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Application of encapsulated tomato by-product extract as a colorant in mascarpone

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ABSTRACT

Agricultural by-products, often underutilized, represent a significant opportunity to obtain sustainable value-added products to develop new functional foods. In this context, the (typically discarded) aerial parts of tomato plants (*Solanum lycopersicum* L. var. cerasiforme), were studied as new sources of natural bioactive compounds, focusing the extraction and encapsulation (using spray drying with maltodextrin as the encapsulant) of chlorophylls. The encapsulated natural colorant was subsequently incorporated into mascarpone cheese to assess its stability and effect on nutritional and antioxidant properties. The obtained extracts were mainly characterized by high concentrations of bioactive compounds, particularly quercetin-3-*O*-deoxyhexoside (14.4 ± 0.3 mg/g). The colorant imparted a greener hue to the mascarpone (a* –6.0 ± 0.05 vs. –1.7 ± 0.05 in control, $p < 0.001$) and maintained antioxidant activity (OxHLIA EC_{50} = 1440 ± 72 to 1166 ± 66 μg/mL, $p = 0.030$) without altering the nutritional profile ($p > 0.05$). Moreover, no cytotoxicity was observed, and the fatty acid composition remained unchanged. Accordingly, the studied encapsulated colorant might be used as a functional ingredient in other dairy products, imparting an attractive color while preserving nutritional integrity. In conclusion, the incorporation of TAPC is an effective strategy to enrich dairy products with natural pigments and bioactive compounds without compromising quality. Future research is needed to explore its application in other food matrices and assess long-term storage effects.

1. Introduction

Agro-food industry faces a significant challenge in managing crop-derived waste. Tomatoes (*Solanum lycopersicum* L.), the most widely cultivated vegetable worldwide, is a paramount example of this problem. In 2022, global tomato production reached 186,107,972 metric tons, with an average yield of 37 tons per hectare. The leading producing countries include the United States (particularly California), China, Italy, Turkey, and Spain. In Europe, tomato production for industrial purposes is primarily concentrated in the Mediterranean region, with Italy, Spain, and Portugal as prominent producers (*Food and Agriculture Organization of the United Nations, FAO*, 2023). This extensive production generates considerable vegetable by-products, such as stems, leaves, and branches, which are mostly discarded (Almeida, Rodrigues, Gaspar, Braga, & Quina, 2021). Actually, the aerial parts of tomato plant have long been underestimated despite their notable content of bioactive compounds. The leaves and stems contain diverse phenolics, alkaloids, and carotenoids, which possess antioxidant, and antimicrobial properties and potential health benefits (Añibarro-Ortega et al., 2020). However, the lack of information and limited research on these green tissues have restricted their industrial use. Nonetheless, there is growing recognition that these residues can be transformed into valuable functional ingredients with useful application in food, cosmetic, and pharmaceutical industries (Chabi et al., 2024). In particular, the extraction of chlorophylls, the predominant green pigments in these parts, has emerged as an innovative solution. These pigments can be used as natural colorants in various products, including dairies and baked goods, providing a potentially attractive green and a beneficial consuming

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trend (Mohammad Azmin et al., 2022).

However, the industrial application of these by-products imposes specific challenges. The efficiency of extraction methods and the stability of the extracted pigments are critical aspects to achieve the commercial viability of the whole process. Recent advances in extraction technologies, such as ultrasound- and microwave-assisted processes, have significantly improved the recovery of bioactive compounds from agricultural by-products, offering sustainable and economically viable solutions. Moreover, protecting these compounds from degradation is essential to preserve their functional properties. In this context, spray drying has shown promising results in stabilizing different pigments (Freixo et al., 2016; Jurić et al., 2022; Molina et al., 2019). Despite these developments, few studies have explored the application of chlorophyll-rich encapsulated extracts from tomato aerial parts in real food systems, and even fewer have assessed their nutritional and functional impact in dairy matrices.

As the agro-food industry moves towards higher circularity and more efficient production models, the valorization of vegetable residues, such as the aerial parts of tomatoes, represents a response to environmental challenges and an opportunity for developing innovative products. Accordingly, this study focused on the extraction and spray-drying encapsulation of chlorophylls from tomato leaves and secondary stems, followed by their incorporation into mascarpone cheese. The aim was to characterize the nutritional and chemical composition of the extract, assess its chlorophyll content and stability, and evaluate its antioxidant potential and color performance in the dairy matrix, highlighting its potential as a natural colorant and functional food ingredient.

2. Materials and methods

2.1. Samples

The aerial parts of *Solanum lycopersicum* L. var. cerasiforme (cherry tomato) were harvested at late summer in Bragança (northeast Portugal), after collecting the edible fruits (any remaining fruit was carefully removed before collecting the green aerial parts). The collected samples were cleaned to remove soil and impurities, frozen, lyophilized and reduced to a fine (20 mesh), uniform powder.

2.2. Tomato aerial parts extraction

For ultrasound-assisted extraction, 10 g of the sample were mixed with 500 mL of a hydroethanolic solution (90 %, *v/v*) and subjected to ultrasonication at 400 W (5 min at 25 °C) using an ultrasonic homogenizer (CY-500, OpticIvymen Systems, Barcelona, Spain) with a titanium probe operating at 20 kHz. The mixture was then centrifuged (4000 *g* for 10 min) and filtered to remove solid residues. The resulting extract was designated as TAP (tomato aerial parts extract).

2.3. Encapsulation of extracts from tomato aerial parts

The microencapsulation of TAP was conducted using the method described by Freixo et al. (2016), with maltodextrin (MD) as the encapsulating agent, which was selected due to its high solubility, low viscosity at high concentrations, neutral taste, low cost, and excellent performance in spray-drying processes. A solution was prepared by dissolving 166 g of maltodextrin (95 % *w*/w) in 6 L of extract solution (5 L of ultrapure water), corresponding to a target concentration of 5 % (*w/v*) of encapsulating material in the total feed solution (6 L), immediately before atomization. The atomization process was performed using a spray dryer (BUCHI B-191, Laboratory-Techniques LTD, Flawil, Switzerland) equipped with a 1 mm diameter spray nozzle. The inlet air temperature was set to 130 °C with a pressure of 6.5 bar, while the outlet air temperature was maintained at 71 °C. The liquid feed rate to the dryer was approximately 6 mL/min, with a feed temperature of 40 °C. The process was conducted under controlled and constant conditions. The yield of the spray drying process was calculated as the ratio between the weight of the obtained powder (dry basis) and the total weight of solids present in the initial atomized solution (67.9 %). The encapsulated colorant was designated with the acronym TAPC.

2.4. Mascarpone preparation

Mascarpone cheese was prepared using a Thermomix by combining 1200 g of whole cow's milk, 1200 g of heavy cream (minimum 35 % fat), 30 g of lemon juice, and a teaspoon (6 g) of salt. The mixture was initially blended at speed 3 for 15 s and further heated to 90 °C for 25 min at speed 1. After heating, the mixture was allowed to rest in the Thermomix bowl for 10–15 min to cool, creating favorable conditions for mascarpone formation. The mixture was then transferred to a strainer using a spatula and left to drain for 24 h to ensure gradual whey removal. This method achieved the desired texture and consistency for the mascarpone cheese. The cheese was divided into two parts: one was used as the control (MC) and the other was added with 3 % TAPC (corresponding to 0.02 % TAP, considering the obtained extraction yield of 11.28 %) (MT). Finally, each of these samples was further subdivided: one part was immediately used for the corresponding analyses (MC t_0 and MT t_0), and the other was stored (MC t_3 and MT t_3) under refrigeration (5 ± 2 °C, for 3 days) for subsequent analysis.

2.5. Nutritional and chemical characterization

The nutritional profile and chemical characterization of the TAP, TAPC, and mascarpone cheese, both with and without the encapsulated colorant, were evaluated. For the cheese samples, analyses were conducted on the day of preparation (t_0) and after three days of cold storage (t_3) to assess their stability under refrigerated conditions. Results were expressed as grams per 100 g of fresh weight (g/100 g fw) for TAP and mascarpone cheese samples (MC and MT) at t_0 and t_3. Energy content was reported in kilocalories per 100 g (kcal/100 g), tocopherols were measured in milligrams per 100 g of fresh weight (mg/100 g fw), and fatty acids were expressed as relative percentages (%). For TAPC samples, all results were reported on a dry weight (dw) basis.

2.5.1. Nutritional value

The nutritional profile was assessed following the procedures established by the AOAC (Association of Official Analytical Chemists, 2005).

2.5.2. Free sugars

Free sugars were extracted from the lyophilized samples and analyzed using an HPLC system (Knauer, Smartline system 1000, Berlin, Germany) equipped with a refractive index detector (RI detector, Knauer Smartline 2300), following the methodology described by Barros et al. (2013). Separation was achieved using a Eurospher 100–5 NH2 column (250 mm × 4.6 mm, 5 µm, Knauer) with isocratic elution employing acetonitrile/deionized water (70,30, *v*/v) at a flow rate of 1 mL/min, operating at 35 °C.

2.5.3. Organic acids

Organic acids were extracted and analyzed using ultra-fast liquid chromatography (UFLC) coupled with a photodiode array (PDA) detector, following the method described by Barros et al. (2013). The analysis was performed on a Shimadzu 20 A series UFLC system (Shimadzu Corporation) using a reverse-phase C18 SphereClone column (250 mm × 4.6 mm, 5 µm, Phenomenex, Torrance, CA, USA). Separation was achieved with isocratic elution using sulfuric acid (3.6 mM) at a flow rate of 0.8 mL/min, operating at 35 °C. For quantitative analysis, the following calibration curves were used: oxalic acid ($y = 1 \times 10^7 x +$ 432,019; R^2: 0.9938; LOD: 0.0544; LOQ: 0.1648), malic acid ($y =$ 930,178× + 62,016; R^2: 0.9991; LOD: 0.1616; LOQ: 0.4896), citric acid

($y = 1 \times 10^6 x + 65{,}994$; R^2: 0.9974; LOD: 0.1393; LOQ: 0.4222; fumaric acid ($y = 4 \times 10^7 x + 119{,}019$; R^2: 0,9997; LOD: 0.0066; LOQ: 0.0200), and lactic acid ($y = 590{,}749 + 2177.2$; R^2: 0.9999; LOD: 0.0288; LOQ: 0.0871).

2.5.4. Tocopherols

Following the extraction method described by C. Pereira, Barros, Carvalho, and Ferreira (2013), tocopherols were analyzed using a Knauer Smartline system 1000 HPLC (Berlin, Germany) with a fluorescence detector (FP-2020; Jasco, Easton, USA). Separation was performed on a Polyamide II normal-phase column (250 mm × 4.6 mm, 5 μm, YMCWaters, Milford, MA, USA) with isocratic elution using n-hexane and ethyl acetate (70:30, *v*/v) at 1 mL/min and 35 °C.

2.5.5. Fatty acids

To determine the compounds, extraction and derivatization procedures were first performed. Subsequently, analysis was conducted using a DANI model GC 1000 gas chromatograph with a split/splitless injector and flame ionization detector (FID) at 260 °C. Separation was achieved on a Zebron-Kame column (30 m × 0.25 mm ID × 0.20 μm d_f, Phenomenex). Fatty acids were identified by comparing their retention times with those of fatty acid methyl ester (FAME) standards. Data were analyzed using Clarity DataApex 4.0 Software.

2.6. Extraction of tomato leaf-based encapsulated colorant and mascarpone formulations for phenolic compounds and antioxidant activity evaluation

The extraction of the encapsulated colorant from tomato leaves, as well as from the control cheese and the cheese with added colorant, was carried out using 1 g of each sample dissolved in 50 mL of solvent, employing an ultrasonic treatment as detailed in **Section 2.2.** The extracts were then freeze-dried for subsequent analyses.

2.7. Phenolic compounds

The TAP, TAPC, and cheese extracts were resuspended in the same solvent, with TAP at a concentration of 12 mg/mL and the other samples at 25 mg/mL. They were then filtered through a 0.2 μm membrane and injected into an HPLC system (Dionex Ultimate 3000 UPLC, Thermo Scientific, San Jose, CA, USA). The system, equipped with a diode array detector (DAD) set to wavelengths of 280, 330, 370, and 520 nm, was coupled to an electrospray ionization mass spectrometer (Linear Ion Trap LTQ XL, Thermo Scientific, San Jose, CA, USA) operating in negative mode, as described by Bessada, Barreira, Barros, Ferreira, and Oliveira (2016). Data acquisition and analysis were performed using Xcalibur® software (Thermo Finnigan, San Jose, CA, USA). Chromatographic separation was achieved using a reverse-phase C18 column, Water Spherisorb S3 ODS-2 (3 μm, 4.6 mm × 150 mm, Waters, Milford, MA, USA). The compounds were identified based on their UV–Vis spectra, mass spectra, and retention times compared to authentic standards and literature references. Quantification was conducted using calibration curves obtained from seven levels of standard compounds, and the concentrations of individual phenolic compounds were expressed as mg per gram of extract.

2.8. Total chlorophyll

The total chlorophyll content was determined by Añibarro-Ortega et al. (2020) weighing 170 mg of the sample and treating it with 10 mL of a solvent mixture of acetone and hexane (4:6). The mixture was vortexed for 1 min to ensure complete extraction. Subsequently, the solution was filtered, and the absorbance was measured at 645 and 663 nm.

2.9. Color parameters

The color evaluation of extracts and formulated colorant was conducted immediately after encapsulation, while the color analysis of the mascarpone cheese, both with and without colorant, was performed on the day of preparation and after three days of cold storage. This analysis utilized a colorimeter (model CR-400, Konica Minolta Sensing, Inc., Osaka, Japan) equipped with an adapter for granular materials (model CR-A50), with color parameters assessed in the CIE Lab* color space (E. Pereira et al. (2015). Measurements were taken using illuminant C and an 8 mm aperture. Data interpretation was carried out using the "Spectra Magic Nx" software (version CM-S100W 2.03.0006, Konica Minolta).

2.10. Antioxidant activity

The antioxidant activity of the samples was assessed using two methods: lipid peroxidation inhibition (TBARS) and oxidative hemolysis inhibition (OxHLIA), following the protocols outlined by C. Pereira, Calhelha, Barros, Queiroz, and Ferreira (2014) and Lockowandt et al. (2019), respectively. The results were expressed as EC_{50} values, representing the concentration required to achieve 50 % antioxidant activity, measured in μg/mL. Trolox served as the positive control for both assays.

2.11. In vitro simulation of the gastrointestinal tract

To assess the bioaccessibility of phenolic compounds in mascarpone cheese, an in vitro gastrointestinal digestion process was performed, following the method outlined by Brodkorb et al. (2019). The cheese underwent simulated salivary, gastric, and intestinal digestion using corresponding synthetic fluids, including α-amylase, rabbit gastric extract, lipase, pepsin, bile salts, and pancreatin. Aliquots were collected from each digestion phase for subsequent analysis using HPLC-DAD-ESI/MS to identify and quantify the phenolic compounds (**see section 2.7**). Additionally, a sample from the intestinal phase was preserved for fatty acid analysis (**see section 2.5.5**).

2.12. Cytotoxic activity

The cytotoxicity of the samples was evaluated using the MTT assay, following the protocol outlined by Machado et al. (2022). Briefly, cells (1.0×10^4 per well) were seeded in 96-well tissue culture plates (Thermo Scientific, Denmark) and allowed to incubate for 24 h. After this period, the medium was carefully exchanged with 100 μL of the encapsulated colorant sample, rich in anthocyanins, diluted at 1:10 (*v*/v) and 1:20 (v/v) in growth medium. Dimethyl sulfoxide (DMSO) served as the negative control, while fresh cell culture medium was used as the positive control (representing cells under optimal growth conditions with high redox potential). The plates were then incubated for an additional 24 h. Following incubation, 100 μL of MTT solution (0.5 mg/mL) were added to each well, and the plates were incubated again for 2 h. The supernatant was then carefully removed, and 100 μL of DMSO were added to dissolve the formazan crystals, with gentle agitation (protected from light). Absorbance was subsequently measured at 570 nm using a microplate reader (Synergy H1, Biotek Instruments, USA).

2.13. Statistical analysis

For each mascarpone formulation (MC and MT) and storage time (0 and 3 days), three independent samples were analyzed in triplicate. Data were expressed as mean ± standard deviation. The statistical tests were applied considering a value of $\alpha = 0.05$ (95 % confidence) using the IBM SPSS Statistics for Windows software version 25.0 (IBM Corp., Armonk, NY, USA).

An analysis of variance (ANOVA) was performed with type III sum of squares, using the GLM (Generalized Linear Model) procedure.

Compliance with the ANOVA requirements, specifically the normality of the distribution of results and the homogeneity of variances, was verified through the Shapiro-Wilk and Levene tests, respectively.

All dependent variables were analyzed using a 2-factor ANOVA, specifically the formulation (F) and storage time (ST). In cases with a significant interaction between the two factors, the results were compared through the estimated marginal means, in all cases where the effect of each individual factor was statistically significant.

3. Results and discussion

3.1. Nutritional and chemical composition of Solanum lycopersicum L. var. cerasiforme by-products

3.1.1. Nutritional composition

The nutritional profile, expressed in fresh weight (fw) of the aerial parts of *Solanum lycopersicum* L. var. cerasiforme, a by-product that has been scarcely explored in the scientific literature, is detailed in Table 1. The results reveal that the material is predominantly aqueous, with a moisture content of 80.3 ± 0.5 g/100 g, which is expected given the fresh nature of the plant material. Additionally, 2.57 ± 0.07 g/100 g of protein and 3.4 ± 0.2 g/100 g of ash were detected, indicating a relevant nutrient contribution, while the low lipid content (0.43 ± 0.04 g/100 g) underscores its lean nature. The carbohydrate content, 13.3 ± 0.2 g/100 g, reflects the presence of sugars and dietary fiber.

Although specific comparative studies on this type of plant biomass are difficult to find, results are similar to the ones obtained in other Solanaceae species. For example, the leaves of *Solanum macrocarpon* exhibit a protein content of 4.3 ± 0.1 g/100 g, 0.6 ± 0.0 g/100 g of lipids, and 1.3 ± 0.0 g/100 g of ash (Oboh, Ekperigin, & Kazeem, 2005), while *Solanum nigrum* L. shows protein values of 3.81 ± 0.02 g/100 g of protein, 0.70 ± 0.01 g/100 g of lipids, 1.56 ± 0.02 g/100 g of ash, and 8.19 ± 0.01 g/100 g of carbohydrates (Akubugwo, Obasi, & Ginika, 2007).

3.1.2. Chemical composition

The chemical composition of the aerial parts of *S. lycopersicum* L. var. cerasiforme reveals potentially significant properties for the food industry. In fw basis (Table 1), a total sugar content of 7.66 ± 0.02 g/100 g was quantified, with glucose (3.0 ± 0.3 g/100 g) and sucrose (3.1 ± 0.2 g/100 g) as the main individual sugars.

In what concerns the organic acids profile (Table 1), which may offer acidulant properties and contribute to the preservation and safety of foods, besides contributing to their, malic acid (1.53 ± 0.04 g/100 g) and citric acid (0.68 ± 0.02 g/100 g) were the predominant forms.

Despite the scarcity of studies on the sugar and organic acid profiles of TAP are limited, works conducted with related wild tomato species (*S. pimpinellifolium*, *S. neorickii*, *S. chmielewskii*, *S. habrochaites*, and *S. pennellii*) identified glucose (0.20 ± 0.04 to 1.08 ± 0.01 g/100 g), fructose (0.0010 ± 0.0001 to 0.52 ± 0.01 g/100 g), and sucrose (0.61 ± 0.01 to 1.44 ± 0.01 g/100 g) as the main sugars, somehow corroborating the predominance of glucose and sucrose, although having been quantified with different methodologies. Additionally, malic acid (0.23 ± 0.01 to 0.53 ± 0.01 g/100 g) and citric acid (0.020 ± 0.001 to 0.11 ± 0.01 g/100 g) were the predominant organic acids (Schauer, Zamir, & Fernie, 2005), in line, again, with the results presented herein.

The tocopherol (Table 2) content (per fw) in the aerial parts of *S. lycopersicum* L. var. cerasiforme reached 5.2 ± 0.1 mg/100 g, with γ-tocopherol (3.9 ± 0.1 mg/100 g) and α-tocopherol (1.42 ± 0.05 mg/100 g) as the predominant vitamers, which are generally lower values than those reported in tomato leaves grown in Argentina (Quadrana et al., 2013).

The fatty acid (FA) profile (Table 2) of TAP reveals a balanced composition, with 11 identified fatty acids. Among saturated fatty acids (SFA), palmitic acid (C16:0) stands out, representing 37.3 ± 0.02 % of all quantified fatty acids; in turn, polyunsaturated fatty acids (PUFA) constitute 29.8 ± 0.1 %, with linoleic acid (C18:2n6c) accounting for 23.4 ± 0.1 %, while monounsaturated fatty acids (MUFA) corresponded to 19.7 ± 0.2 %, with oleic acid (C18:1n9c) as the major MUFA (12.3 ± 0.2 %). In a related study on *Solanum lycopersicum* L. cv. Chebli, a different FA profile was observed, with linolenic acid (C18:3; 43.6 ± 0.3 %) as dominant acid, was followed by linoleic acid (C18:2; 22.4 ± 0.5 %), and palmitic acid (C16:0; 19.8 ± 0.2 %) (Ammar, Zarrouk, & Nouairi, 2015).

3.2. Phenolic compounds and chlorophyll in Solanum lycopersicum L. var. cerasiforme by-products

The phenolic compound profile of TAP was characterized using HPLC-DAD-ESI/MS, as shown in Fig. 1. The corresponding identification data, including retention time, λ_{max} in the UV–vis region, the pseudomolecular ion, and the main fragment MS^2 ions, are summarized in Table 3.

Six compounds, primarily phenolic acids and flavonoids, were identified. Compound 1, with a pseudomolecular ion $[M-H]^-$ at *m/z* 499 and fragment ions at *m/z* 353, 191, and 179, was tentatively identified

Table 1
Nutritional value and individual hydrophilic compounds of *Solanum lycopersicum* var. *cerasiforme* by-products.

Macronutrients (g/100 g fw) and energy value (kcal/100 g fw)	
Moisture	80.3 ± 0.5
Protein	2.57 ± 0.07
Ash	3.4 ± 0.2
Lipids	0.43 ± 0.04
Carbohydrates	13.3 ± 0.2
Energy value	67.3 ± 0.8
Individual sugars (g/100 g fw)	
Fructose	1.60 ± 0.01
Glucose	3.0 ± 0.3
Sucrose	3.1 ± 0.2
Total sugars	7.66 ± 0.02
Organic acids (g/100 g fw)	
Oxalic acid	0.139 ± 0.002
Malic acid	1.53 ± 0.04
Citric acid	0.68 ± 0.02
Fumaric acid	0.000020 ± 0.000001
Total organic acids	2.4 ± 0.1

Table 2
Individual lipophilic compounds evaluated in *Solanum lycopersicum* var. *cerasiforme* by-products.

Tocopherols (mg/100 g fw)	
α-Tocopherol	2.83 ± 0.04
γ-Tocopherol	3.9 ± 0.1
Total tocopherols	6.6 ± 0.1
Fatty acids (relative %)	
Capric acid (C10:0)	0.952 ± 0.003
Lauric acid (C12:0)	1.02 ± 0.02
Myristic acid (C14:0)	4.84 ± 0.02
Pentadecanoic acid (C15:0)	0.688 ± 0.007
Palmitic acid (C16:0)	37.3 ± 0.02
Stearic acid (C18:0)	5.71 ± 0.05
Elaidic acid (C18:1n9t)	7.46 ± 0.01
Oleic acid (C18:1n9c)	12.3 ± 0.2
Linoleic acid (C18:2n6c)	23.4 ± 0.1
Linoelaidic acid (C18:2n6t)	2.96 ± 0.05
α-Linolenic acid (C18:3n3)	3.40 ± 0.02
Saturated fatty acids	**50.5 ± 0.1**
Monounsaturated fatty acids	**19.7 ± 0.2**
Polyunsaturated fatty acids	**29.8 ± 0.1**

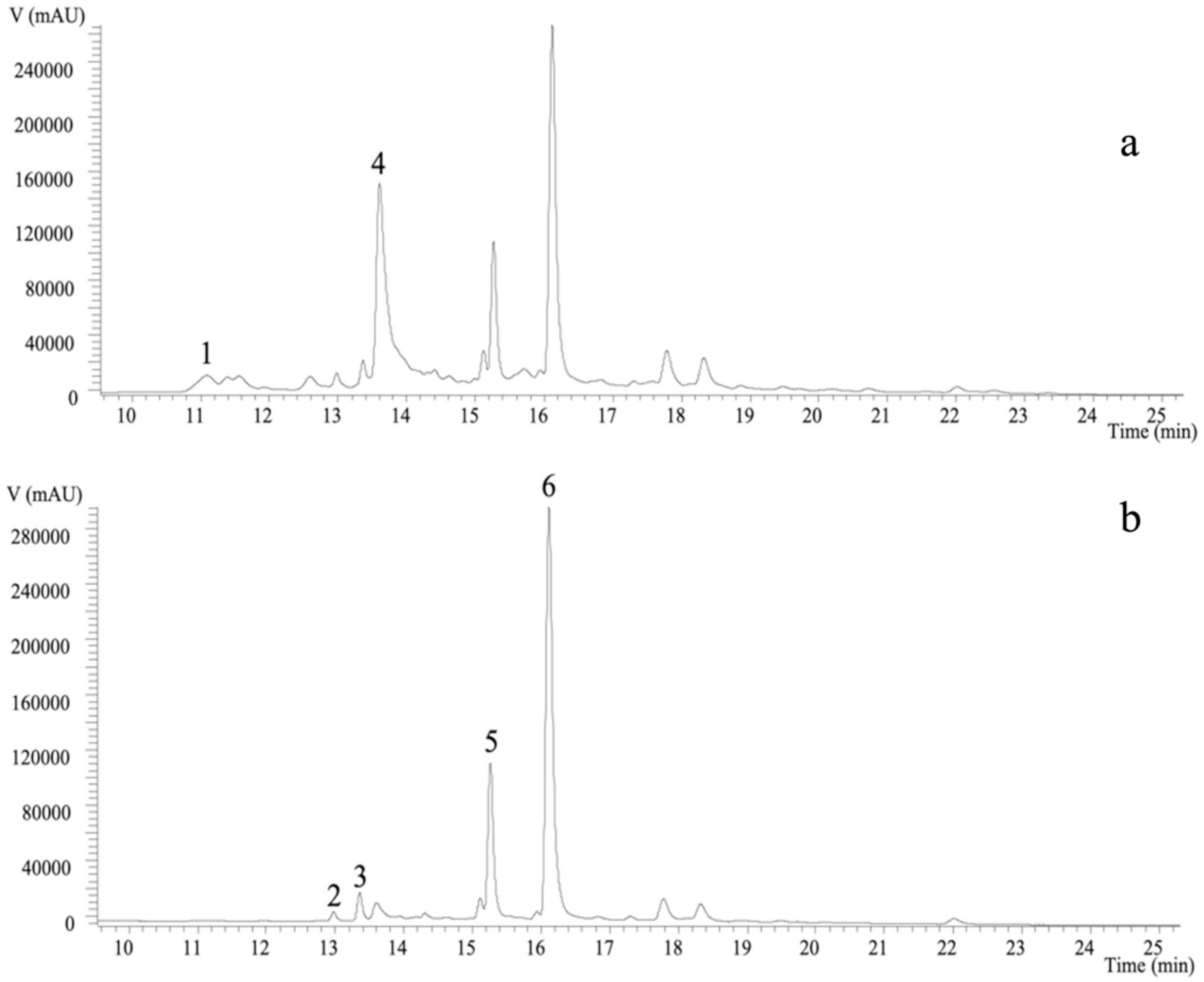

Fig. 1. HPLC phenolic profile of the extract of the aerial parts of *Solanum lycopersicum* L. var. cerasiforme, recorded at (a) 330 nm and (b) 370 nm.

Table 3
Phenolic composition of *Solanum lycopersicum* var. *cerasiforme* by-products.

Peak	Rt (min)	λ_{max} (nm)	$[M-H]^-/[M]^+$ *m/z*	MS^2	Tentative Identification	Concentration (mg/g ext)
1	11.16	320	499	353(100), 191(45), 179(5)	*p*-Coumaroyl-caffeoylquinic acid	1.21 ± 0.06
2	12.98	323	903	741(100), 609(38), 301(43)	Quercetin-*O*-pentosyl-hexocyl-deoxyhexoside	0.34 ± 0.01
3	13.36	352	771	609(100), 301(30)	Quercetin-*O*-deoxyhexoside-*O*-glucoside	0.68 ± 0.01
4	13.60	321	707	MS2: 353(100) MS3: 191(100), 179(10), 135(<5)	3-*O*-caffeoylquinic acid dimer	9.8 ± 0.2
5	15.26	354	741	609(100), 301(70)	Quercetin-pentosyl-deoxyhexoside	4.36 ± 0.02
6	16.11	355	609	301(100)	Quercetin-3-*O*-deoxyhexoside	14.4 ± 0.3
					Total	**30.7 ± 0.6**

Calibration curves used for quantification: Chlorogenic acid ($y = 168{,}823\times - 161{,}172$; R^2: 0.9999; limit of detection (LOD): 0.20 μg/mL; limit of quantitation (LOQ): 0.68 μg/mL); Quercetin-3-*O*-glucoside ($y = 34{,}843\times - 160{,}173$; R^2: 0.9998; LOD: 0.21 μg/mL; LOQ: 0.71 μg/mL); Quercetin-3-*O*-rutinoside ($y = 13{,}343\times + 7675$, R^2: 0.9998, LOD: 0.18 μg/mL; LOQ: 0.65 μg/mL); nq: not quantified.

as *p*-coumaroyl-caffeoylquinic acid, based on its fragmentation pattern, where the ion at *m/z* 353 corresponds to the loss of a coumaroyl group (Clifford, Zheng, & Kuhnert, 2006). In contrast, compound 2 exhibited a pseudomolecular ion $[M-H]^-$ at *m/z* 903 and fragment ions at *m/z* 741, 609, and 301, should be a quercetin-*O*-pentosyl-hexoxyl-desoxyhexoside, but the nature and position of the sugar units could not be confirmed; either way, the fragment at *m/z* 301, indicates the loss of multiple sugar moieties and confirms the presence of the quercetin aglycone (Vallverdú-Queralt, Jáuregui, Medina-Remón, Andrés-Lacueva, & Lamuela-Raventós, 2010).

Moving on, compound 3, with a pseudomolecular ion $[M-H]^-$ at *m/z* 771 and prominent fragment ions at *m/z* 609 and 301, was identified as quercetin-*O*-desoxyhexoside-*O*-glucoside, supported by the presence of the aglycone fragment at *m/z* 301, which reflects the cleavage of sugar units, based on MS/DAD data. Compound 4 showed a deprotonated ion $[M-H]^-$ at *m/z* 707, with fragment ions at *m/z* 353, 191, and 135, which led to its tentative identification as a 3-*O*-caffeoylquinic acid dimer, a structure confirmed by the presence of the characteristic ion at *m/z* 353, corresponding to the caffeoylquinic acid core (Gómez-Romero, Segura-Carretero, & Fernández-Gutiérrez, 2010).

For compound 5, a pseudomolecular ion $[M-H]^-$ at *m/z* 741 was observed, with fragment ions at *m/z* 609 and 301. This fragmentation pattern suggested it to be quercetin-*O*-pentosyl-deoxyhexoside, with the ion at *m/z* 301 corresponding to the quercetin aglycone, indicating the presence of sugar groups attached to the flavonoid structure. Similarly, Compound 6 was characterized by its pseudomolecular ion $[M-H]^-$ at *m/z* 609, with a prominent fragment at *m/z* 301, leading to its identification as quercetin-*O*-deoxyhexoside, another glycosylated derivative of quercetin, where the loss of the sugar group reveals the quercetin backbone (Añibarro-Ortega et al., 2020).

The quantitative analysis of phenolic compounds in the TAP revealed a total phenolic content of 30.7 ± 0.6 mg/g of extract (Table 3), with quercetin-3-*O*-deoxyhexoside as the most abundant (14.4 ± 0.3 mg/g) compound, followed by 3-*O*-caffeoylquinic acid dimer (9.8 ± 0.2 mg/g), quercetin-pentosyl-deoxyhexoside (4.36 ± 0.02 mg/g), and *p*-coumaroyl-caffeoylquinic acid (1.21 ± 0.06 mg/g). In turn, quercetin-*O*-deoxyhexoside-*O*-glucoside (0.68 ± 0.01 mg/g) and quercetin-*O*-pentosyl-hexosyl-deoxyhexoside (0.34 ± 0.01 mg/g) were present in significantly lower amounts, while the triterpenoid glycoside residue was present in trace amounts. Overall, these results highlight the predominance of quercetin and caffeoylquinic derivatives, in variable concentrations, reflecting the complexity of the corresponding biosynthetic processes.

Other studies investigating phenolic compounds in tomato plant leaves from different cultivars (Caramba, Valentine, Negro, Abuela, Río Alto, Anairis, Rosa, and Yack) have reported similar profiles, with quercetin-3-*O*-rutinoside, quercetin-3-*O*-pentosyl-rutinoside, and chlorogenic and neochlorogenic acids identified as the main compounds, and overall concentrations ranging from 3774 to 9252 μg/g (Figueiredo-González, Valentão, & Andrade, 2016).

These findings are further supported by studies on tomato by-products, where quercetin-3-*O*-rutinoside was the principal phenolic compound, with concentrations ranging between 5.8 and 17 mg/g of extract (Añibarro-Ortega et al., 2020). Similarly, Taveira et al. (2012) found quercetin-3-*O*-rutinoside to be the main phenolic compound present in the leaves of *Lycopersicon esculentum* Mill., in both Cherry and Bull's Heart varieties. Taken together, these findings emphasize the importance of quercetin derivatives and caffeoylquinic acids in various tomato cultivars and products, although the exact concentrations and profiles may vary depending on the cultivar and analyzed part of the plant.

Likewise, the aerial parts *of S. lycopersicum* L. exhibited a significant content of chlorophyll *a* (20.0 ± 0.1 mg/100 g) and chlorophyll *b* (12.82 ± 0.08 mg/100 g), totaling 32.8 ± 0.2 mg/100 g in fw basis. The dominance of chlorophyll *a* was also reported in a previous studies, which also quantified similar concentrations of both chlorophyll types, despite using different analytical techniques (Galdino, De Barros, Santos, Mendes, & Das Neves, 2023; Molina et al., 2022).

3.3. Analysis of encapsulated colorant (TAPC) and different mascarpone formulation

Considering the two sources of variability (formulation and storage time), the main purpose of this comparative study was verifying differences among MC and MT, independently of the storage time (ST), as well verify significant changes induced by storage time (0 days and 3 days), regardless of the formulation type (F). All acquired conclusions (except L^* value and SFA in the digestion assay) were obtained from the estimated marginal means plots, since the interaction among F and ST was significant ($p < 0.05$).

3.3.1. Nutritional value

Mascarpone is an Italian cheese renowned for its high fat and moisture content, produced through thermal acid coagulation. In general, the differences among MC and MT were significant ($p < 0.05$) mostly in the nutritional parameters (Table 4), while ST affected only ash content (higher in non-stored samples). Except for moisture content, which indicates that TAPC addition may favor the dynamics of water retention, all nutritional parameters presented slightly higher contents in MC.

The measured values showed some variation in comparison to those reported by United States Department of Agriculture (*FoodData Central U.S. Department of Agriculture*, 2023), which may be attributed to differences in the recipe and production process (Zade & Ghosh, 2016).

3.3.2. Sugars

Lactose was clearly the dominant sugar (Table 4), but its concentration (≈5 g/100 g fw) was relatively low compared to other dairy products, as it is typical in mascarpone (Zade & Ghosh, 2016). In what concerns the F effect, glucose and sucrose were only detected in MT, which, in turn, did not affect the lactose content among MC and MT. On the other hand, glucose and sucrose were nearly the same in non-stored and stored samples, while lactose suffered a significant decrease (≈20 %) from 0 to 3 days. This reduction should not be explained by hydrolysis (since glucose concentration did not increase), nor by fermentative processes, because lactic acid (please see next section) showed only a slight increase after 3 days of storage.

3.3.3. Organic acids

The organic acid profile identified the presence of lactic acid and citric acid (Table 4) without significant differences induced by F, in contrast with the ST, which exerted a slight increasing effect in both

Table 4
Nutritional profile and individual hydrophilic compounds of TAPC (g/100 g dw) and different mascarpone formulations (g/100 g dw).

	TAPC (g/100 g dw)	Formulation (F)		*p*-value ($n = 18$)[A]	Storage time (ST)		*p*-value ($n = 18$)[B]	F × ST *p*-value ($n = 36$)[C]
		MC	MT		0 days	3 days		
Moisture	–	54 ± 1	61 ± 1	<0.001	57 ± 3	58 ± 4	0.801	<0.001
Protein	nd	4.5 ± 0.1	3.3 ± 0.1	<0.001	3.8 ± 0.5	3.9 ± 0.5	0.464	<0.001
Ash	0.036 ± 0.002	0.36 ± 0.05	0.30 ± 0.05	0.037	0.40 ± 0.02	0.26 ± 0.04	<0.001	<0.001
Lipids	0.20 ± 0.01	29.7 ± 0.4	27.8 ± 0.2	<0.001	29 ± 1	29 ± 1	0.737	0.023
Carbohydrates	99.4 ± 0.1	11 ± 1	8 ± 1	<0.001	10 ± 2	9 ± 2	0.550	0.022
Energy[D]	400 ± 0.1	330 ± 4	294 ± 4	<0.001	312 ± 16	312 ± 22	0.994	<0.001
Glucose	0.59 ± 0.05	nd	0.21 ± 0.01	–	0.1 ± 0.1	0.1 ± 0.1	0.765	<0.001
Sucrose	1.73 ± 0.05	nd	0.47 ± 0.04	–	0.2 ± 0.2	0.2 ± 0.2	0.664	<0.001
Lactose	nd	5 ± 1	5 ± 1	0.235	5.8 ± 0.2	4.3 ± 0.3	<0.001	<0.001
Sugars	**2.3 ± 0.1**	**5 ± 1**	**6 ± 1**	**0.188**	**6.1 ± 0.3**	**4.6 ± 0.1**	**<0.001**	**0.001**
Lactic acid	nd	0.55 ± 0.03	0.6 ± 0.1	0.206	0.5 ± 0.1	0.6 ± 0.1	0.001	<0.001
Citric acid	nd	0.22 ± 0.01	0.23 ± 0.05	0.268	0.21 ± 0.02	0.24 ± 0.04	0.002	<0.001
Organic acids	**–**	**0.77 ± 0.04**	**0.8 ± 0.2**	**0.219**	**0.7 ± 0.1**	**0.9 ± 0.1**	**0.001**	**<0.001**

[A] Values of *p* lower than 0.05 indicate that the two formulations are significantly different. [B]Values of *p* lower than 0.05 indicate that the two storage times are significantly different. [C]Values of *p* less than 0.05 indicate a significant interaction between the factors, not allowing to classify the differences induced by each individual factor. [D]Energy is given in kcal/100 g dw for TAPC and in kcal/100 g fw for mascarpone.

organic acids, although not as high as it could be expected in the case of lactic acid, considering the relevant decrease in lactose concentration observed after 3 days. The presence of citric acid is typical in mascarpone cheese and its origin should be linked to the lemon juice added during the production process. The increase in organic acid concentrations in stored samples can contribute to the texture of the cheese (more intense coagulation) and to a higher stability and microbiological safety (by lowering the pH) (Moula Ali, Sant'Ana, & Bavisetty, 2022).

3.3.4. Tocopherols

The detected isoforms were α-tocopherol and γ-tocopherol (Table 5), which showed opposite behavior among both formulations: α-tocopherol was lower (0.84 ± 0.02 mg/100 g fw) in MT, while γ-tocopherol was detected in higher concentration (2.5 ± 0.1 mg/100 g fw) in the same formulation, most likely due to the predominance of this vitamer in TAPC. In what concerns ST effect, it was only significant for γ-tocopherol, that tended to decrease with storage.

3.3.5. Fatty acids

Besides the tabled fatty acids (Table 5), C11:0, C13:0, C15:1, C17:1, C18:2n6t, C20:0, and C20:1 were also detected, but in percentages lower than 0.5 %.

In general, saturated fatty acids (SFAs) were predominant, particularly due to palmitic acid (C16:0) followed by polyunsaturated fatty acids (PUFAs), among which linoleic acid (C18:2n6c) was predominant, and monounsaturated fatty acids (MUFA), as it is typically observed in mascarpone (Buratto, 2010).

The differences found among individual fatty acids were more evident among formulations, being statistically significant in all cases (as also in SFA and PUFA), and generally inducing a slight raise in SFA and light decrease in PUFA. In turn, there was no significant effect induced by storage time in C6:0, C8:0, C10:0, C15:0, C16:0, which is in agreement with the higher stability of saturated fatty acids.

3.3.6. Phenolic compounds

The phenolic compounds identified in the TAPC (Table 6) showed lower concentrations when compared to the original extract (**section 3.2**), which was expected, as the encapsulated product consisted of only 5 % extract. In MT, which contained only 3 % colorant, the phenolic content decreased further, as anticipated, while no phenolic compounds were detected in MC. The three detected compounds, 3-*O*-caffeoylquinic acid, quercetin-pentosyl-deoxyhexoside and quercetin-3-*O*-deoxyhexoside, were not affected by ST, remaining constant along the three days of storage. Accordingly, the incorporation of TAPC enriches the product with three important bioactive compounds, and this important property was kept throughout the whole ST, highlighting the potential of using encapsulated phenolics.

The prevalence of quercetin derivatives and chlorogenic acids observed in the present study agrees with previous reports. Figueiredo-González et al. (2016) highlighted the variability in phenolic profiles among tomato varieties, while consistently identifying quercetin glycosides and chlorogenic acids as major constituents. Similarly, Añibarro-Ortega et al., 2020 and Taveira et al. (2012) reported quercetin-3-*O*-rutinoside as the principal phenolic compound in tomato by-products and leaves, reinforcing the relevance of these molecules in the aerial tissues of tomato plants.

As previously described, the phenolic profile of the TAPC extract was dominated by quercetin derivatives and chlorogenic acids. During refrigerated storage, no significant differences were observed in the concentrations of these main phenolic compounds. This apparent stability may be related to their glycosylated structure, as flavonol glycosides such as quercetin-3-*O*-rutinoside are generally more resistant to degradation than their corresponding aglycone forms. However, transformations such as oxidation, hydrolysis, or interaction with milk proteins may still occur in dairy systems. These interactions can reduce the detectable concentration of free phenolics without necessarily compromising their antioxidant potential, as protein–polyphenol complexes may retain bioactivity (Yu, & wei, & Hu, Q. rui., 2024). Factors such as pH, temperature, and the structural characteristics of both phenolics and proteins influence the extent of these interactions (van de Langerijt, O'Mahony, & Crowley, 2023). Consequently, the phenolic stability observed in our study may reflect a balance between limited degradation and complex formation. Further studies are warranted to explore these mechanisms under varied storage and processing conditions.

3.3.7. Chlorophylls and color parameters

The results obtained for chlorophyll *a* and chlorophyll *b* (Table 6) are very similar with the ones observed with phenolic compounds, since these compounds were only present in MT and no changes were observed along ST. In dry weight basis, TAPC contained 101.90 ± 0.02 mg/100 g of chlorophyll *a* and 65.23 ± 0.02 mg/100 g of chlorophyll *b*. However, after encapsulation with maltodextrin, where only 5 % of the

Table 5
Tocopherols (g/100 g dw) and fatty acids (relative percentage) of TAPC and different mascarpone formulations (g/100 g dw).

	TAPC (g/100 g dw)	Formulation (F)		*p*-value (n = 18)[A]	Storage time (ST)		*p*-value (n = 18)[B]	F × ST *p*-value (n = 36)[C]
		MC	MT		0 days	3 days		
α-Tocopherol	–	1.06 ± 0.04	0.84 ± 0.02	<0.001	0.9 ± 0.1	1.0 ± 0.1	0.163	<0.001
γ-Tocopherol	3.5 ± 0.1	1.6 ± 0.5	2.5 ± 0.1	<0.001	2.3 ± 0.1	1.8 ± 0.5	0.008	<0.001
Tocopherols	**3.5 ± 0.1**	**2.7 ± 0.5**	**3.3 ± 0.1**	**<0.001**	**3.3 ± 0.1**	**2.8 ± 0.5**	**0.005**	**<0.001**
C6:0	–	2.8 ± 0.3	3.3 ± 0.5	0.010	3.0 ± 0.2	3.1 ± 0.5	0.300	<0.001
C8:0	–	1.5 ± 0.1	1.7 ± 0.2	0.010	1.6 ± 0.1	1.7 ± 0.3	0.168	<0.001
C10:0	–	3.2 ± 0.2	3.4 ± 0.3	0.035	3.3 ± 0.1	3.3 ± 0.4	0.743	<0.001
C12:0	–	3.7 ± 0.1	4.1 ± 0.4	0.002	3.8 ± 0.1	4.0 ± 0.4	0.040	<0.001
C14:0	3.00 ± 0.01	11.2 ± 0.1	11.8 ± 0.4	<0.001	11.4 ± 0.1	11.7 ± 0.5	0.041	<0.001
C14:1	–	1.31 ± 0.01	1.38 ± 0.05	0.001	1.32 ± 0.01	1.37 ± 0.05	0.013	<0.001
C15:0	–	1.15 ± 0.01	1.17 ± 0.02	<0.001	1.16 ± 0.02	1.16 ± 0.02	0.726	0.004
C16:0	55.2 ± 0.2	36.4 ± 0.3	37.6 ± 0.2	<0.001	36.8 ± 0.5	37.2 ± 0.5	0.072	0.015
C16:1	–	1.90 ± 0.03	1.81 ± 0.02	<0.001	1.83 ± 0.04	1.87 ± 0.05	0.017	0.002
C17:0	–	0.58 ± 0.01	0.3 ± 0.3	0.001	0.56 ± 0.01	0.3 ± 0.3	0.001	<0.001
C18:1n9c	12.6 ± 0.2	11.9 ± 0.2	12.1 ± 0.1	<0.001	12.0 ± 0.3	12.1 ± 0.1	0.048	<0.001
C18:2n6c	20.5 ± 0.1	20.2 ± 0.5	18 ± 2	<0.001	19.6 ± 0.2	18 ± 2	0.054	<0.001
C18:3n3	8.7 ± 0.1	2.3 ± 0.2	2.2 ± 0.1	0.013	2.3 ± 0.1	2.2 ± 0.1	0.001	<0.001
SFA	**58.2 ± 0.2**	**61 ± 1**	**64 ± 2**	**<0.001**	**62.0 ± 0.1**	**63 ± 2**	**0.187**	**<0.001**
MUFA	**12.6 ± 0.2**	**15.8 ± 0.3**	**16.0 ± 0.2**	**0.176**	**15.7 ± 0.1**	**16.1 ± 0.2**	**<0.001**	**0.005**
PUFA	**29.2 ± 0.4**	**23.0 ± 0.5**	**21 ± 2**	**<0.001**	**22.3 ± 0.1**	**22 ± 2**	**0.049**	**<0.001**

[A] Values of *p* lower than 0.05 indicate that the two formulations are significantly different. [B]Values of *p* lower than 0.05 indicate that the two storage times are significantly different. [C]Values of *p* less than 0.05 indicate a significant interaction between the factors, not allowing to classify the differences induced by each individual factor.

Table 6
Phenolic compounds (mg/g extract), chlorophylls (mg/100 g fw), and antioxidant activity (EC_{50}, μg/mL)[A] of different mascarpone formulations.

	Formulation (F)		*p*-value (n = 18)[B]	Storage time (ST)		*p*-value (n = 18)[C]	F × ST *p*-value (n = 36)[D]
	MC	MT		0 days	3 days		
3-*O*-caffeoylquinic acid	nd	0.043 ± 0.002	–	0.02 ± 0.02	0.02 ± 0.02	0.793	<0.001
Quercetin-pentosyl-rutinoside	nd	0.18 ± 0.02	–	0.1 ± 0.1	0.1 ± 0.1	0.804	0.048
Quercetin-3-*O*-rutinoside	nd	0.15 ± 0.04	–	0.06 ± 0.06	0.1 ± 0.1	0.165	<0.001
Phenolic compounds	**–**	**0.36 ± 0.05**	**–**	**0.2 ± 0.2**	**0.2 ± 0.2**	**0.524**	**<0.001**
Chlorophyll *a*	nd	2.0 ± 0.1	–	1 ± 1	1 ± 1	0.300	<0.001
Chlorophyll *b*	nd	2.6 ± 0.1	–	1 ± 1	1 ± 1	0.168	<0.001
Chlorophylls	**nd**	**4.6 ± 0.2**	**–**	**2 ± 2**	**2 ± 2**	**0.743**	**<0.001**
OxHLIA	1307 ± 199	1299 ± 98	0.890	1440 ± 72	1166 ± 66	<0.001	<0.001
TBARS	2524 ± 228	2718 ± 45	0.005	2474 ± 172	2767 ± 133	<0.001	0.002
L	92 ± 1	86 ± 1	<0.001	89 ± 3	89 ± 3	0.885	0.777
*a**	−1.70 ± 0.05	−6.0 ± 0.4	<0.001	−4 ± 2	−4 ± 2	0.596	<0.001
*b**	0.58 ± 0.01	0.3 ± 0.3	0.001	10.8 ± 0.2	10.0 ± 0.5	<0.001	<0.001

[A] Extract concentration providing 50 % of antioxidant activity. [B]Values of *p* lower than 0.05 indicate that the two formulations are significantly different. [C]Values of *p* lower than 0.05 indicate that the two storage times are significantly different. [D]Values of *p* less than 0.05 indicate a significant interaction between the factors, not allowing to classify the differences induced by each individual factor.

TAP was used, the resulting TAPC contained only 5.5 ± 0.5 mg/100 g dw of chlorophyll *a* and 6.9 ± 0.5 mg/100 g dw of chlorophyll *b*, which, even in this scenario, was high enough to impart color and potentially provide functional benefits to the prepared mascarpone, which remained stable during the 3 days of storage.

As expected, the presence of chlorophylls was reflected in the colorimetric analysis (Table 6). MT exhibited slightly lower luminosity (86 ± 1 versus 92 ± 1 in MC), but greener hue (−6.0 ± 0.05 versus −1.7 ± 0.05) and similar blueness (t_0 10.8 ± 0.2; t_3 10.0 ± 0.5). All color parameters were kept nearly unchanged along ST, despite the significant, but minor, change in blueness parameter (Fig. 2), demonstrating the colorant's stability and the efficacy of the encapsulation within the mascarpone cheese matrix. However, as the storage evaluation was limited to three days, which corresponds to the expected shelf life of fresh mascarpone without preservatives, these findings reflect short-term behavior only. Therefore, longer storage assessments would be required to fully understand the stability and performance of the encapsulated colorant under extended conditions and across different food systems.

3.3.8. Antioxidant activity

The antioxidant activity was assessed using the OxHLIA (oxidation-induced hemolysis inhibition) assay at 60 min and the TBARS (thiobarbituric acid-reactive substances) assay (Table 6). In the OxHLIA assay, there was no significant difference among MC and MT, whereas activity measured with TBARS assay should slightly higher values in MC, which could somehow be anticipated considering the poor activity showed by TAPC (EC_{50} = 3236 ± 190 μg/mL for OxHLIA and EC_{50} = 4832 ± 266 μg/mL for TBARS).

In what concerns ST effect the evolution for each antioxidant activity type was dissimilar: while OxHLIA values significantly decreased from 1440 ± 72 μg/mL to 1166 ± 66 μg/mL, reflecting the putative release of TAP (EC_{50} = 92 ± 5 μg/mL for OxHLIA and EC_{50} = 78 ± 3 μg/mL for TBARS) from its encapsulated form, the same was not observed in the case of TBARS assay.

Although mascarpone cheese has not been widely studied in terms of antioxidant activity, it is known that dairy products generally possess some level of antioxidant capacity, attributed to compounds such as proteins, bioactive peptides, and certain vitamins (such as vitamins A and E) (Stobiecka, Król, & Brodziak, 2022). The antioxidant activity observed in TAPC and MT formulations is consistent with their higher phenolic and chlorophyll content, suggesting a relationship between bioactive compound levels and oxidative inhibition capacity.

3.4. Impact of gastrointestinal digestion on the fatty acid profile of mascarpone samples

This study was conducted to attain a more realistic approach on the potential effects of the prepared mascarpone formulations. As show in in Table 7, there were no significant differences among MC and the MT for the majority (except C12:0, C15:0, and C16:0) of the quantified fatty acids; in turn, GD induced alterations with statistical significance, despite being minor in absolute terms, in all tabled fatty acids except C14:0 (p = 0.310). Nonetheless, the profiles were highly similar among control and functionalized formulations, as well as in fresh (0 days) and stored (3 days) samples. Accordingly, the incorporation of encapsulated colorant in mascarpone did not affect its fatty acid profile, suggesting that encapsulated colorants can be used without changing these molecules.

Although the INFOGEST model has been widely adopted to evaluate the digestive fate of bioactive compounds in various food systems, there are currently no studies applying this protocol to mascarpone cheese. Nevertheless, comparisons can be drawn with similar dairy matrices. For instance, Tibaquira-Pérez et al. (2025) reported that the incorporation of encapsulated high oleic palm oil in cottage cheese resulted in a fatty acid release of approximately 72 % after in vitro digestion, particularly of oleic and linoleic acids. Similarly, Machado, Sousa, Rodríguez-Alcalá, Pintado, and Gomes (2023) observed that probiotic cheeses fortified

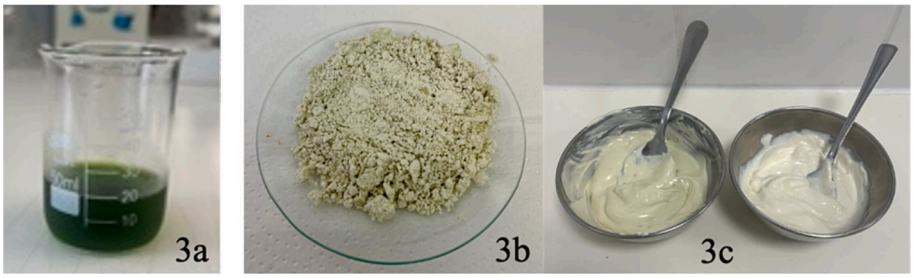

Fig. 2. Visual appearance of TAP (a), TAPC (b), MT and MC (c).

Table 7
Fatty acids (relative percentage) before and after in vitro simulated gastrointestinal of mascarpone formulations.

	Formulation (F)		*p*-value (n = 18)[A]	Gastrointestinal digestion (GD)		*p*-value (n = 18)[B]	F × GD *p*-value (n = 36)[C]
	MC	MT		Non-digested	Digested		
C6:0	3.1 ± 0.1	3.0 ± 0.3	0.183	3.0 ± 0.2	3.1 ± 0.1	0.016	<0.001
C8:0	1.7 ± 0.1	1.6 ± 0.1	0.085	1.6 ± 0.1	1.7 ± 0.1	<0.001	<0.001
C10:0	3.5 ± 0.1	3.4 ± 0.2	0.035	3.3 ± 0.1	3.6 ± 0.1	<0.001	<0.001
C12:0	3.9 ± 0.1	3.8 ± 0.1	0.005	3.8 ± 0.1	4.0 ± 0.1	<0.001	<0.001
C14:0	11.4 ± 0.1	11.4 ± 0.1	0.640	11.4 ± 0.1	11.4 ± 0.1	0.310	<0.001
C14:1	1.5 ± 0.1	1.4 ± 0.1	0.596	1.32 ± 0.01	1.56 ± 0.02	<0.001	<0.001
C15:0	1.14 ± 0.01	1.16 ± 0.02	<0.001	1.16 ± 0.02	1.14 ± 0.01	0.001	<0.001
C16:0	36 ± 1	36 ± 1	0.022	37 ± 1	35.1 ± 0.2	<0.001	<0.001
C16:1	2.1 ± 0.2	2.0 ± 0.2	0.430	1.83 ± 0.04	2.28 ± 0.03	<0.001	0.014
C17:0	0.53 ± 0.02	0.54 ± 0.03	0.108	0.56 ± 0.01	0.51 ± 0.01	<0.001	<0.001
C18:1n9c	11 ± 1	11 ± 1	0.340	11.9 ± 0.3	10.2 ± 0.1	<0.001	<0.001
C18:2n6c	20 ± 1	20 ± 1	0.956	19.6 ± 0.2	21.2 ± 0.2	<0.001	<0.001
C18:3n3	2.5 ± 0.3	2.5 ± 0.2	0.982	2.3 ± 0.1	2.7 ± 0.1	<0.001	<0.001
SFA	**61.5 ± 0.5**	**61.4 ± 0.5**	**0.671**	**62.0 ± 0.1**	**60.9 ± 0.1**	**<0.001**	**0.782**
MUFA	**15.2 ± 0.4**	**15.2 ± 0.5**	**0.882**	**15.7 ± 0.1**	**14.7 ± 0.1**	**<0.001**	**<0.001**
PUFA	**23 ± 1**	**23 ± 1**	**0.887**	**22.3 ± 0.1**	**24.4 ± 0.2**	**<0.001**	**<0.001**

[A] Values of *p* lower than 0.05 indicate that the two formulations are significantly different. [B]Values of *p* lower than 0.05 indicate that the two storage times are significantly different. [C]Values of *p* less than 0.05 indicate a significant interaction between the factors, not allowing to classify the differences induced by each individual factor.

with avocado and coconut oils preserved 50–70 % of relevant bioactive lipids throughout the gastrointestinal simulation. In our study, although the target matrix was mascarpone—a high-fat, low-protein fresh cheese—chlorophyll-rich encapsulates contributed measurable levels of free fatty acids after digestion, notably oleic and linoleic acids. These findings support the feasibility of incorporating functional lipid compounds in soft dairy matrices and highlight the need for further studies comparing digestive behavior across different cheese types.

3.5. Cytotoxicity

The cytotoxicity analysis of TAPC (Fig. 3) underscores its safety profile for potential food applications. The death control, as expected, exhibited significant cytotoxicity, with cell viability reaching approximately 80–90 %, serving as a benchmark for maximal cell damage. In contrast, all tested concentrations of TAPC showed low cytotoxicity levels, with cell viability remaining close to the baseline levels. This pattern suggests that TAPC has minimal cytotoxic effects and maintains cellular integrity even at the highest concentration tested. According to the available literature, there are no published studies assessing the cytotoxicity of encapsulated extracts from tomato aerial parts in Caco-2 cells. Previous work has focused mainly on leaf extracts applied to gastric cell lines (e.g., AGS), showing varying degrees of toxicity depending on the cultivar and solvent system (Figueiredo-González, Valentão, Pereira, & Andrade, 2017). In contrast, our results showed no cytotoxic effects for the encapsulated extract tested in Caco-2 cells, suggesting that, under the tested conditions, the TAPC colorant presents good preliminary safety for potential use in food applications.

4. Conclusions

The study confirms that the use of encapsulated colorant derived from the aerial parts of the tomato plant (TAPC) presents a promising opportunity for the food industry. The encapsulation process with maltodextrin effectively preserved the color intensity and stability of the colorant in mascarpone cheese throughout refrigerated storage. This approach an innovative visual appearance to this particular type of cheese, besides maintaining its nutritional and chemical integrity. The

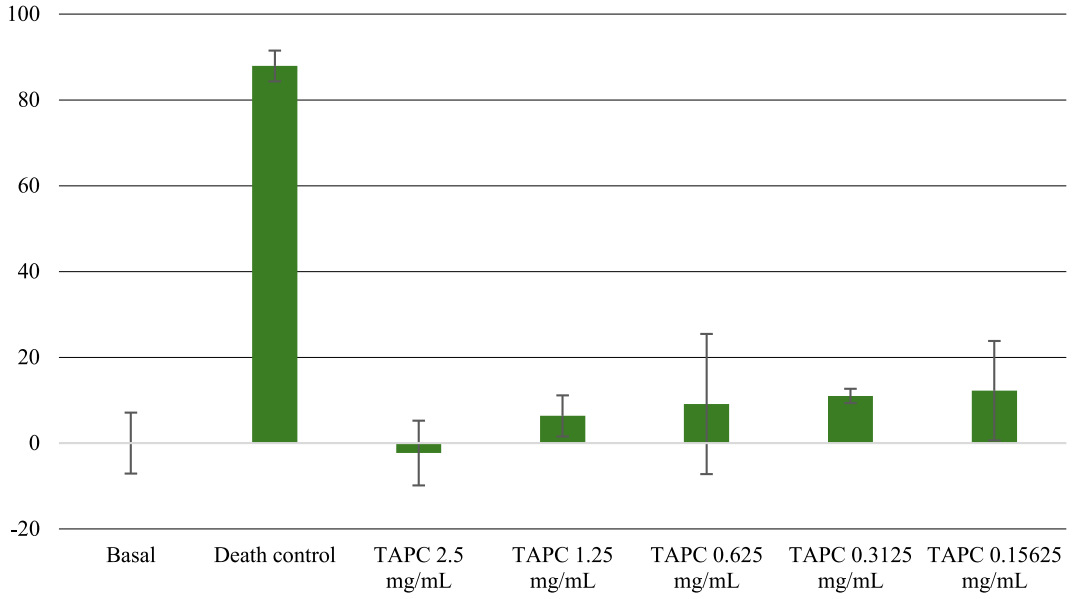

Fig. 3. Cytotoxicity assessment of encapsulated colorant at various concentrations.

gradual release of bioactive compounds from the encapsulated colorant during storage underscores the method's effectiveness in delivering sustained functional benefits. Given that the mascarpone cheese was formulated without preservatives and intended for short-term consumption, the 3-day storage period reflects realistic conditions for this type of fresh product. To advance its industrial application, further research should address the long-term stability of the encapsulated colorant, include sensory evaluation to assess consumer acceptability, and explore its performance in various food systems such as yogurt, butter, or plant-based alternatives, where natural pigments and functional additives are increasingly in demand. Such efforts will help ensure the practical viability of this natural colorant as a multifunctional ingredient in clean-label food development.

CRediT authorship contribution statement

Adriana K. Molina: Writing – original draft, Methodology, Investigation, Formal analysis. **Maria G. Leichtweis:** Writing – original draft, Methodology, Investigation, Formal analysis. **Manuela Machado:** Methodology, Formal analysis. **Sara Silva:** Methodology, Formal analysis. **Manuela Pintado:** Resources, Investigation, Conceptualization. **João C.M. Barreira:** Writing – review & editing, Validation, Conceptualization. **Maria Inês Dias:** Supervision, Investigation, Formal analysis. **Miguel Á. Prieto:** Validation, Supervision, Investigation. **Lillian Barros:** Writing – review & editing, Validation, Supervision, Resources, Conceptualization. **Carla Pereira:** Writing – review & editing, Validation, Supervision, Resources, 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

Data will be made available on request.

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