq./g according to the FRAP assay (Table 2).

3.2. Characterization of tomato by-product extract-loaded vesicles

Using 120 mg/mL of phospholipid S75 and 20 mg/mL of tomato by-product extract, homogeneous vesicles were obtained (PDI ~0.229), with a small size (~88 nm) and negative surface charge (−40 mV) (Table 3). The addition of Nutriose® FM06 (120 mg/mL) did not significantly change the size of vesicles (94 nm, $p > 0.05$), which remained homogeneously dispersed (PDI ~0.198, $p > 0.05$) and negatively charged (−46 mV, $p > 0.05$). Empty liposomes showed a smaller mean diameter (~70 nm, $p < 0.05$) and a higher PDI (~0.306, $p < 0.05$) than extract-loaded liposomes. Empty nutriosomes showed a mean diameter of ~85 nm and a PDI of ~0.289. Overall, the incorporation of the phytocomplex did not impair vesicle formation and was associated with a narrower size distribution.

Liposomes and nutriosomes retained approximately 76% and 75% of their initial antioxidant activity, respectively, after purification by dialysis.

The stability study conducted over a period of 180 days demonstrated that both liposomes and nutriosomes maintained consistent physicochemical properties (Fig. 1). Measurements of mean diameter, PDI, and zeta potential were performed every 30 days for up to 180 days, showing no significant variations throughout the entire evaluation period ($p > 0.05$ among values of each parameter of each formulation).

The stability of both liposomes and nutriosomes during freeze-drying was assessed by measuring the mean diameter, PDI, and zeta potential before and after the process (Fig. 2). Liposomes had a significant increase in size, from ~88 nm to ~547 nm ($p < 0.05$), following freeze-drying and redispersion. Accordingly, the PDI increased to ~0.368 ($p < 0.05$ versus the pre-freeze-drying value), indicating greater variability in size distribution and reduced vesicle homogeneity due to instability. By contrast, nutriosomes exhibited only a slight increase in size after rehydration, remaining relatively small, with an average diameter of ~159 nm ($p < 0.05$ versus the value before freeze-drying) and a PDI of ~0.130 ($p < 0.05$ versus the value before freeze-drying). Zeta potential was not significantly affected by freeze-drying and remained highly negative in both formulations ($p > 0.05$).

Table 1
Carotenoid content (ppm) determined by indirect method, and polyphenol content (ppm) in the tomato by-product extract.

	Carotenoids (ppm)	Polyphenols (ppm)
	Indirect method	
Lutein	0.23	
Zeaxanthin	1.41	
Lycopene	11.29	
β-carotene	39.93	
Phytoene	23.92	
3-OH-benzaldehyde		0.043
3,5-diOH-benzoic acid		0.008
Protocatechuic acid		0.084
Caffeic acid		0.069
Neochlorogenic acid		0.227
Cryptochlorogenic acid		0.011
Chlorogenic acid		0.201
Naringenin		0.049
Quercetin-3-galactoside		10.150

Table 2
Antioxidant activity and total phenolic content of tomato by-product extract, expressed as mg of Trolox equivalents/g of extract (mg TE/g), μmol of Fe^{2+} equivalents/g of extract (μmol Fe^{2+} eq./g), and mg of gallic acid equivalents/g of extract (mg GAE/g). Mean values ± standard deviations are reported (n = 3).

	Antioxidant activity		Total phenolic content
	DPPH assay	FRAP assay	Folin-Ciocalteu assay
	(mg TE/g)	(μmol Fe^{2+} eq./g)	(mg GAE/g)
Tomato by-product extract	3.20 ± 0.09	129.01 ± 24.03	27.23 ± 1.13

Table 3
Mean diameter (MD), polydispersity index (PDI), zeta potential (ZP), and retention of antioxidant activity after dialysis (RAA%) of empty and tomato by-product-loaded vesicles. Each value represents the mean ± standard deviation of at least three replicates. The same letter (a, b, c, d, e) indicates values not statistically different from each other ($p > 0.05$) and different from other values ($p < 0.05$).

	MD (nm)	PDI	ZP (mV)	RAA (%)
Empty liposomes	70 ± 5	$0.306^{b} \pm 0.015$	$-39^{d} \pm 9$	-
Empty nutriosomes	$85^{a} \pm 6$	$0.289^{b} \pm 0.024$	$-39^{d} \pm 5$	-
Liposomes	$88^{a} \pm 4$	$0.229^{c} \pm 0.004$	$-40^{d} \pm 5$	$76^{e} \pm 5$
Nutriosomes	$94^{a} \pm 12$	$0.198^{c} \pm 0.027$	$-46^{d} \pm 2$	$75^{e} \pm 3$

3.3. Stability study of tomato by-product-loaded vesicles under simulated gastrointestinal pH and ionic conditions

The stability of liposomes and nutriosomes was assessed in simulated media mimicking the pH and ionic strength conditions of saliva (pH 6.75), gastric fluid (pH 1.20), and intestinal fluid (pH 7.00). After incubation at 37 °C for 10 min, 2 h, and 6 h, respectively, no significant changes were observed in either the mean diameter or the PDI of the vesicles (Fig. 3). Both formulations therefore remained stable under all tested conditions, with no statistically significant differences compared with the initial values ($p > 0.05$).

3.4. Biocompatibility assay and protective effect against oxidative stress

Caco-2 cell viability was assessed after 48 h of exposure to the tomato by-product extract in aqueous dispersion or loaded in liposomes and nutriosomes at different extract concentrations (20, 2, 0.2, and 0.02 μg/mL). None of the tested formulations significantly reduced cell viability compared with untreated control cells (100% viability). Cell viability remained above 90% at all tested concentrations ($p > 0.05$), indicating that the extract, both in free form and when loaded in liposomes or nutriosomes, was well tolerated by Caco-2 cells (Fig. 4, upper panel).

Caco-2 cells exposed to 0.5 mM hydrogen peroxide showed a reduction in viability to approximately 76%, confirming the oxidative damage induced by the treatment. Co-treatment with the tomato by-product extract in aqueous dispersion or in liposomes (at an extract concentration of 20 μg/mL) did not significantly improve cell viability compared with the hydrogen peroxide-treated control ($p > 0.05$). In contrast, treatment with nutriosomes resulted in a statistically

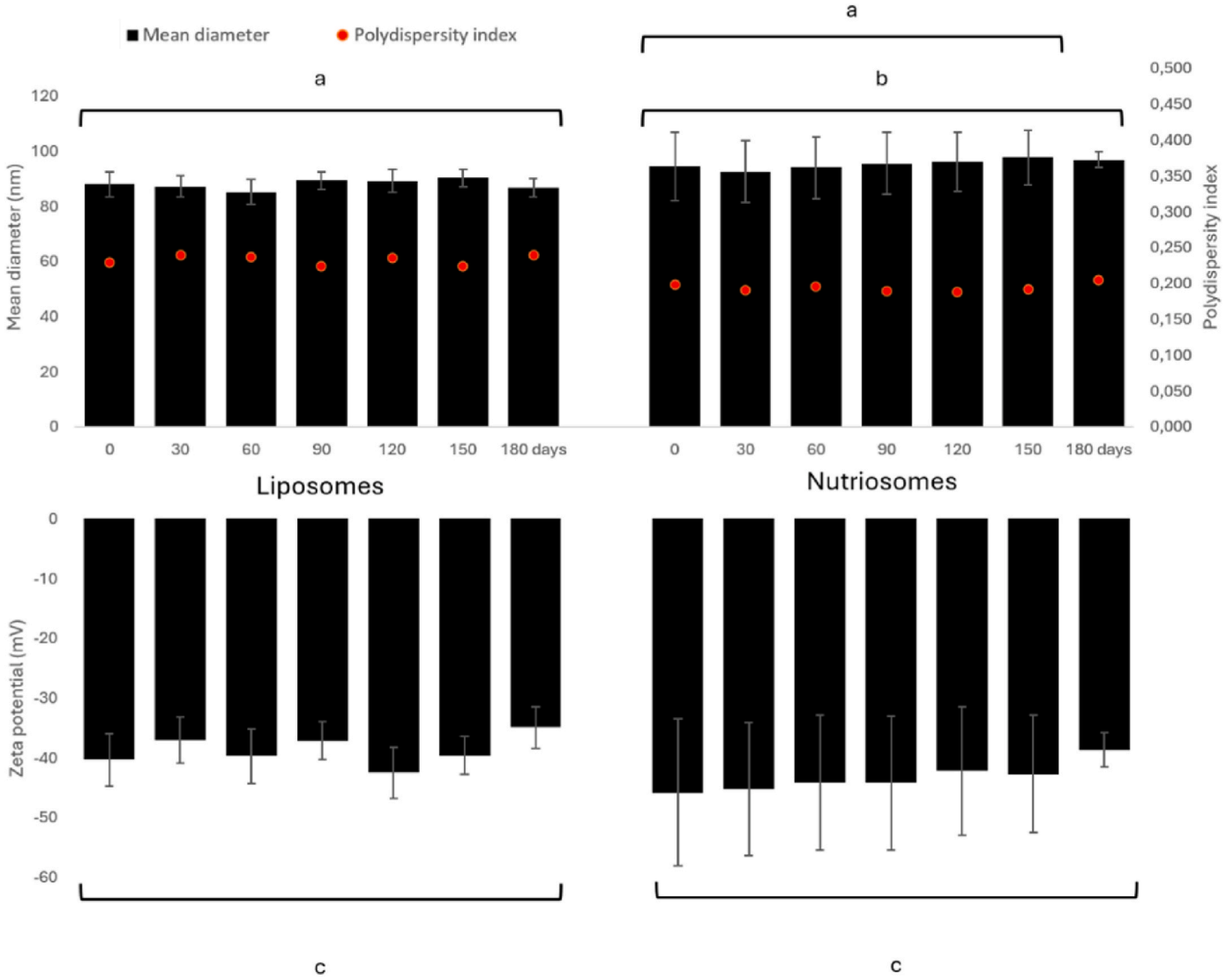


Fig. 1. Mean diameter (nm), polydispersity index, and zeta potential (mV) of tomato by-product extract-loaded liposomes and nutriosomes, stored in the dark at 4 °C for 180 days. Mean values (bars) ± standard deviations are reported (n = 3). The same letter (a, b, c) indicates values not statistically different from each other ($p > 0.05$).

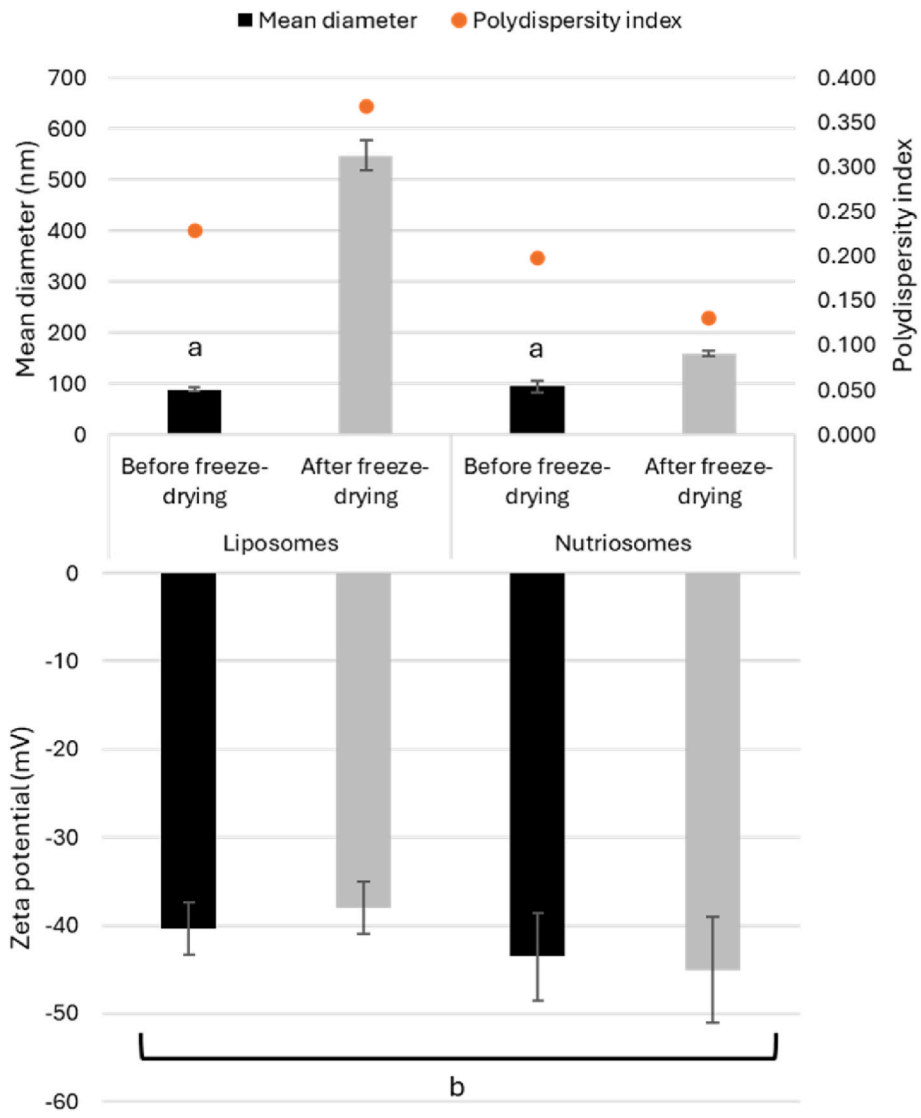


Fig. 2. Mean diameter (nm), polydispersity index, and zeta potential (mV) of tomato by-product extract-loaded liposomes and nutriosomes, before and after freeze-drying. Mean values (bars) ± standard deviations are reported (n = 3). The same letter (a, b) indicates values not statistically different from each other ($p > 0.05$) and different from other values ($p < 0.05$).

significant increase in cell viability, reaching approximately 93% ($p < 0.05$ versus hydrogen peroxide), indicating a protective effect against oxidative stress (Fig. 4, lower panel).

3.5. Characterization of nutriosome-loaded gummy candies

The results of the uniformity of mass test showed that two gummies exceeded the 5% deviation threshold from the average mass, whereas none exceeded the 10% limit. According to the European Pharmacopoeia monograph 'Uniformity of mass of single-dose preparations' of European Pharmacopoeia, the gummies complied with the specified requirements, as no more than two units deviated by over 5%, and none deviated by more than 10% (data not shown).

The water-loss study showed that gummy behaviour depended on the storage condition (Fig. 5). Gummies stored under open-air conditions showed a progressive decrease in weight over 10 days, reaching a final water loss of approximately 28%. By contrast, samples stored in sealed plastic bags showed only minimal weight loss, with a final water loss < 3%. These findings indicate that sealed packaging effectively limited moisture evaporation and preserved the physical stability of the gummy matrix during short-term storage.

Finally, the release behaviour of nutriosome-loaded gummies was evaluated under acidic conditions simulating the gastric environment. After 2 h of incubation at pH 1.20, the gummy matrix was completely dissolved, allowing physicochemical characterization of the resulting dispersion containing the released vesicles (Table 4). The released nutriosomes showed a significant increase in mean diameter compared with the freshly prepared formulation, from 94 ± 12 nm to 249 ± 37 nm ($p < 0.05$). Despite this increase in size, the vesicles remained within the nanometric range and retained a homogeneous size distribution. Indeed, the PDI did not significantly change after release, remaining low and comparable to that of freshly prepared nutriosomes (~0.198, $p > 0.05$ versus the freshly prepared sample). These findings suggest that exposure to the gummy matrix and subsequent release under acidic conditions did not induce marked aggregation or loss of colloidal homogeneity. The observed increase in size may be attributed to partial bilayer rearrangement, vesicle swelling, or interactions with components of the dissolved gummy matrix. Overall, the release assay indicates that the gummy formulation was able to dissolve under gastric-like conditions while allowing the recovery of a dispersion with nanometric size and low polydispersity.

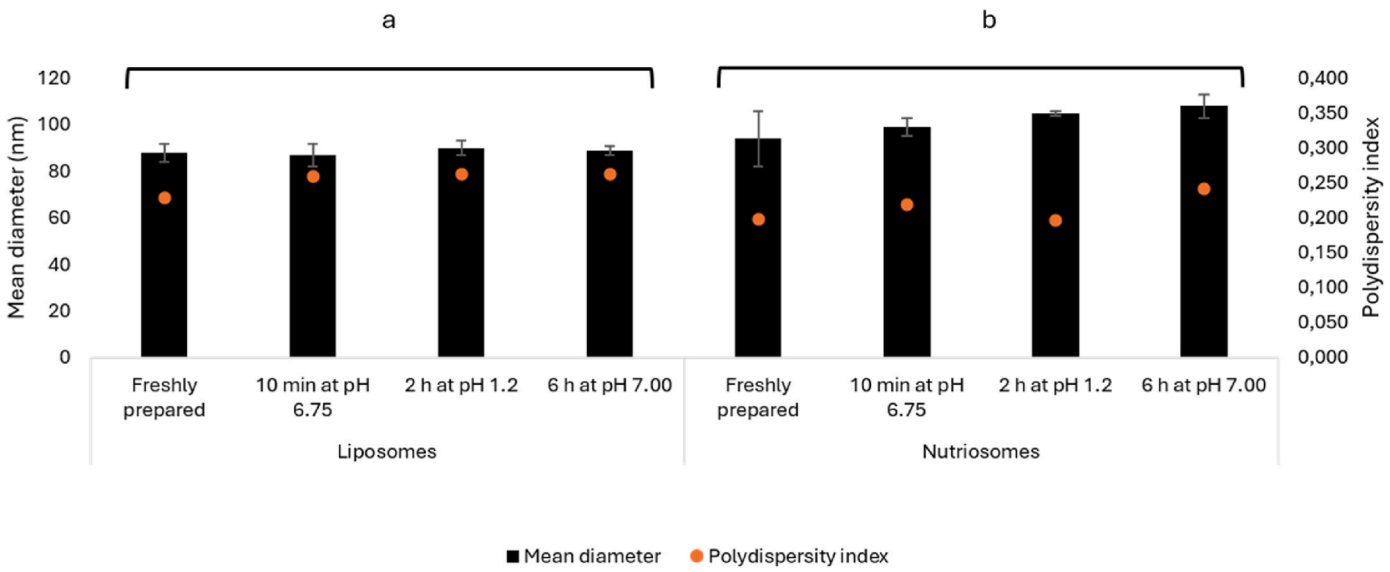


Fig. 3. Mean diameter (nm) and polydispersity index of tomato by-product extract-loaded liposomes and nutriosomes, incubated for 10 min at pH 6.75, for 2 h at pH 1.20, or for 6 h at pH 7.00. Mean values (bars) ± standard deviations are reported (n = 3). The same letter (a, b) indicates values not statistically different from each other ($p > 0.05$).

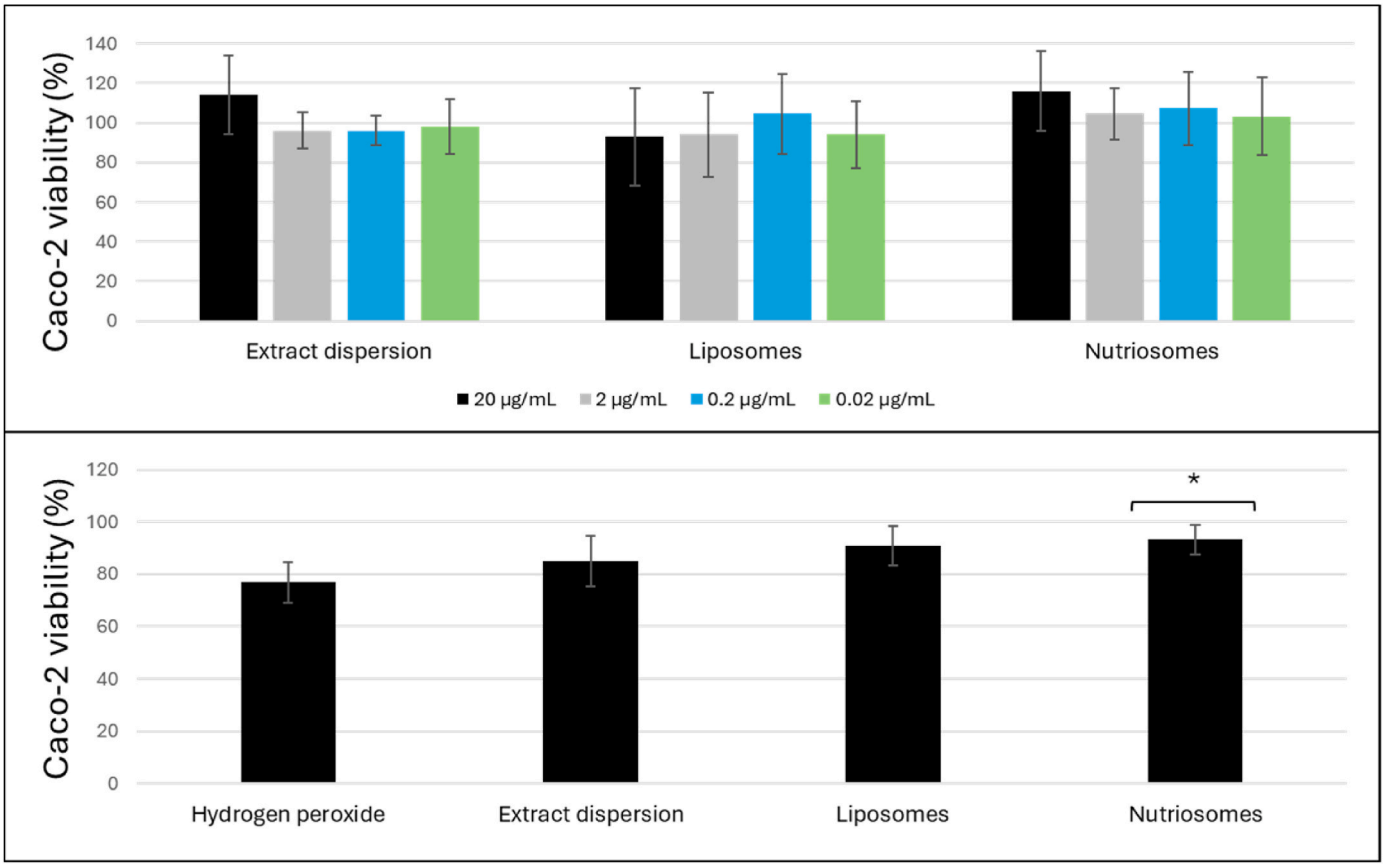


Fig. 4. Upper panel: viability of Caco-2 cells incubated for 48 h with tomato by-product extract, in dispersion or loaded in liposomes and nutriosomes, properly diluted in growth medium up to 20, 2, 0.2, and 0.02 µg/mL. The mean values (bars) ± standard deviations are reported (n = 3). *$p < 0.05$ compared to the untreated control cells (100% viability). **Lower panel**: viability of Caco-2 cells stressed with hydrogen peroxide (0.5 mM) or with hydrogen peroxide (0.5 mM) and simultaneously treated with samples diluted in growth medium up to 20 µg/mL of extract, either free or encapsulated. The mean values (bars) ± standard deviations are reported (n = 3). *$p < 0.05$ compared to hydrogen peroxide.

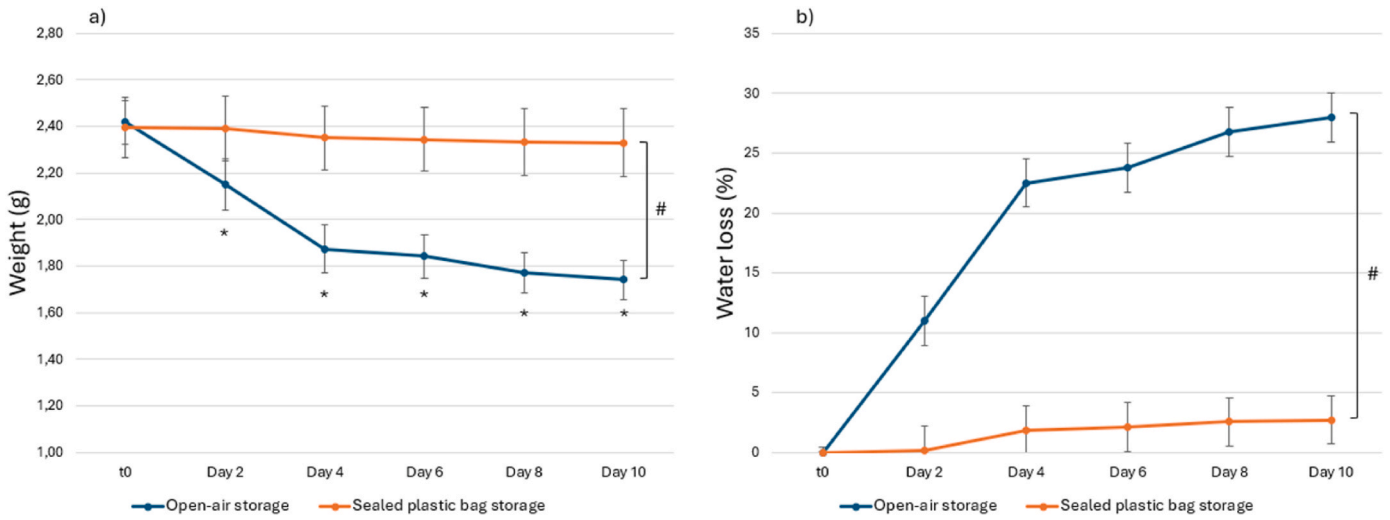


Fig. 5. Evaluation of **a)** weight (g) and **b**) water loss (%) of the gummies during the 10-day storage performed under open-air conditions (grey) or in sealed plastic bags (dark). Data are reported as mean ± standard deviation (n = 10). $p < 0.05$ compared with the corresponding value at day 0; #$p < 0.05$ between open-air and sealed-bag storage conditions at day 10.

Table 4
Mean diameter (nm) and polydispersity index (PDI) of nutriosomes loaded with tomato by-product extract and released from gummy candies after 2 h at pH 1.20. Mean values (bars) ± standard deviations are reported (n = 3). The same letter (a) indicates values not statistically different from each other ($p > 0.05$).

	Time	Mean diameter (nm)	PDI
Nutriosomes	Freshly prepared	94 ± 12	0.198^{a} ± 0.027
	2 h (pH 1.20)	249 ± 37	0.109^{a} ± 0.089

4. Discussion

The present work addresses two interconnected aspects: the valorisation of tomato processing by-products as a sustainable source of antioxidant bioactives and the development of a vesicular delivery strategy to convert this complex phytocomplex into a functional oral ingredient. Tomato by-products are relevant within a circular economy perspective because they contain high-value compounds, including carotenoids and polyphenols, which have been associated with antioxidant, anti-inflammatory and intestinal protective effects [11,34]. However, their practical application requires not only efficient recovery but also appropriate formulation strategies. Carotenoids and phenolic compounds differ markedly in polarity, aqueous solubility and susceptibility to degradation, making the extract a promising but challenging ingredient to formulate [35,36]. In this context, phospholipid-based vesicles were investigated as carriers able to improve the technological properties, stability, and functional performance of the tomato by-product phytocomplex.

The phytochemical characterization of the extract provided the basis for the formulation strategy. The main identified compounds were β-carotene, lycopene, phytoene, and quercetin-3-galactoside, confirming the coexistence of lipophilic carotenoids and more polar phenolic compounds. This mixed composition supports the use of phospholipid-based vesicular systems, which can incorporate lipophilic molecules within the bilayer and more hydrophilic compounds in the aqueous domains [37]. The phytochemical and antioxidant profile of the extract, assessed by the Folin-Ciocalteu, DPPH, and FRAP assays, was consistent with the presence of these bioactive classes. The extract showed a total phenolic content of 27.23 ± 1.13 mg GAE/g, together with antioxidant activity corresponding to 3.20 ± 0.09 mg TE/g and 129.01 ± 24.03 μmol Fe^{2+} eq./g. These data support the functional potential of the phytocomplex and provide a rationale for the use of a delivery system designed to protect sensitive compounds and support their incorporation into an oral formulation.

The preformulation study showed that the phospholipid concentration was critical for the incorporation of the tomato by-product extract into vesicular systems. At low phospholipid concentrations (60, 80, 100 mg/mL), the phytocomplex was not effectively incorporated, and phase separation was observed immediately after preparation (data not shown), suggesting that the lower phospholipid concentrations were insufficient to maintain the phytocomplex within a stable vesicular structures. This observation is consistent with previous reports showing that lecithin concentration can influence the incorporation of food-derived compounds in liposomal systems, since a higher phospholipid amount increases the vesicular phase available for compound incorporation [38]. Using 120 mg/mL of phospholipid S75 and 20 mg/mL tomato by-product extract, homogeneous liposomes were obtained, with a mean diameter of ~88 nm, a low PDI (~0.229), and a negative zeta potential (~−40 mV). These values indicate the formation of small and relatively homogeneous vesicles with a surface charge compatible with colloidal stabilization [39]. The comparable RAA% values of liposomes and nutriosomes suggest a similar overall incorporation of the antioxidant phytocomplex into the two vesicular systems.

The comparison with the corresponding empty vesicles suggests that the phytocomplex influenced vesicle assembly. Empty liposomes were smaller (~70 nm) and more polydisperse (PDI ~0.306) than extract-loaded liposomes, indicating that extract incorporation was not associated with a loss of colloidal homogeneity. Instead, the extract-loaded liposomes showed a narrower size distribution (PDI ~0.229). A plausible explanation is that lipophilic constituents, such as carotenoids, may have interacted with or partitioned into the phospholipid bilayer, while phenolic compounds may have interacted with the polar head groups of phospholipids, thereby modulating lipid packing and bilayer curvature, which may have contributed to the slightly larger vesicle size and narrower size distribution observed [40,41]. Similar effects on vesicle size, homogeneity, and bioactive incorporation have been described by different authors for liposomal systems containing plant-derived bioactives [36,42].

The addition of Nutriose® FM06 did not substantially modify the initial physicochemical properties of the extract-loaded vesicles. Previous structural studies indicate that Nutriose® FM06 is not simply arranged as an external coating but is distributed within the interlamellar and intervesicular aqueous regions of the phospholipid system, where it may interact with the polar phospholipid head groups and contribute to

vesicle stabilization [17]. More recently, Paul et al. reported that additional polymeric surface modification of nutriosomes with guar gum can further modulate their physicochemical stability, controlled release, and cellular uptake, highlighting the relevance of polysaccharide–vesicle interactions in determining the functional properties of these systems [43].

Nutriosomes showed a mean diameter of ~94 nm, a PDI of ~0.198 and a zeta potential of ~ −46 mV, values not significantly different from those of liposomes ($p > 0.05$). Both formulations also showed high and comparable retention of antioxidant activity after dialysis, with RAA values of ~76% for liposomes and ~75% for nutriosomes, suggesting that the presence of Nutriose® FM06 did not markedly affect the overall retention of the antioxidant phytocomplex within or associated with the vesicular system [17].

During 180 days of storage at 4 °C, both liposomes and nutriosomes maintained their mean diameter, PDI and zeta potential without significant variations, suggesting good colloidal stability under refrigerated storage conditions [39,44]. The highly negative zeta potential of both formulations (~−40 mV for liposomes and ~−46 mV for nutriosomes) may have contributed to colloidal stability by promoting electrostatic repulsion among vesicles and limiting their aggregation during storage.

Freeze-drying was carried out to further extend the shelf-life of the formulations [45]. After freeze-drying and rehydration, liposomes showed a significant increase in mean diameter, from ~88 nm to ~547 nm, together with an increase in PDI to ~0.368. These changes indicate a loss of colloidal homogeneity, likely associated with vesicle fusion, aggregation or partial bilayer destabilization during the process. Similar destabilization phenomena have been reported for liposomal systems exposed to freezing and dehydration in the absence of adequate cryoprotection [45,46]. In contrast, nutriosomes showed a more limited increase in mean diameter, reaching ~159 nm after rehydration, and maintained a low PDI (~0.130). The decrease in PDI observed after rehydration may indicate that Nutriose® FM06 favoured the recovery of a relatively narrow vesicle population during freeze-drying. However, this finding should not be interpreted as an intrinsic improvement in vesicle homogeneity, and further structural investigations would be required to clarify the underlying mechanism. This behaviour agrees with previous studies on nutriosomes, in which the inclusion of a soluble dextrin improved the structural stability of phospholipid vesicles during processing [17,18]. The markedly better preservation of vesicle size and size distribution observed for nutriosomes after freeze-drying may be related to molecular interactions between Nutriose® FM06 and the phospholipid bilayer. Nutriose® FM06 is a soluble dextrin rich in hydroxyl groups and has previously investigated for its potential functional properties [17,47]. Carbohydrate-based lyoprotectants can stabilise phospholipid membranes during dehydration according to the water-replacement hypothesis, whereby hydroxyl groups form hydrogen bonds with the polar regions of phospholipids, particularly phosphate and carbonyl groups, partially replacing the interactions normally established with water. This can preserve the spacing and organization of phospholipid head groups and reduce dehydration-induced alterations in lipid packing and phase behaviour. In parallel, the formation of a carbohydrate-rich matrix with reduced molecular mobility may further limit vesicle fusion and aggregation during freezing and drying [48]. These mechanisms could therefore explain the markedly lower increase in size observed for nutriosomes compared with conventional liposomes after rehydration. However, since phospholipid–dextrin interactions and membrane fluidity were not directly investigated in the present study by techniques such as Fourier-transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), or fluorescence anisotropy, this mechanistic interpretation remains hypothetical and warrants further investigation. However, the better preservation of size and PDI after freeze-drying supports the potential use of nutriosomes as powder intermediates for subsequent incorporation into solid or semi-solid oral matrices.

The stability of the formulations under simulated salivary, gastric, and intestinal pH and ionic conditions provided complementary information on their behaviour under conditions relevant to oral gastrointestinal transit. Both liposomes and nutriosomes maintained their mean diameter and PDI after incubation at pH 6.75 for 10 min, pH 1.20 for 2 h, and pH 7.00 for 6 h, suggesting that the vesicles preserved their colloidal organization under pH and ionic strength conditions relevant to oral administration. These findings are consistent with previous data obtained with grape pomace extract-loaded nutriosomes, which showed sizes of ~100 nm, PDI values below 0.2 and stability in simulated salivary, gastric, and intestinal fluids [16]. However, the present model did not include digestive enzymes, bile salts, or the dynamic conditions of gastrointestinal digestion and should therefore be regarded as a preliminary stability assessment rather than a complete simulation of gastrointestinal transit. Future studies using standardized digestion models, such as the INFOGEST protocol, will be required to more accurately evaluate vesicle integrity, bioactive release, and bioaccessibility under physiologically relevant conditions [49].

The biological assays were carried out in Caco-2 cells as an intestinal cell model to evaluate the appropriateness of the formulations for oral delivery. The free extract, as well as the extract-loaded liposomes and nutriosomes, were well tolerated by the cells at all tested concentrations, with cell viability remaining > 90% after 48 h of exposure, providing preliminary evidence of biocompatibility in an intestinal epithelial cell model. The oxidative stress assay represented the main functional test of the study. Hydrogen peroxide is widely used to induce oxidative damage in Caco-2 cells, where it can impair membrane integrity, induce DNA damage, and alter antioxidant enzyme responses [50]. Exposure of Caco-2 cells to 0.5 mM hydrogen peroxide reduced cell viability to ~76%, confirming the induction of oxidative damage. Under the same conditions, the free extract and liposomal formulation did not significantly improve cell viability. In contrast, nutriosomes significantly restored viability to approximately 93%, indicating a protective effect against oxidative stress. Comparable cytoprotective effects have been reported for biopolymer-associated vesicular systems. Sodium alginate- and gum Arabic-associated liposomes have been shown to improve the performance of grape pomace polyphenols in intestinal models [51], while chitosan-coated liposomes containing grape seed proanthocyanidins provided marked protection against oxidant-induced cytotoxicity in Caco-2 cells [52]. More recently, alginate-enriched nutriosomes carrying a polyphenol-rich extract also showed enhanced protection of H_2O_2-stressed Caco-2 cells [53]. Unlike systems requiring an additional polymeric coating, Nutriose® FM06 is an integral component of the nutriosome formulation, potentially combining vesicle stabilization with its functional properties as a soluble dextrin.

The co-treatment model was selected to assess the ability of the formulations to counteract an ongoing acute oxidative insult, rather than to evaluate a prophylactic pre-conditioning effect. The apparent discrepancy between the antioxidant activity measured by DPPH and FRAP assays and the limited protection provided by the free extract and liposomes in Caco-2 cells should be considered in light of the different nature of these experimental models. DPPH and FRAP are cell-free chemical assays that measure radical-scavenging or reducing capacity under controlled conditions, whereas cellular protection additionally depends on the stability and availability of the bioactive compounds in the culture medium, their interaction with the cell membrane, cellular uptake, and intracellular metabolism [54,55]. Thus, the lack of significant protection by the free extract may reflect limited cellular availability of its antioxidant constituents despite their antioxidant activity measured in cell-free assays. Likewise, interactions of conventional liposomes with components of the serum-containing culture medium may affect vesicle integrity, bioactive release, or cellular internalization [56]. The higher protection observed with nutriosomes may therefore result from improved preservation and availability of the phytocomplex at the cell interface rather than simply from its intrinsic antioxidant capacity. However, as vesicle stability in the culture medium, cellular uptake, and intracellular metabolism were not directly investigated, these

mechanisms remain hypothetical and require dedicated studies.

Since liposomes and nutriosomes showed comparable retention of antioxidant activity after dialysis (~76% and ~75%, respectively), the higher protection observed with nutriosomes is unlikely to be explained solely by differences in the overall retention of the antioxidant phytocomplex. Instead, it may be related to improved functional availability of the phytocomplex at the cell interface. This interpretation is consistent with previous studies showing improved biological performance of antioxidant compounds delivered by nutriosomes in intestinal models [17], including grape pomace extract-loaded systems [16,57]. The present results are therefore consistent with the nutriosome literature and extend the nutriosomal approach to a tomato by-product extract characterized by a distinct carotenoid- and polyphenol-rich composition.

Given their improved stability after freeze-drying and their higher protective effect against oxidative stress, nutriosomes were selected for incorporation into gummy formulations. This step assessed whether the carrier could be integrated into an oral matrix with potential advantages in terms of acceptability and ease of administration. Indeed, gummies and other chewable dosage forms are increasingly investigated for paediatric and patient-centred applications because they may improve palatability and ease of intake compared with conventional solid forms [19,20,58].

The release assay showed that the gummy matrix completely dissolved after 2 h at pH 1.20 without preventing vesicle release under gastric-like conditions. The dispersion containing the released nutriosomes showed an apparent increase in mean diameter compared with the freshly prepared formulation, from ~94 nm to ~249 nm, but the measured dispersion remained within the nanometric range and retained a low PDI, not significantly different from the fresh formulation. Therefore, the size increase may reflect vesicle swelling, partial bilayer rearrangement or interactions with components of the dissolved gummy matrix, or contributions of matrix components to the DLS scattering signal. In this respect, the release data are consistent with the previous gastric pH stability results and provide preliminary information on the behaviour of the vesicular formulation after release from the gummy matrix. The release test was limited to gastric-like conditions and did not include a preceding oral phase simulating mastication and exposure to saliva. Therefore, the results should be interpreted as a preliminary assessment of gummy dissolution and vesicle recovery rather than as a complete simulation of oral-to-gastric release.

Overall, this study provides preliminary evidence that nutriosomes are systems able to improve the technological robustness and biological performance of sustainable phytocomplexes intended for functional food and nutraceutical applications and can be easily incorporated into a chewable oral matrix while retaining acceptable colloidal properties.

5. Conclusions

This study demonstrates the feasibility of valorizing tomato processing by-products through their incorporation into phospholipid-based vesicles and subsequent integration into a gummy formulation intended for oral administration. Both liposomes and nutriosomes effectively preserved the antioxidant activity of the extract and exhibited good colloidal stability under the investigated storage and gastrointestinal-relevant conditions. Notably, nutriosomes showed improved stability following freeze-drying and enhanced protection against oxidative stress in Caco-2 cells, highlighting the potential contribution of Nutriose® FM06 to the technological and biological performance of the vesicular system. Furthermore, the successful incorporation of the vesicles into gummies and their subsequent recovery under acidic conditions support the feasibility of this approach for developing innovative oral functional formulations.

Despite these promising findings, some limitations should be acknowledged, including the use of a simplified gastrointestinal model, the lack of direct investigations into the molecular interactions between Nutriose® FM06 and the phospholipid bilayer, and the absence of microscopic characterization of vesicle morphology and lamellarity. Future studies will therefore focus on elucidating the role of Nutriose® FM06 in membrane organization and stabilization, evaluating vesicle behavior using standardized gastrointestinal digestion protocols, such as INFOGEST, and further characterizing the gummy formulation to assess its suitability for oral delivery.

CRediT authorship contribution statement

Federica Fulgheri: Writing – original draft, Formal analysis, Data curation, Conceptualization. **Matteo Aroffu:** Writing – original draft, Formal analysis, Data curation. **Mohammad Firoznezhad:** Writing – original draft, Formal analysis, Data curation, Conceptualization. **Maria Cristina Meloni:** Formal analysis, Data curation. **Andrea Angeli:** Formal analysis, Data curation. **Urska Vrhovsek:** Writing – original draft, Formal analysis, Data curation, Conceptualization. **Maria Letizia Manca:** Writing – review & editing, Writing – original draft, Supervision, Formal analysis, Data curation, Conceptualization. **Maria Manconi:** Writing – original draft, Formal analysis, Data curation, 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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Data availability

The authors are unable or have chosen not to specify which data has been used.

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