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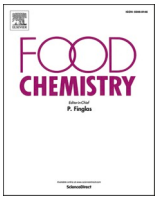

Novel cold and thermally pasteurized cardoon-enriched functional smoothie formulations: A zero-waste manufacturing approach

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ABSTRACT

This study investigated the potential of incorporating cardoon (*Cynara cardunculus* L.) blades as bioactive and dietary fiber ingredients in vegetable/fruit-based smoothies, within a zero-waste approach. The smoothie formulations were pasteurized by high-pressure (550 MPa for 3 min, HPP) and thermal (90 °C for 30 s, TP) treatments and stored at 4 °C for 50 days. Cardoon-fortified smoothies exhibited higher viscosity, darker color, increased phenolic compound levels, and greater anti-inflammatory and antioxidant activities. Furthermore, the cardoon blade ingredients contributed to a more stable dietary fiber content throughout the smoothies' shelf-life. HPP-processed smoothies did not contain sucrose, suggesting enzymatic activity that resulted in sucrose hydrolysis. All beverage formulations had low or no microbial growth within European limits. In conclusion, the fortification of smoothies with cardoon blades enhanced bioactive properties and quality attributes during their shelf-life, highlighting the potential of this plant material as a potential functional food ingredient in a circular economy context.

1. Introduction

The increase in the world's population and the growing need for nutritious foods are one of humanity's main concerns (Fukase & Martin, 2020). Developing emerging strategies to ensure the appropriate balance between the supply and demand for the planet's resources is urgent and extremely necessary. Implementing methodologies that promote the circularity of resources and their efficient use could reduce the biowaste generated during their processing (Bianchi & Cordella, 2023). Indeed, the reuse of biowaste, usually rich in compounds of great potential, and its application in the development of new functional food ingredients, has been increasingly explored (Baker, Lu, Parrella, & Leggette, 2022). This approach fits the objectives of the 2030 Agenda for Sustainable Development proposed by the United Nations.

Smoothies are beverages prepared from a mixture of different fruits and vegetables. These beverages can be rich in nutritional and health-promoting compounds, constituting a healthy, convenient, and ready-to-eat food product that encourages fruit and vegetable dairy intake (Nieva, Jagus, Agüero, & Fernández, 2022; Picouet et al., 2016), so that the consumption of smoothies has increased considerably in recent years. However, the characteristics of this type of product may favor the presence of contaminants and facilitate its rapid degradation. Therefore, smoothies are usually processed by thermal pasteurization, allowing the inactivation of vegetative pathogenic microorganisms, such as yeast and molds, and of enzymes such as peroxidase and polyphenol oxidase. This allows not only to ensure food safety but also to extend the product shelf-life, although it may compromise nutritional and sensory characteristics due to the high temperature (Aaliya et al., 2021). To try and overcome this problem, sustainable nonthermal preservation processing techniques can be used. However, their optimization to ensure the safety of

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the different types of formulations without interfering with sensory, nutritional, and chemical characteristics, as well as the optimization for large-scale implementation is complex and there are still parameters that need to be studied (Aaliya et al., 2021; Artés-Hernández, Castillejo, Martínez-Zamora, & Martínez-Hernández, 2021).

Currently, the beverage industry generates considerable amounts of waste and by-products rich in dietary fiber, such as fruit and vegetable peels, vegetable stalks, and fruit pulp after juicing. Dietary fiber is a macronutrient that can be valorized and upcycled within the food value chain as a food and beverage ingredient ('Aqilah, Rovina, Felicia, & Vonnie, 2023). This approach can contribute to the circularity and resource efficiency of the agri-food systems, promoting adequate human intake of dietary fiber, especially considering that a significant proportion of the world's population does not meet the recommended daily intake (25 g per day) (EFSA, 2014). Dietary fiber plays a protective role against cardiovascular disease, type 2 diabetes, and cancer, and contributes to improving gastrointestinal health and controlling body weight (Wang, Li, Wang, Liu, & Ni, 2021). The use of vegetable species has recently been studied to improve both the nutritional content and microbiological safety of smoothies, thus avoiding the use of synthetic additives. This approach makes it possible to obtain a product with natural characteristics and even potential health benefits (Artés-Hernández et al., 2021; Casco, Jagus, Agüero, & Fernández, 2022). Simultaneously, the incorporation of ingredients obtained from biowaste in smoothies' formulations promotes self-sufficiency in a zero-waste context.

Cynara cardunculus L. var. *altilis*, commonly called cardoon, is widespread in the countries of the Mediterranean basin. This species is usually included in the Mediterranean diet due to its nutritional value and phytochemical composition (Mandim, Santos-Buelga, Ferreira, & I., Petropoulos, S. A., & Barros, L., 2023; Zayed, Serag, & Farag, 2020). Although it has been recognized as a promising species in different industrial sectors over the last decade, a significant amount of plant material is discarded every year. The quantity of generated biomass is determined by various factors, such as environmental conditions, maturity stage, or plant size. Studies comparing the potential of cardoon tissues highlight a great diversity of bioactive compounds and the promising biological potential demonstrated by this plant tissue (Mandim et al., 2023; Zayed et al., 2020). For instance, the outer stalks and blades of the cardoon can be hard and fibrous in texture, so they are often removed and discarded before using the tender inner parts. However, due to their high fiber content, these tissues show great potential for being reused as a source of dietary fiber while simultaneously being rich in compounds of phytochemical interest (Mandim et al., 2023).

This study aims to develop a novel and healthy potential functional drink enriched with cardoon blades, which are usually discarded, through the implementation of emerging processing methodologies. The formulations obtained can contribute positively to a circular and more sustainable economy while simultaneously promoting public health and helping prevent different diseases, owing to their content of valuable bioactive compounds.

2. Material and methods

2.1. Plant samples

Cardoon (*Cynara cardunculus* L. var. *altilis* DC., cv. Bianco Avorio) blades were gathered in the experimental fields of the Thessaly University in Velestino (22.756° E, 39.396° N), Greece. In previous studies, our research team evaluated the influence of the maturation cycle on the possible application of this plant tissue as a potential functional ingredient (Mandim et al., 2022). Based on data already obtained, blades in the principal growth stages (PGS) 1 (sample B3), 4/5 (sample B8), and 7/8 (sample B13) were selected as the most promising material for the present study. All blades' samples were lyophilized, reduced to a ~ 20 mesh particle size with a domestic blender, and stored in a deep freezer (−80 °C) in air-sealed bags until use.

2.2. Extraction

Blade samples (PGS 1, PGS 4/5, and PGS 7/8) were combined and processed according to the procedure described by Mandim et al. (2022). Briefly, equal proportions of each samples were mixed with a hydroethanolic solution (EtOH:H_2O, 80:20, *v/v*) and stirred for 1 h. The resulting solution was filtered, and the remaining residue was re-extracted under the same conditions. The ethanolic fraction was evaporated and the aqueous phase was lyophilized. The solid residue resulting from the extraction procedure was oven-dried (30 °C) and stored protected from light until further use as a dietary fiber ingredient.

2.3. Extract bioactivity assessment

2.3.1. Antioxidant activity

To confirm the antioxidant activity previously attributed to each cardoon blade sample, the extract herein prepared was analyzed by the thiobarbituric acid reactive species (TBARS) formation inhibition assay, using a porcine brain tissue solution, as described by Mandim et al. (2022). The extract was re-dissolved in distilled water and successively diluted to obtain the working concentrations (1–31 μg/mL). The commercial antioxidant Trolox (3.13–250 μg/mL) was used as the positive control. Results were given as half-maximum extract concentrations (μg/mL) responsible for inhibiting lipid peroxidation (IC_{50} values).

2.3.2. Antimicrobial activity

The natural extract was evaluated for its effectiveness against the foodborne bacterial and fungal strains listed in supplementary **Table S3**. The experimental protocol and results presentation format followed the methodology outlined by Mandim et al. (2022).

2.4. Analysis of total dietary fiber in the extraction residue

The fiber content of the solid residue resulting from cardoon blade extraction was determined following the enzymatic-gravimetric method AOAC 985.29 (Association of Official Analytical Chemists, 2019).

2.5. Smoothies' formulation and processing

2.5.1. Smoothies' preparation

The prepared smoothies consisted of pineapple (54%, *w/w*), spinach (4%, *w/w*), mint (2%, *w/w*), and autoclaved water (40%, *w/w*). All fresh fruits and vegetables used were obtained from local markets in Bragança, Portugal. Before use, spinach and mint were washed by dipping in 0.1% sodium erythorbate for 5 min and dried. Smoothies enriched with bioactive cardoon blade extract (2 mg per mL of smoothie) and fiber (7 mg of cardoon blades per mL of smoothie). The added 7 mg/mL of fiber were calculated based on the fiber content determined in the solid residue obtained after the extraction procedure (section 2.4.). All the constituents were blended with a Thermomix device (Bimby TM6, Vorwek, Wuppertal, Germany) for 60 s. Two types of smoothies were obtained, one without added cardoon and another one with cardoon blade extract and fiber-rich residue. After preparation, the smoothies were transferred to flexible polyamide-polyethylene bags (Penta Ibérica Lda, Torres Vedras, Portugal) (20 mL per bag) and heat-sealed. The smoothies were stored at 4 °C for the different processing treatments.

2.5.2. High-pressure and thermal pasteurization processing

Each of the prepared smoothies (with and without cardoon) were submitted to either thermal pasteurization (TP) or high-pressure processing (HPP). Four smoothies were, thus, obtained, designated as 1, 2, 3, and 4. Smoothies 1 and 2 did not contain cardoon ingredients and were treated by TP and HPP, respectively. Smoothies 3 and 4 contained

cardoon extract and fiber-rich residue and were processed by TP and HPP, respectively. The previously packed smoothies were submitted to TP or HPP as follows:

Thermal pasteurization. The smoothie packages were placed into a water bath at 90 °C. To estimate the time required for the smoothies to reach 90 °C, a package containing water was also placed in the bath, and the rise of temperature was monitored with a thermometer; the intended temperature was reached after 3.15 min. Afterwards, the samples were maintained in the water bath for 30 s, to be further quickly cooled in an ice bath.

High-pressure processing. The smoothie packages underwent treatment at 550 MPa for 3 min in a 55-L high-pressure unit (Hiperbaric 55, Hiperbaric S.A, Spain), using water as the pressure-transmitting medium at a rate of 250 MPa/min, followed by decompression in <2 s. Cold pressurizing water was utilized to uphold consistent temperature conditions (15 °C) during HPP processing.

After thermal and cold processing, the smoothie samples were stored at 4 °C for 50 days.

2.6. Smoothies' stability and quality assessment

2.6.1. Physicochemical parameters

Selected physical parameters of smoothies were analyzed during shelf-life. Briefly, a viscosimeter B-one plus Lamy Rheology Instruments (Champagne au Mont d'Or, France), using the LV2 sensor at 250 rpm for 30 s, was used to analyze the smoothies' viscosity. The pH was measured using a pH meter (Hanna Instruments, HI5521–02 Model, Smithfield, Rhode Island, USA). The smoothies' color was assessed using a CR400 Konica Minolta colorimeter (Chiyoda, Tokyo, Japan) featuring a D65 standard illuminant. Measurements were recorded in the CIE L^*, a^*, and b^* color, where L^* represents lightness, a^* indicates redness, and b^* denotes yellowness. Finally, total soluble solids (TSS) were quantified using a HI 96801digital refractometer (Hanna Instruments, Woonsocket, Rhode Island, USA). The physical parameters were evaluated in triplicate at days 0, 3, 7, 10, 14, 22, 35, and 50 (T0, T3, T7, T10, T14, T22, T35, and T50, respectively) of shelf-life.

2.6.2. Proximate composition and energy

The smoothies were lyophilized and characterized in terms of protein, crude fat, ash, and fiber contents according to the official food analytical methods of AOAC International (Association of Official Analytical Chemists, 2019). The carbohydrates content was determined by different. Results were expressed as g per 100 mL of smoothie. The energy was calculated using the conversion factors: 9 kcal/g for crude fat, 4 kcal/g for protein and carbs, and 2 kcal/g for fiber (European Parliament and Council of the European Union, 2011).

2.6.3. Soluble sugars

The free sugars composition of the smoothies was analyzed in a Knauer Smartline 1000 HPLC (Berlin, Germany) coupled to a refractive index detector as described by Pereira et al. (2019). Identification and quantification procedure were achieved using standards of (D-(−)-fructose, D-(+)-sucrose, D-(+)-glucose, D-(+)-trehalose, and D-(+)-raffinose pentahydrate, acquired from Sigma-Aldrich, St. Louis, MO, USA). Results were expressed as g per 100 mL of filtered smoothie.

2.6.4. Fatty acids

After extracting the lipid fraction of the smoothies with petroleum ether in a Soxhlet apparatus, a transesterification process was carried out and the obtained fatty acid methyl esters were analyzed by in a Gas-liquid Chromatography (GC 1000, DANI Instruments, Contone, Gambarogno, Switzerland) system coupled with a Flame Ionization Detector (FID), as described by Pereira et al. (2019). Qualitative and quantitative analyses were performed by comparing the retention time and the area of the peaks of the samples with those of the commercial standard mixture 47,885-U (Sigma-Aldrich, St. Louis, MO, USA). Results were expressed as relative percentages of each identified fatty acid.

2.6.5. Phenolic compounds

The smoothies were filtered through 0.22 μm syringe filters for subsequent analysis. The profile of extractable phenolic compounds was characterized in a HPLC coupled to a diode array detector and an electrospray ionization mass spectrometer (HPLC-DAD-ESI/MS). The chromatographic conditions employed in separation and detection were previously described by Pereira et al. (2019). The tentative identification of the detected compounds was achieved through the comparison of chromatographic information with available commercial standards and relevant literature data. Individual compounds quantification was carried out by the extrapolation of the peak area values in calibration curves of available standard compounds (see supplementary **Table S4**). The Excalibur™ software was used for data collection and processing. Results were expressed as mg per 100 mL of filtered smoothie.

2.6.6. Antioxidant activity

The antioxidant activity of the smoothies was assessed using the TBARS (described in section 2.3.1.) and cellular antioxidant activity (CAA) assays. Briefly, a smoothie aliquot was subject to successive dilutions with water to obtain a range of concentrations for testing. Ten dilutions were tested for TBARS, while three dilutions were tested for CAA. Both assays follow procedures described by Besrour et al. (2022), using the commercial antioxidants trolox and quercetin as positive controls, respectively. Results were expressed as the percentage of oxidation inhibition at a specific smoothie dilution.

2.6.7. NO-production inhibition capacity

The anti-inflammatory capacity of the smoothies was assessed by the lipopolysaccharide-induced nitric oxide (NO) production inhibition method employing a murine macrophage cell line (RAW 264.7) (European Collection of Authenticated Cell Cultures - ECACC), following the procedure described by Mandim et al. (2022). The cells were routinely maintained in an incubator (Heal Force CO_2 Incubator, Shanghai Lishen Scientific Equipment Co, Ltd., China) at 37 °C with 5% CO_2 and a humidified atmosphere, using Dulbecco's Modified Eagle Medium (DMEM) supplemented with fetal bovine serum (10%), glutamine (2 mM), penicillin (100 U/mL) and streptomycin (100 mg/mL). Dexamethasone (50 mM) was used as the positive control, and samples in without lipopolysaccharide were used as the negative control. The NO concentration was assessed using a nitrite assay kit (Griess Reagent, Sigma-Aldrich, St. Louis, MO, USA) and by comparation with the nitrite standard curve ($y = 0.0068\times + 0.0951$, $R^2 = 0.9864$). Results were expressed as the percentage of NO production inhibition at a specific smoothie dilution.

2.6.8. Hepatotoxicity

The hepatotoxicity of the smoothies was assessed in a primary culture of non-tumoral cells obtained from pig liver. The cells were routinely maintained with RPMI-1640 medium supplemented as described above (section 2.6.7). The cells were used when a confluence of around 80 to 90% was reached. Different dilutions of smoothies were incubated with the cells for 72 h under the conditions above. After the incubation period, the influence of the smoothies on cell proliferation was determined using the sulforhodamine B colorimetric assay Mandim et al. (2022). Ellipticine was the positive control and cells without sample were used as the negative control. Results were expressed as the percentage of cell proliferation inhibition at a specific smoothie dilution.

2.6.9. Microbiological quality

The microbial stability of the obtained smoothies was analyzed over the shelf-life. The microbial analyses were performed after smoothies processing (T0), as well as at days 10, 22, 35, and 50 (T10, T22, T35, and T50, respectively) of shelf-life. Briefly, 1 mL of each smoothie formulation was mixed with 9 mL of peptone water (PW, Liofilchem, Italy).

From this solution, serial dilutions were prepared until the concentration of 10^{-3}. Following that, several types of microorganisms were tested: total aerobic mesophiles, coliforms, yeasts, molds, *Bacillus cereus*, and *Staphylococcus* spp.

In the case of aerobic mesophiles (ISO 4833-2:2013) and coliforms (ISO 4832:2006), 1 mL of each smoothie's suspension was inoculated in melting plate count agar (PCA, 15 mL; Liofilchem) and violet-red bile lactose agar (VRBLA, 15 mL; Liofilchem), respectively. After homogenization and solidification, all the plates were incubated in the reverse position at 30 °C for 72 h and 48 h, for aerobic mesophiles and coliforms respectively. Both types of microorganisms were tested using the pour plate technique (LOQ = 1 log CFU/mL). After the incubation period, only plates with colonies between 15 and 300 for aerobic mesophiles and between 10 and 150 for coliforms were counted.

In the case of yeasts and molds (ISO 21527-1/2:2008), *B. cereus* (ISO 7932:2004), and *Staphylococcus* spp. (ISO), 0.2 mL of the prepared smoothie's suspension was pipetted onto a plate containing dichloran rose bengal chloramphenicol (DRBC, 15 mL; Liofilchem), mannitol yolk polymyxin (MYP, 15 mL; Liofilchem), and Baird Parker agar (BPA, 15 mL; Liofilchem). All these microorganisms were analyzed using the spread plate methodology (LOQ = 1.7 log CFU/g). For yeasts and molds, the plates were incubated at 25 °C for 5 days in the upright position and the counting was performed in plates with <150 colonies. In the case of *B. cereus* and *Staphylococcus* spp., the plates were incubated at 37 °C for 24 h in reverse position, and the counting was performed when the plates had between 10 and 150 colonies.

2.7. Statistical analyses

All assays were performed in triplicate ($n = 3$), and data were expressed as mean values ± standard deviation (SD), except for antimicrobial activity. Results were processed using Microsoft Excel (Microsoft Corporation, Redmond, WA, USA). Statistical analyses of data were performed using SPSS Statistics (IBM SPSS Statistics for Mac, Version 28.0. Armonk, NY, USA). The Tukey's HSD test ($p = 0.05$) was used to compare the means and identify any significant variations between the samples (ANOVA).

3. Results and discussion

3.1. Bioactivity of cardoon blade extract and fiber content of the extraction residue

The antioxidant and antimicrobial properties of the extract from the mixture of cardoon blades and the fiber content of the extraction residue were investigated. The blades extract showed the ability to inhibit lipidic peroxidation, as evaluated by the TBARS assay, exhibiting an IC_{50} of 14 ± 1 μg/mL, a value close to the one of Trolox (IC_{50} = 9.1 ± 0.3 μg/mL) used as a reference antioxidant. In our previous study, the individual samples corresponding to each of the three maturation stages selected to prepare the extract used in this work exhibited different TBARS antioxidant values, with sample B3 showing the highest activity (IC_{50} 1.61 μg/mL), followed by B8 (11.6 μg/mL) and B13 (81.9 μg/mL) (Mandim et al., 2022). Therefore, the result obtained for the combination of the individual samples extract is not only close to the most active individual samples but also considerably increases that of the least active one. This enhancement could be associated with the polyphenolic profile obtained from the mixture and possible synergistic effects between the compounds present.

The results of the antimicrobial effects of the extract against bacterial strains and two fungi are shown in supplementary **Table S1**. The obtained extract exhibited higher efficiency against all bacterial and fungal strains tested than the individual samples (Mandim et al., 2022). Those values do not differ significantly ($p > 0.05$) from the ones obtained for the extract prepared from the mixture of the blades, with only slightly higher MFC values being obtained for the antifungal activity.

Cardoon blades are highly appreciated in traditional medicine for their high dietary fiber content. In this study, the cardoon blades residue resulting from the extraction methods contained 37.6 ± 0.1% of dietary fiber. This result is in the range of the values described by Escobar-Ledesma et al. (2020) for the fiber content of different plant tissues of the artichoke, a subspecies of *Cynara*, with contents of 13.07, 27.79, and 44.23 g/100 g dw of dietary fiber for receptacles, spikes, and bracts, respectively. The observed variability in fiber content among distinct plant parts could be associated with the intricate demands of plants for biosynthetic processes. Inulin, identified as the primary form of dietary fiber in cardoon, holds particular significance as an essential energy reserve (Escobar-Ledesma et al., 2020). A high fiber content in foods can reduce or delay the bioaccessibility of polyphenols in the upper digestive tract. Pacheco, González, Robert, and Parada (2018) suggest that the inclusion of inulin, which can be obtained from cardoon blades, in food products could contribute to increasing the availability of polyphenols in the lower digestive tract, with potential benefits for human health.

3.2. Smoothies' stability and quality attributes

3.2.1. Physicochemical parameters

The results obtained in the analyses of the physical parameters of the prepared smoothies, i.e., pH, viscosity, color (L^*, a^*, and b^* values), and TSS content, are shown in Table 1. The initial pH values for the smoothies without cardoon processed by conventional TP and HPP were 4.10 and 4.05, respectively; however, the fortification with cardoon blade extract decreased the pH value of both smoothies to 3.80. The processing method had a small impact on the pH during storage at 4 °C for 50 days of the smoothies, keeping them stable and exhibiting fluctuating values. Except for smoothie 4 (HPP processed with cardoon extract), for which the pH increased slightly during the storage period (from 3.80 to 4.10).

Regarding viscosity, the initial values were 72, 67, 79, and 91 MPa/s for smoothies 1, 2, 3, and 4, respectively. TP processing resulted in an increase in viscosity in smoothies 1 and 3, probably due to the chemical changes induced by heat, such as the breakdown of pectin and protein molecules and the gelatinization of starches (Hurtado et al., 2019). In contrast, for HPP processing viscosity was less affected, since this processing did not involve high temperatures, and the molecular structure of the ingredients would be less affected; thus, the smoothies exhibited a texture more similar to the fresh product. This particular processing method involves high pressures, which can interfere with cell structures and, for example, reduce the molecular mass of molecules such as carbohydrates and protein complexes, resulting in a less viscous product, as observed by Kumar et al. (2023) and Ribeiro et al. (2019). On the other hand, the fortification of smoothies 3 and 4 with cardoon increased viscosity, possibly due to a higher degree of fiber-water interactions that create a gelatinous matrix (Mehta, Kumar, & Sabikhi, 2017). It is to take into account that cardoon possessed high fiber content, but that this latter also contained natural sugars, which could also contribute to enhanced water retention and, therefore, to the increase in smoothies' viscosity (Mehta et al., 2017).

The CIE L^*, a^*, and b^* color parameters were analyzed to characterize the color of the prepared smoothies. The fortification with cardoon resulted in smoothies exhibiting a darker color, with lower L^* values and higher a^* and b^* values (Table 1). In all smoothies, an increase in a^* values was observed over time, suggesting a decrease in green tones and improved preservation of red coloration, particularly in the smoothies fortified with cardoon. However, b^* values did not change significantly over time. The results obtained are in line with those described by Fernández et al. (2018 and 2020) for fruit and vegetable smoothies processed by HPP at different pressures (between 350 and 650 MPa). Those authors suggested that the differences observed during storage were due to enzymatic or nonenzymatic processes, as well as the breakdown and polymerization of the major pigments (Fernández, Denoya, Agüero, Vaudagna, & Jagus, 2020). Changes in the color and

Table 1
Physicochemical attributes of the smoothie formulations during storage at 4 °C for 50 days (T0 to T50).

Samples		pH	Viscosity (MPa/s)	TSS (°Brix)	Color parameters		
					L^*	a^*	b^*
Smoothie 1	T0	$4.1 \pm 0.1^{\text{bcdefgh}}$	$72 \pm 4^{\text{mno}}$	$7.3 \pm 0.1^{\text{hi}}$	$50 \pm 1^{\text{cde}}$	$-3.4 \pm 0.2^{\text{s}}$	$29 \pm 2^{\text{ghij}}$
	T3	$4.06 \pm 0.02^{\text{cdefgh}}$	$86 \pm 2^{\text{ghijk}}$	$7.4 \pm 0.2^{\text{fghi}}$	$46 \pm 2^{\text{efgh}}$	$-2.2 \pm 0.1^{\text{q}}$	$25 \pm 1^{\text{lm}}$
	T7	$4.14 \pm 0.02^{\text{bcdefgh}}$	$98 \pm 4^{\text{cdef}}$	$7.2 \pm 0.1^{\text{hi}}$	$54 \pm 2^{\text{ab}}$	$-1.4 \pm 0.1^{\text{p}}$	$31 \pm 1^{\text{efgh}}$
	T10	$4.3 \pm 0.1^{\text{a}}$	$80 \pm 2^{\text{ijklmn}}$	$7.3 \pm 0.1^{\text{ghi}}$	$49.2 \pm 0.5^{\text{cde}}$	$-1.0 \pm 0.1^{\text{o}}$	$30 \pm 1^{\text{efghij}}$
	T14	$4.23 \pm 0.1^{\text{abc}}$	$100 \pm 7^{\text{bcde}}$	$7.2 \pm 0.1^{\text{i}}$	$42 \pm 1^{\text{ijk}}$	$-0.64 \pm 0.02^{\text{klmn}}$	$29 \pm 1^{\text{ghij}}$
	T22	$4.09 \pm 0.01^{\text{bcdefgh}}$	$89 \pm 9^{\text{efghij}}$	$7.2 \pm 0.1^{\text{i}}$	$49 \pm 2^{\text{cdefg}}$	$-0.52 \pm 0.01^{\text{jkl}}$	$30 \pm 1^{\text{efghij}}$
	T35	$4.17 \pm 0.01^{\text{abcdef}}$	$112 \pm 3^{\text{a}}$	$7.2 \pm 0.1^{\text{hi}}$	$49 \pm 3^{\text{cde}}$	$-0.52 \pm 0.01^{\text{jkl}}$	$27 \pm 1^{\text{jkl}}$
	T50	$4.13 \pm 0.01^{\text{bcdefgh}}$	$79 \pm 1^{\text{jklmn}}$	$7.2 \pm 0.3^{\text{i}}$	$55 \pm 1^{\text{a}}$	$-0.34 \pm 0.02^{\text{jk}}$	$28.1 \pm 0.2^{\text{ijk}}$
Smoothie 2	T0	$4.05 \pm 0.04^{\text{cdefgh}}$	$67 \pm 1^{\text{o}}$	$6.6 \pm 0.1^{\text{k}}$	$42 \pm 2^{\text{ijk}}$	$-3.8 \pm 0.1^{\text{t}}$	$29 \pm 2^{\text{fghij}}$
	T3	$4.19 \pm 0.04^{\text{abcde}}$	$71 \pm 1^{\text{no}}$	$6.6 \pm 0.1^{\text{jk}}$	$52 \pm 1^{\text{bc}}$	$-5.3 \pm 0.2^{\text{u}}$	$37 \pm 3^{\text{a}}$
	T7	$4.0 \pm 0.1^{\text{cdefgh}}$	$97 \pm 6^{\text{cdefg}}$	$6.9 \pm 0.3^{\text{j}}$	$49 \pm 1^{\text{cdef}}$	$-3.8 \pm 0.3^{\text{t}}$	$35 \pm 1^{\text{ab}}$
	T10	$4.2 \pm 0.1^{\text{abcdef}}$	$101 \pm 7^{\text{bcd}}$	$6.8 \pm 0.1^{\text{jk}}$	$47.9 \pm 0.4^{\text{cdefg}}$	$-2.5 \pm 0.1^{\text{r}}$	$32 \pm 2^{\text{cdef}}$
	T14	$4.1 \pm 0.1^{\text{bcdefgh}}$	$74 \pm 2^{\text{lmno}}$	$6.7 \pm 0.1^{\text{jk}}$	$43 \pm 2^{\text{hij}}$	$-2.1 \pm 0.1^{\text{q}}$	$31 \pm 1^{\text{efghi}}$
	T22	$3.98 \pm 0.02^{\text{efghi}}$	$66 \pm 2^{\text{o}}$	$6.6 \pm 0.1^{\text{jk}}$	$38 \pm 2^{\text{kl}}$	$-1.0 \pm 0.1^{\text{o}}$	$25 \pm 1^{\text{lm}}$
	T35	$4.09 \pm 0.05^{\text{bcdefgh}}$	$66 \pm 5^{\text{o}}$	$6.7 \pm 0.1^{\text{jk}}$	$40 \pm 3^{\text{jk}}$	$-0.29 \pm 0.02^{\text{j}}$	$24 \pm 1^{\text{lm}}$
	T50	$3.9 \pm 0.1^{\text{hij}}$	$86 \pm 8^{\text{ghijk}}$	$6.8 \pm 0.1^{\text{jk}}$	$50 \pm 4^{\text{cd}}$	$-0.9 \pm 0.1^{\text{no}}$	$29 \pm 1^{\text{fghij}}$
Smoothie 3	T0	$3.8 \pm 0.2^{\text{ij}}$	$79 \pm 6^{\text{jklmn}}$	$7.4 \pm 0.2^{\text{efghi}}$	$39.3 \pm 0.4^{\text{jk}}$	$-0.84 \pm 0.03^{\text{mno}}$	$31 \pm 3^{\text{efghij}}$
	T3	$4.07 \pm 0.03^{\text{bcdefgh}}$	$83 \pm 2^{\text{hijklm}}$	$7.7 \pm 0.1^{\text{abc}}$	$48 \pm 3^{\text{cdefg}}$	$-0.71 \pm 0.02\ 1^{\text{mno}}$	$32 \pm 1^{\text{efgh}}$
	T7	$4.0 \pm 0.1^{\text{fghi}}$	$86 \pm 7^{\text{hijk}}$	$7.7 \pm 0.1^{\text{abc}}$	$42 \pm 1^{\text{ijk}}$	$-0.55 \pm 0.03^{\text{jklm}}$	$32 \pm 2^{\text{defg}}$
	T10	$4.16 \pm 0.05^{\text{abcdefg}}$	$91 \pm 8^{\text{defghi}}$	$7.7 \pm 0.1^{\text{abc}}$	$47 \pm 2^{\text{defgh}}$	$-0.90 \pm 0.04^{\text{no}}$	$33 \pm 3^{\text{bcde}}$
	T14	$4.1 \pm 0.1^{\text{bcdefgh}}$	$110 \pm 5^{\text{ab}}$	$7.4 \pm 0.1^{\text{defgh}}$	$47 \pm 1^{\text{defg}}$	$0.011 \pm 0.001^{\text{i}}$	$35 \pm 2^{\text{abc}}$
	T22	$4.0 \pm 0.2^{\text{defghi}}$	$106 \pm 8^{\text{abc}}$	$7.6 \pm 0.1^{\text{abcd}}$	$45 \pm 3^{\text{ghi}}$	$0.9 \pm 0.1^{\text{g}}$	$31 \pm 2^{\text{efgh}}$
	T35	$4.1 \pm 0.1^{\text{bcdefgh}}$	$108 \pm 8^{\text{abc}}$	$7.8 \pm 0.1^{\text{a}}$	$39 \pm 2^{\text{jk}}$	$1.8 \pm 0.2^{\text{ef}}$	$28 \pm 1^{\text{jkl}}$
	T50	$3.95 \pm 0.02^{\text{ghij}}$	$93 \pm 5^{\text{defgh}}$	$7.8 \pm 0.1^{\text{ab}}$	$45 \pm 4^{\text{fghi}}$	$2.0 \pm 0.2^{\text{e}}$	$31.4 \pm 0.2^{\text{efgh}}$
Smoothie 4	T0	$3.8 \pm 0.1^{\text{j}}$	$91 \pm 9^{\text{defghi}}$	$7.5 \pm 0.1^{\text{cdef}}$	$35 \pm 1^{\text{lm}}$	$-0.75 \pm 0.03^{\text{lmno}}$	$25 \pm 1^{\text{lm}}$
	T3	$4.2 \pm 0.1^{\text{abcdef}}$	$85 \pm 1^{\text{hijkl}}$	$7.7 \pm 0.1^{\text{abc}}$	$41 \pm 2^{\text{ijk}}$	$0.44 \pm 0.04^{\text{h}}$	$35 \pm 1^{\text{abcd}}$
	T7	$4.2 \pm 0.1^{\text{abcdef}}$	$86 \pm 5^{\text{ghijk}}$	$7.6 \pm 0.1^{\text{abcd}}$	$34.6 \pm 0.3^{\text{m}}$	$1.7 \pm 0.1^{\text{f}}$	$29 \pm 1^{\text{hij}}$
	T10	$4.3 \pm 0.1^{\text{ab}}$	$89 \pm 3^{\text{efghij}}$	$7.6 \pm 0.2^{\text{abcde}}$	$41 \pm 1^{\text{ijk}}$	$2.7 \pm 0.1^{\text{d}}$	$25 \pm 1^{\text{klm}}$
	T14	$4.2 \pm 0.1^{\text{abcd}}$	$101 \pm 2^{\text{bcd}}$	$7.5 \pm 0.1^{\text{cdefg}}$	$34.5 \pm 0.2^{\text{m}}$	$3.5 \pm 0.1^{\text{c}}$	$28 \pm 1^{\text{ijkl}}$
	T22	$4.0 \pm 0.1^{\text{cdefgh}}$	$76 \pm 1^{\text{klmno}}$	$7.6 \pm 0.1^{\text{bcde}}$	$40 \pm 2^{\text{jk}}$	$5.2 \pm 0.2^{\text{b}}$	$36 \pm 2^{\text{ab}}$
	T35	$4.2 \pm 0.1^{\text{abcdef}}$	$82 \pm 8^{\text{hijklmn}}$	$7.7 \pm 0.1^{\text{abc}}$	$46 \pm 1^{\text{efgh}}$	$6 \pm 1^{\text{a}}$	$37.6 \pm 0.5^{\text{a}}$
	T50	$4.1 \pm 0.2^{\text{bcdefgh}}$	$89 \pm 8^{\text{fghij}}$	$7.7 \pm 0.2^{\text{abc}}$	$40 \pm 1^{\text{jk}}$	$5.4 \pm 0.3^{\text{b}}$	$33 \pm 2^{\text{bcde}}$

Results are presented as mean ± SD ($n = 3$). Different letters in the same column correspond to significant differences ($p < 0.05$) between samples. Smoothie 1 – pasteurized formulation; Smoothie 2 – formulation subjected to high-pressure processing; Smoothie 3 – pasteurized formulation with cardoon; Smoothie 4 – formulation with cardoon subjected to high-pressure processing.

viscosity of smoothies influence consumers' visual appeal, potentially affecting their acceptability. In future studies, it will be important to optimize formulation, processing, and storage conditions, with the simultaneous development of sensory analysis studies to gain deeper insights into consumer perceptions of the beverage.

The initial TSS content varied among smoothies, with T0 values ranging between 6.6°Brix for smoothie 2 and 7.5°Brix for smoothie 4. Smoothies 3 and 4 had the highest °Brix values, which can be explained by the enrichment with cardoon blades. Moreover, some of the observed differences may also be explained by the ability that thermal treatment has to enhance TSS extraction (Castillejo, Martínez-Hernández, Gómez, Artés, & Artés-Hernández, 2016). In general, the TSS content did not change significantly over the 50 days of the shelf-life, except in smoothie 3 where a significant increase from 7.4 to 7.8°Brix was recorded over the storage time. Similar results were reported by Castillejo et al. (2016), who observed that TSS content did not differ in vegetable smoothies subjected to thermal processing and stored at 5 and 20 °C for 21 days.

3.2.2. Nutritional composition

The proximate constituents and energy value of the prepared smoothie formulations are presented in Table 2, and the free sugar and fatty acid composition are shown in Table 3.

No significant changes were observed in the caloric value of the fruit/vegetable-based smoothies fortified with cardoon blade ingredients, regardless of the processing technology employed for pasteurization. It remained consistent, ranging between 23 and 35 kcal/100 mL. The unchanged caloric content of smoothies aligns with the growing of low-calorie diets in recent times, gained in recent times, potentially improving their acceptability among consumers.

Carbohydrates were the most abundant macronutrients in all smoothies' formulations (between 5.2 and 7.9 g/100 mL), followed by proteins (0.28 to 0.50 g/100 mL), fat (0.014 to 0.113 g/100 mL), and ash (0.0026 to 0.0058 g/100 mL). Although smoothie 4 (enriched with cardoon blades and processed by HPP) exhibited a higher fiber content (0.68 g/100 mL at day 0), non-significant differences ($p < 0.05$) were noted with the other smoothies' preparations, with levels ranging between 0.51 and 0.66 g/100 mL. However, while the fiber content in the smoothies enriched with cardoon remained constant over the shelf-life, the formulations without cardoon exhibited a significant decrease, with a reduction of around 24% and 16% from day 0 to 50, for smoothies 1 and 2, respectively (Table 2). It has been reported that long storage periods can disintegrate the fibers, reducing their ability to provide nutritional benefits, such as reducing blood cholesterol levels and the incidence of heart disease, diabetes, colon cancer, or obesity (Wang et al., 2021). The addition of cardoon blades seems to help maintain fiber integrity, possibly owing to its potential functional properties and its capacity to protect the fibers from degradation caused by phenomena such as oxidation. Studies on fiber degradation and the protective effect that natural extracts have been scarce. Exploring this subject and understanding the possible associated mechanisms would be an interesting topic for future research.

In the two preparations without cardoon, the protein contents

Table 2
Proximate composition and energetic value of the smoothie formulations during storage at 4 °C for 50 days (T0 to T50).

Samples		Proximate constituents (g/100 mL)						Energy (kcal/100 mL)
		Moisture	Ash	Proteins	Fat	Dietary fiber	Carbohydrates	
Smoothie 1	T0	92.8 ± 0.3^{bcde}	0.049 ± 0.003^{bc}	0.34 ± 0.02^{efgh}	0.045 ± 0.004^{g}	0.66 ± 0.02^{bc}	6.1 ± 0.2^{bcdef}	28 ± 1^{cdefg}
	T10	92 ± 1^{cdef}	0.044 ± 0.004^{cde}	0.335 ± 0.002^{fghi}	0.109 ± 0.003^{b}	0.55 ± 0.01^{ef}	6.5 ± 0.5^{bcde}	29 ± 3^{bcde}
	T22	93 ± 1^{abc}	0.039 ± 0.004^{ef}	0.28 ± 0.01^{i}	0.021 ± 0.002^{k}	0.43 ± 0.01^{i}	5.8 ± 0.5^{defg}	25 ± 2^{fgh}
	T35	92.2 ± 0.3^{def}	0.053 ± 0.004^{ab}	0.371 ± 0.002^{efg}	0.036 ± 0.002^{i}	0.5 ± 0.1^{fghi}	6.9 ± 0.2^{b}	30 ± 1^{bcd}
	T50	92 ± 1^{def}	0.053 ± 0.005^{ab}	0.33 ± 0.01^{ghi}	0.034 ± 0.003^{i}	0.5 ± 0.1^{efg}	7 ± 1^{b}	30 ± 2^{bcd}
Smoothie 2	T0	93 ± 1^{ab}	0.048 ± 0.002^{bcd}	0.29 ± 0.01^{hi}	0.026 ± 0.002^{j}	0.51 ± 0.01^{efgh}	$5.3 \pm 0.1f^{g}$	24 ± 1^{h}
	T10	93.3 ± 0.3^{abcd}	0.026 ± 0.002^{g}	0.31 ± 0.02^{hi}	0.104 ± 0.003^{c}	0.55 ± 0.01^{ef}	5.7 ± 0.2^{efg}	26 ± 1^{efgh}
	T22	94.0 ± 0.3^{a}	0.0371 ± 0.0002^{f}	0.50 ± 0.05^{ab}	0.053 ± 0.002^{f}	0.45 ± 0.02^{ghi}	5.2 ± 0.3^{g}	23 ± 1^{h}
	T35	93.1 ± 0.4^{abcde}	0.044 ± 0.001^{cde}	0.37 ± 0.03^{efg}	0.028 ± 0.002^{j}	0.44 ± 0.05^{ghi}	6.0 ± 0.2^{cdefg}	27 ± 1^{defgh}
	T50	93 ± 1^{abc}	0.043 ± 0.002^{cdef}	0.31 ± 0.01^{hi}	0.014 ± 0.001^{l}	0.43 ± 0.01^{hi}	5.9 ± 0.5^{defg}	26 ± 2^{fgh}
Smoothie 3	T0	93.6 ± 0.3^{abc}	0.042 ± 0.001^{cdef}	0.33 ± 0.02^{fghi}	0.040 ± 0.002^{h}	0.570 ± 0.003^{de}	5.4 ± 0.3^{fg}	25 ± 1^{gh}
	T10	93.9 ± 0.1^{a}	0.044 ± 0.002^{cde}	0.30 ± 0.02^{hi}	0.026 ± 0.002^{j}	0.46 ± 0.02^{ghi}	5.2 ± 0.1^{g}	23.3 ± 0.2^{h}
	T22	90.8 ± 0.3^{g}	0.0414 ± 0.0002^{def}	0.45 ± 0.04^{bc}	0.055 ± 0.001^{f}	0.7 ± 0.1^{ab}	7.9 ± 0.3^{a}	35 ± 1^{a}
	T35	93.5 ± 0.5^{ab}	0.042 ± 0.002^{def}	0.30 ± 0.02^{hi}	0.033 ± 0.001^{i}	0.46 ± 0.02^{ghi}	5.6 ± 0.4^{efg}	25 ± 2^{gh}
	T50	92 ± 1^{fg}	0.054 ± 0.004^{ab}	0.43 ± 0.02^{cd}	0.077 ± 0.002^{d}	0.79 ± 0.01^{a}	6.9 ± 0.5^{b}	32 ± 2^{b}
Smoothie 4	T0	93.1 ± 0.2^{abcde}	0.045 ± 0.001^{cde}	0.46 ± 0.01^{bc}	0.113 ± 0.003^{a}	0.68 ± 0.01^{bc}	5.7 ± 0.1^{efg}	27 ± 1^{defgh}
	T10	92 ± 1^{fg}	0.056 ± 0.005^{a}	0.54 ± 0.05^{a}	0.036 ± 0.002^{i}	0.7 ± 0.1^{bc}	7 ± 1^{b}	31 ± 3^{bc}
	T22	92 ± 1^{cdef}	0.058 ± 0.005^{a}	0.39 ± 0.03^{de}	0.033 ± 0.002^{i}	0.6 ± 0.1^{cd}	6.4 ± 0.4^{bcde}	29 ± 2^{bcdef}
	T35	92.1 ± 0.2^{ef}	0.053 ± 0.003^{ab}	0.39 ± 0.04^{de}	0.035 ± 0.002^{i}	0.64 ± 0.02^{cd}	6.8 ± 0.2^{bc}	30 ± 1^{bcd}
	T50	92.2 ± 0.4^{cdef}	0.052 ± 0.004^{ab}	0.38 ± 0.03^{def}	0.059 ± 0.001^{e}	0.66 ± 0.04^{c}	6.6 ± 0.3^{bcd}	30 ± 2^{bcde}

Results are presented as mean ± SD ($n = 3$). Different letters in the same column correspond to significant differences ($p < 0.05$) between samples. Smoothie 1 – pasteurized formulation; Smoothie 2 – formulation subjected to high-pressure processing; Smoothie 3 – pasteurized formulation with cardoon; Smoothie 4 – formulation with cardoon subjected to high-pressure processing.

(Table 2) remained stable with oscillations over the storage period, while the opposite behavior was observed in the smoothies enriched with cardoon. Thus, in the smoothie 4, the one with the higher initial protein content (0.46 g/100 mL), a decrease was observed throughout the storage (0.38 g/100 mL, at T50); by contrast, a protein increase was produced in the smoothie 3 (from 0.33 to 0.43 g/100 mL, at T0 and T50, respectively). Quite possibly these fluctuations may be rather explained by the precision of the analytical method at low protein contents than by the existence of real changes. Similar observations can be made regarding the low levels of the lipidic fraction. The addition of cardoon blades did not significantly influence the carbohydrate content of the smoothies, which ranged between 5.2 and 7.9 g/100 mL. However, prolonged storage resulted in a decrease in total carbohydrate content in most of the preparations, except for smoothie 2, where no significant changes were produced.

A total of 17 fatty acids were identified in the studied fruit/vegetable-based smoothies. Table 3 summarizes the fatty acids detected with the highest relative percentages, as well as the saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acid content; the remaining are shown in Supplementary Material (**Table S1**). Saturated fatty acids (SFA) were the most representative fatty acid class in all cases, with relative percentages ranging from 78 to 94.1%, followed by polyunsaturated fatty acids (PUFA; from 1.66 to 17%) and monounsaturated fatty acids (MUFA; 1.36 to 6.4%). Palmitic (C16:0) and stearic (C18:0) acids were the most abundant ones in all the samples, with percentages between 46 and 57.5% and 10 and 27.2%, respectively.

Regarding the effect of the processing method and fortification with cardoon blades, a varied response was recorded. For example, HPP processing increased the stearic acid level compared to TP, in both enriched and non-enriched formulations, while fortification resulted in an increase in stearic acid in both processing methods. On the other hand, HPP increased the palmitic acid content in smoothies fortified with cardoon, while no significant differences ($p > 0.05$) were recorded between processing treatments for non-fortified formulations. Similar behavior was described by Tsai, Cheng, Chen, and Wang (2018) that noted no significant difference in the fatty acid composition of hazelnut milk processed by HPP and TP. Also, Salvia-Trujillo, Morales-de la Peña, Rojas-Graü, Welti-Chanes, and Martín-Belloso (2017) found that the fatty acid profile of fruit drinks was not affected by high-intensity pulse electric field thermal processing, although the content of individual compounds may vary depending on the stage of fruit ripeness and the addition of fortifying agents.

Fructose, glucose, and sucrose were the simple sugar molecules detected in the prepared smoothie formulations (Table 3). The processing methods used and the fortification with the cardoon extract influenced the sugar composition. An increase in glucose and fructose content was verified in HPP-processed smoothies (smoothies 2 and 4), whereas sucrose was absent in these formulations. Sucrose was only detected in smoothies processed by TP (smoothies 1 and 3) and was the most abundant sugar in both formulations, with a significant increase being recorded for the fortified smoothie (from 3.81 to 5.42 g/mL for smoothies 1 and 3, respectively). The absence of sucrose in the smoothies processed by HPP suggests that total hydrolysis of sucrose occurred and, therefore, that β-fructosidase enzymes were active. This result indicates that enzymatic activity was not effectively inactivated under high pressure conditions, unlike the smoothies processed by the conventional heat treatment (90 °C for 30 s) where sucrose was the most abundant detected sugar. Sucrose inversion has already been described in smoothies processed by HPP in several studies. For example, Picouet et al. (2016) described similar results with a drastic decrease in sucrose content between day 0 and day 7 of storage in a multi-fruit smoothie. Hurtado et al. (2015) also described significantly lower sucrose concentrations in smoothies based on apple and orange juice, apple, banana, and strawberry subjected to HPP. High hydrostatic pressure, an emerging nonthermal pasteurization method, requires careful optimization of processing conditions tailored to diverse food matrices to effectively inactivate food-borne pathogens and detoxify enzymes (Artés-Hernández et al., 2021).

The smoothies fortified with cardoon blades exhibited a slightly higher total sugar content than smoothies without cardoon, although the contents of individual free sugars showed a varied response depending

Table 3
Composition of free sugars and major fatty acids of the smoothie formulations during storage at 4 °C for 50 days (T0 to T50).

Samples		Free sugars (g/100 mL)				Fatty acids (relative percentage, %)						
		Fructose	Glucose	Sucrose	Σ Free sugars	C16:0	C18:0	C18:3n3	C24:0	SFA	MUFA	PUFA
Smoothie 1	T0	1.51 ± 0.04jk	2.0 ± 0.1 fg	3.81 ± 0.03 cd	7.3 ± 0.1efg	53 ± 2cde	16 ± 1 h	3.7 ± 0.2 g	5.9 ± 0.5bc	93 ± 4b	3.3 ± 0.3j	3.7 ± 0.2 g
	T10	1.6 ± 0.1ij	1.9 ± 0.1 g	3.5 ± 0.1e	6.92 ± 0.05 h	53 ± 1 cdef	27.2 ± 0.2a	1.66 ± 0.01i	1.0 ± 0.1j	92 ± 1 cd	6.4 ± 0.2a	1.66 ± 0.01i
	T22	1.71 ± 0.03 h	1.99 ± 0.02 fg	3.91 ± 0.05c	7.6 ± 0.1bcd	46 ± 1 k	15.75 ± 0.26 h	3.7 ± 0.3 g	5.5 ± 0.3 cd	91 ± 2fgh	5.4 ± 0.1c	3.7 ± 0.3 g
	T35	1.7 ± 0.1 hi	1.9 ± 0.1 g	3.79 ± 0.05d	7.4 ± 0.1def	52.95 ± 0.05cdef	19.09 ± 0.03de	4.31 ± 0.01f	4.0 ± 0.1f	91 ± 1 fg	4.7 ± 0.1f	4.31 ± 0.01f
	T50	1.6 ± 0.1ij	2.05 ± 0.03f	3.5 ± 0.1e	7.2 ± 0.1 fgh	53.5 ± 0.4 cd	22.1 ± 0.4c	4.3 ± 0.2f	2.4 ± 0.2 h	90 ± 1 h	5.3 ± 0.1d	4.3 ± 0.2f
Smoothie 2	T0	2.6 ± 0.1f	3.76 ± 0.04d	n.d.	6.35 ± 0.04ij	52 ± 2cdef	20 ± 1d	3.7 ± 0.3 g	5.1 ± 0.5de	92 ± 4de	4.7 ± 0.1f	3.7 ± 0.3 g
	T10	3.0 ± 0.02 cd	3.4 ± 0.1e	n.d.	6.4 ± 0.1i	57.5 ± 0.1a	27.08 ± 0.04a	1.871 ± 0.004i	1.00 ± 0.02j	94.1 ± 0.3a	4.00 ± 0.03i	1.871 ± 0.004i
	T22	2.82 ± 0.04e	3.5 ± 0.1e	n.d.	6.3 ± 0.1ij	48 ± 3ijkk	18 ± 1efg	3.5 ± 0.3 g	5.1 ± 0.1de	92 ± 5 cd	4.5 ± 0.3 g	3.5 ± 0.3 g
	T35	2.60 ± 0.05f	3.5 ± 0.1e	n.d.	6.1 ± 0.1jk	51.0 ± 0.3efgh	18 ± 1 fg	7.2 ± 0.1c	1.7 ± 0.2i	88 ± 3 k	4.8 ± 0.2f	7.2 ± 0.1c
	T50	2.41 ± 0.04 g	3.6 ± 0.1e	n.d.	6.0 ± 0.1 k	53.6 ± 0.3 cd	11.9 ± 0.2i	6.9 ± 0.1c	6.6 ± 0.1a	89 ± 2j	4.3 ± 0.1 h	6.9 ± 0.1c
Smoothie 3	T0	1.23 ± 0.04 l	1.09 ± 0.03 h	5.42 ± 0.03b	7.7 ± 0.1b	51 ± 2fgh	22 ± 1c	4.6 ± 0.4f	3.0 ± 0.3 g	92 ± 4c	3.3 ± 0.2j	4.6 ± 0.4f
	T10	1.4 ± 0.1 k	1.2 ± 0.1 h	5.7 ± 0.1a	8.3 ± 0.2a	46.6 ± 0.4jk	18.3 ± 0.5ef	11.8 ± 0.1b	3.81 ± 0.02f	83 ± 2 m	5.1 ± 0.1e	11.8 ± 0.1b
	T22	1.19 ± 0.02 l	1.20 ± 0.03 h	5.4 ± 0.2b	7.8 ± 0.2b	47 ± 1jk	22.8 ± 0.5c	5.11 ± 0.04e	3.1 ± 0.1 g	90 ± 2i	5.4 ± 0.1 cd	5.11 ± 0.04e
	T35	1.2 ± 0.1 l	1.1 ± 0.1 h	5.4 ± 0.1b	7.7 ± 0.1bc	49.6 ± 0.2 ghi	16.7 ± 0.1 gh	11.7 ± 0.1b	4.0 ± 0.4f	83 ± 1 m	5.0 ± 0.1e	11.7 ± 0.1b
	T50	1.20 ± 0.05 l	1.2 ± 0.1 h	5.5 ± 0.2b	7.9 ± 0.3b	52 ± 1def	25 ± 1b	6.9 ± 0.1c	1.8 ± 0.2i	87 ± 3 l	6.2 ± 0.2b	6.9 ± 0.1c
Smoothie 4	T0	3.2 ± 0.1ab	4.3 ± 0.1b	n.d.	7.5 ± 0.1 cde	55 ± 2bc	26 ± 1ab	2.9 ± 0.1 h	1.6 ± 0.1i	93 ± 4b	4.5 ± 0.2 g	2.9 ± 0.1 h
	T10	3.2 ± 0.1a	4.1 ± 0.1bc	n.d.	7.4 ± 0.1defg	46.08 ± 0.02 k	10 ± 1j	17 ± 1a	6.29 ± 0.05ab	78 ± 1n	4.4 ± 0.2 gh	17 ± 1a
	T22	3.11 ± 0.04abc	4.56 ± 0.04a	n.d.	7.68 ± 0.01bc	52 ± 1defg	13.15 ± 0.02i	5.0 ± 0.2e	6.5 ± 0.5a	91 ± 2gh	4.2 ± 0.1 h	5.0 ± 0.2e
	T35	3.1 ± 0.1bc	4.2 ± 0.1bc	n.d.	7.3 ± 0.2efg	49 ± 2hij	20 ± 1d	6.9 ± 0.3c	4.7 ± 0.4e	91 ± 5ef	1.8 ± 0.1 k	6.9 ± 0.3c
	T50	3.0 ± 0.1d	4.1 ± 0.1c	n.d.	7.1 ± 0.2 gh	56.0 ± 0.1ab	25.3 ± 0.2b	5.56 ± 0.04d	2.1 ± 0.1 hi	93 ± 1b	1.36 ± 0.01 l	5.56 ± 0.04d

Results are presented as mean ± SD ($n = 3$). Each fatty acid is presented as relative percentages. Different letters in the same column correspond to significant differences ($p < 0.05$) between samples. n.d. – not detected; Smoothie 1 – pasteurized formulation; Smoothie 2 – formulation subjected to high-pressure processing; Smoothie 3 – pasteurized formulation with cardoon; Smoothie 4 – formulation with cardoon subjected to high-pressure processing. C14:0—myristic acid; C16:0—palmitic acid; C18:0—stearic acid; C18:3n3—linolenic acid; C24:0—lignoceric acid; SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids.

on the processing method. Thus, in the case of HPP processing formulations, the addition of the cardoon extract led to an increase in the levels of glucose and fructose with no detection of sucrose; however, in thermally processed smoothies only the sucrose content increased, confirming the inefficient inactivation of enzymatic activity in HPP processed products, as discussed above. Additionally, in TP formulations (smoothies 1 and 3) the free sugar content remained stable over the storage time. By contrast, the prolonged storage led to a slight decrease in the sugar content in HPP processing smoothies (smoothies 2 and 4), suggesting possible residual glycosidase activity.

3.2.3. Phenolic composition

The smoothie formulations were also characterized for their composition in extractable phenolic compounds. **Table S5** presents the chromatographic data used in the tentative identification of the detected compounds and Table 4 shows the quantitative results of the tentatively identified phenolic compounds.

Seven compounds were tentatively identified in the studied smoothies, including six phenolic acids (compounds **1** to **6**) and one flavonoid (compound **7**). Compound **1** was tentatively identified as caffeic acid hexoside, previously detected in globe artichoke blades (Ramos et al., 2014). Compound **2** corresponded to caftaric acid, previously identified in leaves of Asteraceae plants (Jaiswal, Kiprotich, & Kuhnert, 2011). Compound **3** was tentatively identified as galloylglucose (Sandhu & Gu, 2010). Compounds **4** and **5** were assigned as *cis* and *trans* 5-*O*-caffeoylquinic acid, respectively, previously identified in cardoon blades and found to be most abundant in the maturity stages selected for this work (Mandim et al., 2022). Compound **6** was coherent with 4-*O*-*p*-coumaroylquinic acid (Del Rio et al., 2004). Finally, compound **7** was tentatively identified as patuletin-3-*O*-β-D-(2-feruloylglucopyranosyl)-(1 → 6)-[β-D-apiofuranosyl-(1 → 2)]-β-D-glucopyranoside, a flavonoid typically found in spinach, one of the ingredients in the smoothie (Pereira et al., 2019).

The reduced number of phenolic compounds detected in the

Table 4
Content of phenolic compounds in the smoothie formulations during storage at 4 °C for 50 days (T0 to T50).

Samples		Contents (mg/100 mL) obtained using the calibration curves presented in supplementary Table S5.								
		Caffeic acid hexoside	Caftaric acid	Galloylglucose	*cis* 5-*O*-Caffeoylquinic acid	*trans* 5-*O*-Caffeoylquinic acid	4-*O*-*p*-Coumaroylquinic acid	Patuletin-3-*O*-β-d-(2-feruloylglucopyranosyl)-(1 → 6)-[β-D-apiofuranosyl-(1 → 2)]-β-D-glucopyranoside	Σ Phenolic acids	Σ Phenolics
Smoothie 1	T0	20.3 ± 00.1a	1.27 ± 0.03c	31.8 ± 0.3a	n.d.	n.d.	12 ± 1b	7.78 ± 0.01c	65 ± 1c	73 ± 1c
	T10	0.328 ± 0.001 h	tr	18.14 ± 0.02i	n.d.	n.d.	n.d.	6.861 ± 0.002 g	18.46 ± 0.02 l	25.32 ± 0.02j
	T22	0.27 ± 0.02 hi	tr	14.90 ± 0.02n	n.d.	n.d.	n.d.	n.d.	15.17 ± 0.01n	15.17 ± 0.01o
	T35	0.066 ± 0.005ij	tr	18.84 ± 0.01f	n.d.	n.d.	n.d.	n.d.	18.91 ± 0.01 l	18.91 ± 0.01 l
	T50	tr	tr	18.66 ± 0.01 fg	n.d.	n.d.	n.d.	n.d.	18.66 ± 0.01 l	18.66 ± 0.01 l
Smoothie 2	T0	11.6 ± 0.2d	0.51 ± 0.01d	26.7 ± 0.3c	n.d.	n.d.	8.6 ± 0.1d	6.53 ± 0.05 h	47.4 ± 0.4d	53.9 ± 0.4e
	T10	0.10 ± 0.01ij	tr	16.54 ± 0.02 k	n.d.	n.d.	n.d.	n.d.	16.65 ± 0.02 m	16.65 ± 0.02 m
	T22	tr	tr	15.793 ± 0.004 m	n.d.	n.d.	n.d.	n.d.	15.793 ± 0.004 mn	15.793 ± 0.004no
	T35	tr	tr	16.23 ± 0.01 l	n.d.	n.d.	n.d.	n.d.	16.23 ± 0.01 m	16.23 ± 0.01 mn
	T50	tr	tr	16.01 ± 0.01 lm	n.d.	n.d.	n.d.	n.d.	16.01 ± 0.01 mn	16.01 ± 0.01 mn
Smoothie 3	T0	14.4 ± 0.4c	1.70 ± 0.02b	17.8 ± 0.3j	85 ± 1a	11 ± 1b	11.5 ± 0.1c	7.3 ± 0.2e	141 ± 2a	149 ± 2a
	T10	0.87 ± 0.03f	tr	14.9 ± 0.1n	11.35 ± 0.01d	1.49 ± 0.04e	0.50 ± 0.01e	5.656 ± 0.002j	29.15 ± 0.05 g	34.81 ± 0.05 g
	T22	1.46 ± 0.03e	tr	23.86 ± 0.01d	18.0 ± 0.2c	2.318 ± 0.004c	0.68 ± 0.01e	8.90 ± 0.03a	46.4 ± 0.2e	55.3 ± 0.2d
	T35	0.46 ± 0.04 gh	tr	15.80 ± 0.01 m	8.6 ± 0.1e	1.445 ± 0.003e	n.d.	6.05 ± 0.02i	26.33 ± 0.01 h	32.38 ± 0.01 h
	T50	0.63 ± 0.02 g	tr	21.2 ± 0.1e	11.4 ± 0.3d	2.03 ± 0.01d	n.d.	8.07 ± 0.02b	35.3 ± 0.2f	43.3 ± 0.2f
Smoothie 4	T0	19.0 ± 0.2b	2.75 ± 0.01a	29 ± 1b	65 ± 1b	12.03 ± 0.03a	12.7 ± 0.3a	7.644 ± 0.001d	140 ± 1b	147 ± 1b
	T10	0.48 ± 0.03gh	tr	18.58 ± 0.01 fg	3.86 ± 0.01f	1.76 ± 0.02de	tr	7.15 ± 0.01f	24.67 ± 0.04i	31.82 ± 0.02 h
	T22	0.054 ± 0.005ij	tr	17.55 ± 0.01j	2.34 ± 0.02 h	n.d.	0.36 ± 0.01ef	6.802 ± 0.003 g	20.307 ± 0.001 k	27.108 ± 0.003i
	T35	tr	tr	18.45 ± 0.03gh	2.98 ± 0.02 g	1.6 ± 0.1e	n.d.	n.d.	23.08 ± 0.04j	23.08 ± 0.04 k
	T50	tr	tr	18.25 ± 0.01 hi	3.14 ± 0.05 g	1.71 ± 0.04e	n.d.	n.d.	23.10 ± 0.02j	23.10 ± 0.02 k

Results are presented as mean ± standard deviation ($n = 3$). Different letters in the same column correspond to significant differences ($p < 0.05$) between samples. n.d. – not detected; tr – traces. Smoothie 1 – pasteurized formulation; Smoothie 2 – formulation subjected to high-pressure processing; Smoothie 3 – pasteurized formulation with cardoon; Smoothie 4 – formulation with cardoon subjected to high-pressure processing. Compound **1** - caffeic acid hexoside; Compound **2** - caftaric acid; Compound **3** – galloylglucose; Compound **4** - *cis* 5-*O*-caffeoylquinic acid; Compound **5** - *trans* 5-*O*-caffeoylquinic acid; Compound **6**–4-*O*-*p*-coumaroylquinic acid; Compound **7** - patuletin-3-*O*-β-d-(2-feruloylglucopyranosyl)-(1 → 6)-[β-D-apiofuranosyl-(1 → 2)]-β-D-glucopyranoside. The chromatographic data used in the tentative identification of phenolic compounds are presented in **supplementary Table S4.**

smoothie samples may be related to the sample preparation method. In this study, the smoothies were directly filtered for compound analysis (section 2.6.5.), without preparing an extract (which is generally very concentrated in this type of compound), nor using an extraction intensification factor, such as temperature, stirring rate, or ultrasound, generally used in solid-liquid extractions. Therefore, it was not possible to extract and detect compounds trapped within cellular compartments or linked to other constituents such as fibers.

From the seven phenolic compounds identified, five were detected in the smoothies without cardoon, while the presence of 5-*O*-caffeoylquinic acid isoforms (compounds **4** and **5**) was only found in the preparations containing cardoon. These compounds had been previously identified as the most abundant phenolics in cardoon and artichoke by-products (Ferioli & D'Antuono, 2022; Mandim et al., 2022; Pandino, Bonomo, Scavo, Mauromicale, & Lombardo, 2022). Thus, *cis* 5-*O*-caffeoylquinic acid (compound **4**) was the most abundant phenolic compound in smoothies 3 and 4 (between 2.34 and 85 mg/100 mL), while galloylglucose (compound **3**) was the one in the highest concentrations in smoothies 1 and 2 (between 14.9 and 31.8 mg/100 mL). Besides contributing a higher variety of phenolic compounds, fortification with cardoon blades resulted in more than the double of total phenolic content in the smoothies. Thus, formulations 1 and 2 exhibited values of 73 and 53.9 mg/100 mL after processing (T0), respectively, while the smoothies fortified with cardoon (3 and 4) contained 149 and 147 mg/

100 mL, respectively.

The content of phenolic compounds notably decreased over the shelf-life, especially in the case of non-fortified smoothies where only galloylglucose (compound **3**) was detected after prolonged storage (T50). The decrease in phenolic content was less severe in the smoothies fortified with cardoon, in which more compounds were also retained throughout storage, especially in the case of smoothie 3 where only two compounds were lost after 50 days (i.e., compounds **2** and **6**). Nevertheless, the phenolic compound decline was still pronounced in these preparations, with a reduction from 3 to 6 times between days 0 and 50 (from 149 to 43.3 mg/100 mL, and 147 to 23.1 mg/100 mL, in smoothies 3 and 4, respectively). Although phenolic compounds are non-nutrients and thus do not contribute to the energy value of smoothies, the identified compounds may contribute to the overall quality attributes of these beverages and provide potential health-promoting effects. Therefore, the reduction in the levels of phenolic compounds, as somehow noted in the TBARS formation inhibition assay, may imply a loss of nutritional value. Although some studies in the literature report that HPP processing may increase the content of bioactive compounds, this was not observed in our study, at least as concerns phenolic compounds. For instance, Andrés, Villanueva, and Tenorio (2016) found that the extractability and subsequent content of different classes of compounds, including polyphenols, increased in milk- and soy-smoothies treated at pressures of 600 MPa for 3 min and 20 °C. For their part, Škegro et al. (2021), applied lower pressures (i.e., 350 and 450 MPa for 5 to 15 min at room temperature) and reported increased stability of bioactive compounds in smoothies prepared from blending fruit juices. The discrepancies among the results obtained might be due to the distinct types of matrices and processing conditions used. Indeed, the authors mentioned the influence that the product matrix may have on the processing and the final characteristics of a smoothie (Aaliya et al., 2021; Kumar et al., 2023). Therefore, optimizing the processing conditions may contribute to enhancing the enriching effect of cardoon blades and thus value their exploitation as a potential functional ingredient in smoothies' formulations.

3.2.4. Bioactive properties

The antioxidant activity was assessed through the ability of the smoothies to inhibit lipidic peroxidation (TBARS assay) and to inhibit the formation of reactive oxygen species in the cellular environment (CAA assay). Table 5 shows the results reached in the TBARS assay with the inhibition percentages corresponding to the 7× dilution. In this dilution, the inhibition values closest to 50% were obtained. Therefore, higher inhibition percentage values at higher dilutions indicate more potent activity. None of the formulations showed antioxidant capacity when evaluated by the CAA assay, suggesting that these formulations did not possess the ability to inhibit this oxidative mechanism.

All smoothie formulations exhibited the capacity to inhibit lipidic peroxidation, with smoothies 3 and 4 (fortified with cardoon) showing, in general, higher activity (i.e., exhibiting higher inhibition percentages at lower dilutions) than smoothies 1 and 2 without cardoon (between 46 and 60% vs 51 and 66%, respectively), considering all the shelf-life. Throughout the storage period, the antioxidant activity was maintained or slightly decreased, when values obtained at T0 and T50 were compared, but for smoothie 4, for which an increase in activity was produced (from 61 to 48.2%).

The reduction in antioxidant activity in the later stages of shelf-life could be justified by the presence of residual oxygen, which causes the oxidization of certain compounds and the consequent reduction in activity (Andrés et al., 2016; Fernández et al., 2020; Hurtado et al., 2019). Although HPP processing interferes minimally with the chemical composition of the products, the processing conditions differentiate the final characteristics. According to the literature, HPP processing either maintains or boosts antioxidant activity, depending on the pressure levels and holding times. Andrés et al. (2016) reported an increase in the antioxidant activity for smoothies processed by HPP at 600 MPa, and, similarly, Fernández et al. (2020) described better results for smoothies processed by HPP at 630 MPa. The same authors also suggested that the

Table 5
Bioactivity and microbiological quality of the smoothie formulations during storage at 4 °C for 50 days (T0 to T50).

Samples		TBARS (% inhibition)*	NO production (% nhibition)**	Microorganisms analyzed (log CFU/mL)					
				Aerobic mesophiles	Coliforms	Yeasts	Molds	*Bacillus cereus*	*Staphylococcus* spp.
Smoothie 1	T0	55.5 ± 0.4^{d}	n.a.	2.3 ± 0.1^{abcd}	n.d.	n.d.	n.d.	n.d.	n.d.
	T10	52.1 ± 0.6^{g}	n.a.	2.4 ± 0.2^{abc}	n.d.	n.d.	n.d.	1.3 ± 0.1^{ab}	n.d.
	T22	58 ± 1^{c}	n.a.	2.4 ± 0.1^{abc}	n.d.	n.d.	n.d.	1.4 ± 0.1^{a}	n.d.
	T35	58.0 ± 0.1^{c}	n.a.	2.5 ± 0.1^{ab}	n.d.	n.d.	n.d.	1.0 ± 0.3^{b}	n.d.
	T50	54 ± 1^{ef}	n.a.	2.5 ± 0.1^{a}	n.d.	n.d.	n.d.	n.d.	n.d.
Smoothie 2	T0	55 ± 1^{de}	n.a.	2.4 ± 0.1^{abc}	n.d.	n.d.	n.d.	n.d.	n.d.
	T10	52.3 ± 0.5^{fg}	n.a.	2.32 ± 0.04^{abcd}	n.d.	n.d.	n.d.	n.d.	n.d.
	T22	51.0 ± 0.1^{g}	n.a.	2.2 ± 0.1^{cd}	n.d.	n.d.	n.d.	1.48 ± 0.02^{a}	n.d.
	T35	55.8 ± 0.3^{d}	n.a.	2.3 ± 0.1^{abcd}	n.d.	n.d.	n.d.	1.0 ± 0.1^{b}	n.d.
	T50	66 ± 3^{a}	n.a.	2.36 ± 0.03^{abc}	n.d.	n.d.	n.d.	n.d.	n.d.
Smoothie 3	T0	48 ± 1^{hi}	61 ± 3^{a}	2.23 ± 0.01^{cd}	n.d.	n.d.	n.d.	n.d.	n.d.
	T10	47 ± 1^{hij}	n.a.	2.1 ± 0.2^{de}	n.d.	n.d.	n.d.	1.5 ± 0.3^{a}	n.d.
	T22	46 ± 1^{j}	n.a.	1.9 ± 0.1^{f}	n.d.	n.d.	n.d.	1.2 ± 0.2^{ab}	n.d.
	T35	60 ± 2^{b}	n.a.	2.0 ± 0.1^{ef}	n.d.	n.d.	n.d.	1.1 ± 0.1^{ab}	n.d.
	T50	51 ± 1^{g}	n.a.	1.9 ± 0.2^{f}	n.d.	n.d.	n.d.	n.d.	n.d.
Smoothie 4	T0	61 ± 3^{b}	68 ± 2^{a}	2.23 ± 0.01^{bcd}	n.d.	n.d.	n.d.	0.2 ± 0.4^{c}	n.d.
	T10	48.2 ± 0.2^{h}	70 ± 1^{a}	2.3 ± 0.1^{bcd}	n.d.	n.d.	n.d.	0.3 ± 0.6^{c}	n.d.
	T22	46 ± 1^{ij}	65.8 ± 0.2^{a}	2.3 ± 0.1^{abcd}	n.d.	n.d.	n.d.	1.3 ± 0.1^{ab}	n.d.
	T35	46.8 ± 0.6^{hij}	54.4 ± 0.2^{a}	2.4 ± 0.1^{abc}	n.d.	n.d.	n.d.	1.15 ± 0.05^{ab}	n.d.
	T50	48.2 ± 0.4^{h}	53 ± 4^{a}	2.4 ± 0.2^{abc}	n.d.	n.d.	n.d.	n.d.	n.d.

*Results corresponding to the 7× dilution of the smoothies; **Results corresponding to the 20× dilution of the smoothies. Results are presented as mean ± SD ($n = 3$). Different letters in the same column correspond to significant differences ($p < 0.05$) between samples. n.a. – no activity; n.d. – not detected; CFU – colony forming unit; Positive control for TBARS – Trolox ($IC_{50} = 9.1$ μg/mL), CAA – Quercetin (% inhibition 95.3 ± 4.6% at 0.3 μg/mL). Smoothie 1 – pasteurized formulation; Smoothie 2 – formulation subjected to high-pressure processing; Smoothie 3 – pasteurized formulation with cardoon; Smoothie 4 – formulation with cardoon subjected to high-pressure processing.

differences in activity could be explained by the increased retention of phenolic compounds.

The anti-inflammatory activity was assessed by measuring the inhibition in the NO production, a proinflammatory mediator, by LPS-stimulated RAW 264.7 macrophages. The results obtained in the smoothies diluted 20-fold are shown in Table 5**.** Only smoothies 3 and 4 enriched with cardoon bracts demonstrated the capacity to inhibit NO production, although smoothie 3 was just detected at T0, while smoothie 4 was maintained without significant changes throughout the shelf-life (50 days). The formulations without cardoon did not show anti-inflammatory capacity in any of the dilutions tested. The obtained results confirmed the functional properties concomitant with the fortification of smoothies with cardoon blades extract, highlighting its use as a potential functional ingredient.

On the other hand, none of the smoothie formulations exhibited the ability to interfere with the non-tumor PLP2 cell proliferation in the dilutions tested (the higher dilution tested was 20×).

3.2.5. *Microbiological quality*

Cardoon blade extract was used to fortify smoothies due to their good antimicrobial properties, thus contributing not only to potential functional qualities, but also to the microbiological safety of this beverage. The smoothies were assessed for their microbial count during their shelf-life to ensure the safety of the formulations. Aerobic mesophilic microorganisms, coliforms, yeasts, molds, *Bacillus cereus*, and *Staphylococcus* sp. were counted over shelf-life at 4 °C for 50 days. The results obtained are shown in Table 5.

Coliforms, yeasts, molds, and coagulase-positive *Staphylococcus* sp. were not detected in none of the formulations analyzed. Regarding the aerobic mesophiles' microorganisms, the total levels ranged from 1.9 to 3.5 log CFU/mL. The fortification of smoothies with cardoon blades caused a slight decrease in the prevalence of aerobic mesophiles, with an average of 2.4 and 2.3 for smoothies 1 and 2, respectively, compared to an average of 2.0 and 2.2 log CFU/mL for smoothies 3 and 4, respectively, considering the whole studied period. *Bacillus cereus* was detected only at intermediate the shelf-life (i.e., days 10, 22, and 35), and only in the case of smoothie 4 minority counts were measured at time 0 (0.2 log CFU/mL). According to European Commission, microbial limits are only set for *Escherichia coli* in unpasteurized juices, which cannot exceed 1000 CFU/mL (European Commission, 2005). In the Portuguese health system, the National Institute of Health "Dr. Ricardo Jorge" (INSA) indicates as satisfactory guide values counts of aerobic mesophiles inferior to 10^6 CFU/mL and of *Bacillus cereus* inferior to 10^3 CFU/mL (Saraiva et al., 2019). Considering those standards, the microbial counts obtained for the studied smoothies would be below the established legal limits.

The use of natural microbial agents in smoothies has begun to be studied in recent years. Green tea, nisin, and natamycin extracts have been shown to increase the shelf-life of smoothies and reduce contamination by *E. coli* and *Listeria* spp., with nisin and natamycin showing the most promising results and increasing the shelf-life by about two weeks (Casco et al., 2022). In the same way, Nieva et al. (2022) described that nisin (12.5 mg/kg), natamycin (200 mg/kg), and citric acid (until pH 3.5) treatment could result in a 14-day shelf-life extension. According to the obtained results, fortifying smoothies with cardoon blades may show a positive effect on reducing contaminants, thereby enhancing the microbiological safety of the beverages. This approach could be an innovative and effective strategy for improving the microbiological safety of these drinks, acting as a natural preservation agent. To the best of our knowledge, the use of cardoon blade extract as a potential functional ingredient in smoothies has not been explored before.

4. Conclusions

Cardoon blade extract was explored as a bioactive ingredient, while the solid residue obtained from the extraction process was studied as a sustainable source of dietary fiber. Results from the fortification of vegetable/fruit-based smoothies with the prepared extract suggested its potential as a promising potential functional ingredient to improve the smoothies' bioactive profile and stability during storage. Meanwhile, the application of HPP was efficient in preserving the nutritional quality attributes of smoothies, thereby maintaining their bioactive properties. This emerging technology offers an innovative alternative that guarantees quality without sacrificing nutritional benefits.

The fortification of the beverage formulations with the fiber residue allowed increasing the dietary fiber content and its stability over shelf-life, although it caused an increase in viscosity. Furthermore, together with the functional extract, a varied and greater phenolic composition was obtained, along with higher antioxidant and anti-inflammatory capacities for the prepared smoothies. Although the microbiological criteria were met in all cases, fortification with cardoon blades seemed to provide more effective microbiological decontamination, with a lower presence of several microbial groups. All in all, the use of cardoon blade extract as a potential functional ingredient, together with their extraction residue as a source of dietary fiber, has proven to be a very promising strategy to fortify smoothies. This approach simultaneously contributes to the implementation of practices within a zero-waste approach in a circular economy context, with benefits for the economy, environment, and different industrial sectors, as well as the respective producing countries and regions.

CRediT authorship contribution statement

Filipa Mandim: Writing – original draft, Investigation, Formal analysis, Conceptualization. **Spyridon A. Petropoulos:** Writing – review & editing, Resources. **Carlos A. Pinto:** Writing – review & editing, Investigation. **Sandrina A. Heleno:** Writing – review & editing, Investigation. **Paula Rodrigues:** Writing – review & editing, Resources, Investigation. **Maria Inês Dias:** Writing – review & editing, Investigation. **Jorge A. Saraiva:** Writing – review & editing, Validation, Resources. **Celestino Santos-Buelga:** Writing – review & editing, Validation, Supervision. **Isabel C.F.R. Ferreira:** Writing – review & editing, Conceptualization. **Lillian Barros:** Writing – review & editing, Validation, Supervision, Resources, Conceptualization. **José Pinela:** Writing – review & editing, Validation, 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.

The authors declare no conflict of interest.

Data availability

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

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Appendix A. Supplementary data

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