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Enhancing shelf life of pumpkin pulp with natural-based preservative and high pressure processing

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Formulated pumpkin pulp fruit was incorporated with natural-based preservative (NBP) extracted from pumpkin peels (Butternut Squash), industrial mix (with and without potassium sorbate), processed by high pressure processing (HPP-600 MPa/4 min) and stored at 5 °C for 45 days. The shelf life was evaluated by microbiological analysis (total aerobic mesophilic, *Enterobacteriaceae* and molds and yeasts), pH, color, texture (TPA) and oxidative stability via changes in β-carotene, γ-tocopherol, total phenolic content (TPC) and antioxidant potential (FRAP). No microbial growth was detected in any formulation throughout the 45-day storage period, likely due to the combined effects of processing, the industrial mix, and the added NBP. Samples containing NBP better preserved color and firmness and exhibited significantly higher total phenolic content compared to other formulations. In contrast, thermally treated samples showed greater changes in texture (firmness, stickiness, chewiness) and in bioactive compounds such as β-carotene, lutein, and γ-tocopherol. Overall, processing method, NBP addition, and storage time did not significantly compromise the nutritional quality of the formulations. The combination of high pressure processing and NBP demonstrated strong potential for extending shelf life, allowing a 50% reduction or complete replacement of potassium sorbate without compromising safety or quality.

KEYWORDS
by-product applications, food safety, non-thermal preservation, pumpkin peel extract, quality retention

Introduction

Pumpkin (*Cucurbita* spp.) is an extraordinary vegetable with great potential as both a nutritious food and a medicinal resource. Its pulp and peel contain essential minerals, as well as valuable phytochemicals such as β-carotene, total flavonoids and phenolic compounds, which can contribute to anti-aging and support the immune system (Hosen et al., 2021). Despite their beneficial properties, pumpkins are one often neglected and underutilized food and medicinal plants (Nyabera et al., 2021). Their production is limited by the lack of genetically improved seeds, which compromises their cultivation on a larger scale. Therefore, research exploring the functionalities of pumpkin is essential to promote its wider use and disseminate its benefits.

When pumpkin is consumed as pulp, i.e., baby food, puree, soups, and others, the processing typically involves the application of high temperatures (e.g., pasteurization, sterilization) to ensure safety, also known for their negative impact, as they can cause undesirable changes in both the nutritional content and sensory quality of the food (Terefe et al., 2013). In this scenario, nonthermal technologies gain space due to their ability to offer simultaneously safety and high-quality products, since less thermal degradation of quality attributes and nutritional compounds its expected. One of these technologies successfully implemented in the food industry is high pressure processing (HPP). In this process, food is subjected to high levels of pressure for a short period (100–800 MPa/1–5 min) instead of high temperatures. The pressure is applied and released uniformly from all directions, regardless of the product's size and geometry (Aganovic et al., 2021), which contrasts with conventional thermal processing and contributes to improved preservation of product quality. As a result, several advantages are reported, including safe and minimally-processed products with extended shelf-life; prepack technique, i.e., the product is packed in their final package (reducing chances of cross contamination); food waste reduction; clean labels; eco-friendliness and a wide range of applications (Houška et al., 2022). However, HPP is a cold pasteurization and alone does not inactivate microbial spores and some enzymes.

Several studies have already demonstrated the potential of the application of HPP in different food products, such as pumpkin puree (García-Parra et al., 2016), pumpkin cubes (Contador et al., 2014; Rinaldi et al., 2023), mango pulp (Ribeiro and Cristianini, 2021) and among others, açaí (*Euterpe oleracea Martius*) (Jesus et al., 2020), mostly demonstrating a better performance of HPP over thermal treatments, particularly in terms of better retention of heat-sensitive nutrients and bioactive compounds, improved preservation of sensory attributes such as color, flavor, and aroma, as well as maintaining product texture while still achieving effective microbial inactivation and shelf-life extension (Goraya et al., 2025).

Additionally, HPP can be combined with other hurdle techniques, such as essential oils or natural-based preservatives extracted from food side streams, to investigate potential synergistic effects (Zou et al., 2023; Melhem et al., 2022). Combining HPP with NBP allows not only an increase in microbial inactivation, but also allows milder HPP conditions, consequently reducing processing costs (Balasubramaniam and Farkas, 2008). Thus, the objective of this study was to evaluate the application of high pressure processing (HPP) at ambient temperature in combination with a natural-based preservative extracted from pumpkin peel, aiming to enhance microbial inactivation while preserving important quality parameters, providing an alternative preservation strategy that avoids the use of chemical preservatives, such as potassium sorbate, and the application of high-temperature treatments (e.g., pasteurization or sterilization).

Materials and methods

Pumpkin-pulp production

Pumpkins (*Cucurbita moschata* butternut squash) were purchased from a local market in Berlin (Germany) and transported to the Innovation laboratory at the Leibniz Institute for Agricultural Engineering and Bioeconomy (ATB). Upon arrival, the pumpkins were carefully cleaned by washing with potable water to remove soil and coarse impurities before processing. Using a blender (Retsch GM 200, Germany), pumpkin pulp (100% pulp) was produced. After peeling, seed removal and cutting, the pulp was homogenized using the blender, resulting in a final yield of 67%.

After production, the pulp was divided into five different sample conditions for subsequent treatments:

A1. Pumpkin-Pulp + mix (without potassium sorbate) + HPP
A2. Pumpkin-Pulp + mix (with potassium sorbate) + Pasteurization
A3. Pumpkin-Pulp + mix (with potassium sorbate) + HPP
A4. Pumpkin-Pulp + mix (without potassium sorbate) + NBP + HPP
A5. Pumpkin-Pulp + mix of ingredients (with 50% potassium sorbate) + NBP + HPP

Natural-based preservative was extracted from pumpkin-pulp peel and incorporated to samples A4 and A5. The mix of ingredients contains natural ß-carotene, starch, citric acid and citrate. Additionally, sample A4 was not added of potassium sorbate, while sample A5 had a 50% reduction of potassium sorbate to evaluate the efficiency of the NBS. The samples were vacuum-packed (Komet Plus Vac20, Germany) in appropriate packages for high pressure processing (Whirl-Pak® Sample Bag) and glass jars for pasteurization. Samples were stored under refrigerated conditions for 45 days, protected from the light.

High pressure process and thermal treatment

HPP treatment was performed at 600 MPa for 4 min at ambient temperature using a U4000 High Pressure system (Unipress, Warsaw, Poland). These conditions were previously shown to have good potential for microbial inactivation and are consistent with industrial practices for cold pasteurization. Thermal treatment was applied using a retort at 90 °C for 3 min, with a total processing time of 15 min, including the heat-up period. The thermal parameters were based on those used for commercial pumpkin pulp processing. After processing, all samples were stored under refrigeration. and shelf-life was evaluated over 45 days with analyses conducted at days 0, 21, and 45.

Microbiology assays

Samples were diluted in peptone water (10^{-1}) and homogenized in a stomacher for 2 min. Serial decimal dilutions were then prepared, and appropriate dilutions were plated in duplicate using the spread plate technique. Total aerobic mesophilic count was enumerated on Plate Count Agar (PCA) (Carl Roth GmbH and Co. KG, Germany) and incubated at 37 °C for 48 h. *Enterobacteriaceae* were determined using Endo agar (Carl Roth GmbH and Co. KG, Germany) supplemented with fuchsin solution (10% in 95% ethanol) and incubated at 37 °C for 48 h. Molds and yeasts were analyzed on DRBC agar (Dichloran Rose-Bengal Chloramphenicol agar) (Carl Roth GmbH and Co. KG, Germany) and incubated at 30 °C for 72 h.

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TABLE 1 Measurement conditions for the texture analyzer.

Measurement	Condition
Test mode	Compression
Speed	50 mm/min
Trigger	0.1 N
Entry depth	10 mm
Beaker	Ø50 mm, height 105 mm
Sample height	50 mm
Adaptor	Ø25 mm, height 40 mm
Load cell	1 kN

Textural properties	Parameter	Sensory feeling
Firmness	F2	Hard/Firm/Soft
Elasticity	t4:5/t1:2	Plastic/Elastic
Cohesion	A4:6/A1:3	Brittle/Crumble
Gumminess	Firmness (F2) x cohesion	-
Chewiness	Gumminess x elasticity	-
Stickiness	A3:4	Sticky/Adhesive

Color and pH measurement

Methods from Gratz, et al. (2021) with minor modification has been conducted for color evaluation. A white light (6000K) reflector from left and right side to guarantee similar light conditions was used. Prior taking the pictures the samples were spread (10 g) evenly in a petri dish. The photos were taken with a Nikon camera (COOLPIXP7700) from the top (20 cm). A simple image processing routine implemented in GNU Octave (Version 4.2.1) was used to determine the L*, a*, b* parameters and RGB of the samples. The pH was measured by benchtop pH-meter (SI Analytics Lab 850 pH-Meter, SI Analytics GmbH, Mainz, Germany).

Profile texture analysis (TPA)

The Profile Texture Analysis of the treated and non-treated samples were conducted using a calibrated Texture Analyzer (TA XT Plus, Stable Micro Systems, UK). Texture parameters such firmness, elasticity, cohesion, gumminess, chewiness and stickiness were measured in triplicate. The following instrumental test parameters were used: mode was forced in compression; load cell value was 1 kN; trigger type was 0.1 N; and a cylindrical aluminum probe (25 mm diameter, 40 mm height) was used. An aliquot of each sample (~50 g) was used. The entry depth of the probe was set to 10 mm using a test speed of 50 mm/min and samples were analyzed at room temperature. The force - time curve was further evaluated to determine the texture parameters using an octave code programmed in-house according to the interpretation rules for textural profile analysis showed in Table 1 (Gratz et al., 2021).

Natural-based preservative (NBP)

NBP was obtained under optimal extraction conditions, previously determined in an optimization study. Briefly, pumpkin peels were freeze-dried, reduced to a powder (~20 mesh) and extracted by heat-assisted extraction using just water as solvent, at a ratio of 1 g/40 mL, for 84 min at 30 °C with constant stirring (Barros et al., 2024). The mixture was filtered through filter paper and dried using a spray dryer (BUCHI, B-191, Laboratory-Techniques LTD, Flawil, Switzerland) with maltodextrin as a carrier agent, in order to reduce stickiness, improving powder recovery, and contributing to the stabilization of bioactive compounds during drying (Freixo et al., 2016).

Extraction of carotenoids and γ-tocopherol

Before vitamin extraction, the pumpkin-pulp samples were freeze-dried. The carotenoid and γ-tocopherol extraction were performed according to the method of Guzman et al. (2012) with slight modifications. Briefly, a sample of 0.01 g of freeze-dried pumpkin-pulp was extracted using 500 µL of pure ethanol. The samples were shaken for 20 min in the dark (80 rpm, room temperature (RT) and then centrifuged (9,000 g, 15 min, RT). The supernatant was collected entirely and transferred to a new 2-mL-reaction tube. The extraction was performed another two times until the pellet was colorless. The solvent was then wholly evaporated using a centrifugal evaporator (no heating, no pulse ventilation). After complete evaporation of ethanol, the residue was taken up in 15 mL isopropanol each for the analysis of β-carotene and 100 µL isopropanol each for the analysis of lutein and γ-tocopherol. The extracts were placed in an ultrasonic bath for 5 min to dissolve the residue completely. The two identical samples from one treatment and 1 day were pooled, and the vitamin content was determined by HPLC-MS/MS.

HPLC-MS/MS

The determination of carotenoid and γ-tocopherol content was performed using an Agilent Infinity 1260 system composed of a binary pump, multicolumn thermostat, and autosampler interfaced with an Agilent G6470A Series triple quadrupole mass spectrometer (Agilent Technologies Sales and Services GmbH and Co. KG, Waldbronn, Germany) coupled with an electrospray (ESI) source in positive ionization mode. MS/MS was operated in the multiple reaction monitoring (MRM) mode. The separation was achieved on a Multospher 120-RP18AQ column (250 × 4.0 mm; 3 µm), and the column temperature was set to 30 °C. The injection volume was 5 µL, and the flow rate was 0.8 mL/min. The sheath gas temperature was set at 275 °C, the sheath gas flow was 11 L/min, the nebulizer pressure was 35 psi, and the collision gas used was nitrogen. The mobile phases were methanol (with 0.1% formic acid) (A) and ethyl acetate (with 0.1% formic acid) B. The elution gradient was as follows: 96% A from 0 to 2 min, 96%–20% A from 2 to 5 min, 20% A from 5 to 10 min and re-equilibrated (post-run) to 96% A for 5 min. The transitions used for quantification, fragmentor voltage, collision energy, and dwell time are listed in Table 2.

The vitamin content was quantified using an external calibration curve. Stock solutions of β-carotene (100 µg/mL), lutein (100 µg/

TABLE 2 Mass spectrometric conditions for the analysis of the selected vitamins.

Compound name	Precursor ion	Product ion	Dwell	Fragmentor	Collision energy
Lutein	568.5	476.4	20	80	20
		430.2	20	80	10
		338.2	20	80	20
		237.2	20	80	20
		145.2	20	80	20
		430.2	20	80	20
β-carotene	536.6	444.6	20	70	20
		269.2	20	70	20
		177	20	70	20
		133.1	20	70	5
γ-tocopherol	416.5	191.1	20	90	26
		164.2	20	90	25
		150.9	20	90	27
		149.8	20	90	25

TABLE 3 Limit of detection (LOD) and quantification (LOQ) for lutein, β-carotene and γ-tocopherol.

Compound name	LOD (ng/mL)	LOQ (ng/mL)
Lutein	19.10	58.00
β-carotene	34.70	105.10
γ-tocopherol	7.70	23.40

mL) and γ-tocopherol (1,000 μg/mL) were prepared in absolute ethanol. The concentration of the working standard solutions was checked photometrically before each HPLC-MS/MS measurement and calculated according to their absorbance values E (1 cm/1%) (β-carotene 233 at 450 nm in acetone, lutein 215 at 450 nm in ethanol (Krajewska et al., 2017), γ-tocopherol 91.4 at 298 nm in ethanol (Chen and Bergman, 2005). A mixture of β-carotene (0.063–4 μg/mL), lutein (1–8 μg/mL) and γ-tocopherol (0.063–1 μg/mL) was prepared. Calibration curves with their corresponding equations were calculated for each standard to determine the concentrations of the freeze-dried pumpkin-pulp samples. The analytical sensitivity was determined by calculating the limit of detection (LOD) and quantification (LOQ) (Table 3).

Preparation of pumpkin-pulp extracts

The preparation of pumpkin-pulp extracts to determine total phenolic contents and antioxidant capacity was according to the method by Mala and Kurian (2016) with slight modifications. Briefly, freeze-dried pumpkin-pulp samples weighing 0.01 g each were extracted with 200 μL of a methanol/water mixture (80/20, v/v). The extraction was carried out by constant shaking (Vitelli mixer, b4-mode, 80 rpm) for 3 h in the dark at RT. After extraction, the samples were centrifuged (10000 x g, RT, 10 min), and the supernatants from the pumpkin-pulp samples were combined.

Determination of total polyphenolic content: Folin-ciocalteau assay

The total phenolic content was determined using the Folin–Ciocalteu method described by Singleton et al. (1999), with minor modifications. Gallic acid was used as the standard for quantification. Briefly, 20 μL of the blank solution (methanol/water, 80:20, v/v) and 20 μL of pumpkin pulp extracts were pipetted into the wells of a 96-well microplate A premixed solution of 150 μL distilled water, 10 μL Folin-Ciocalteu reagent, and 20 μL sodium hydroxide (1 M) was added to each well, and the samples were incubated for 30 min in the dark at RT. The photometric measurement was performed at a wavelength of 765 nm with a Tecan Infinite 200 Pro plate reader. A five-point standard curve with gallic acid (10 μg/mL-100 μg/mL) was used to determine the TPC in all samples. The results were expressed as mg gallic acid equivalents (GAE)/100 g dry weight (dw).

Determination of antioxidative capacity: FRAP

The estimation of antioxidative capacity of pumpkin-pulp extracts was determined by Ferric reducing antioxidant power (FRAP)-Assay. The FRAP solution was prepared by mixing 10 mM TPTZ (in 40 mM HCl) with 20 mM $FeCl_3$ (in 0.25 M acetic acid, pH 3.6) in a ratio of 1:1. Then, 10 μL of a blank with methanol/water (80/20, v:v) or the pumpkin-pulp extracts were pipetted into a 96-well-plate and mixed with 150 μL of the FRAP solution. The samples were incubated for 6 min at RT before measuring the absorbance at 595 nm with a Tecan Infinite 200 Pro plate reader. An external calibration with ascorbic acid (50 μM–1000 μM) was used to determine the antioxidative capacity

of the pumpkin-pulp samples. The results were expressed as mg ascorbic acid equivalents (AAE)/100 g dw (Ou et al., 2002).

Determination of proximate composition

To determine the proximate composition of the pumpkin-pulp formulations, were adhered to the Association of Official Analytical Chemists (AOAC, 2016). Ash content was determined by incinerating the samples in a muffle at 550 °C. Crude protein content was analyzed using the Kjeldahl method, and crude fat was measured by Soxhlet extraction using petroleum ether. The carbohydrate content was calculated by difference, subtracting the sum of ash, protein, and fat content from 100%. The energetic value (kcal) was calculated using the Atwater conversion factors (4 kcal/g for protein and carbohydrates and 9 kcal/g for lipids). The results were expressed in g/100 dry weight (dw).

Determination of chemical composition

The profiles of free sugars and fatty acids were analyzed by chromatography using a HPLC coupled to a refractive index detector (HPLC-RID) and by gas–liquid chromatography with flame ionization detection (GC-FID), respectively.

HPLC-RID analyses were performed using an integrated Knauer Smartline system, following a previously described method (Barros et al., 2013). Separation was achieved on a Eurospher 100–5 NH_2 column (4.6 × 250 mm, 5 µm) maintained at 30 °C. Melezitose was used as the internal standard, and compound identification was based on chromatographic comparison with authentic standards. Results were expressed as g/100 g dry weight (dw).

Fatty acids were analyzed by GC-FID using a DANI GC 1000 equipped with a Macherey–Nagel capillary column, according to Pereira et al. (2011). Identification and quantification were performed by comparison with a commercial fatty acid methyl ester (FAME) standard mixture, and results were expressed as relative percentages.

Statistic evaluation

All experiments were performed in triplicate, and results were expressed as mean ± standard deviation. Data were analysed using two-way analysis of variance (ANOVA) to evaluate the effects of storage time (ST) and preservative type (PT), after verification of homogeneity of variances using Levene's test. Tukey's *post hoc* test was applied to homoscedastic data, while Tamhane's T2 test was used for heteroscedastic samples. Differences between storage times were assessed using Student's t-test. When a significant interaction between ST and PT was observed ($p < 0.05$), trends were explored using estimated marginal means (EMM) and eta squared (PES). In the absence of interaction, factors were analyzed independently. Statistical significance was set at $p = 0.05$, using IBM SPSS Statistics (version 29).

Results and discussion

Microbiology

During the 45 days of the predictive shelf life, no growth of mesophilic bacteria (total plate count), *Enterobacteriaceae*, molds and yeast was observed. This can be attributed not only to the process treatments, but also to the ingredient mix and the NBP (samples A4 and A5) added to the pulp. All five samples studied performed well during their 45 days of shelf life, demonstrating the successful application of the technologies in combination with the mix and the NBP extracted from pumpkin residue, namely, peel.

Sample A1 was processed using high pressure processing (HPP) without the preservative potassium sorbate (PS), while sample A2 was treated with potassium sorbate and pasteurized. Both conditions resulted in no microbial growth, demonstrating the efficacy of HPP alone in inhibiting microbial activity without the need for additional preservatives. To further investigate, sample A3 was processed using HPP and also included PS. As with the other samples, no microbial growth was observed. This indicates that the addition of potassium sorbate could be avoided, as sample A1, processed solely with HPP showed excellent performance during its shelf life without any microbial growth.

It is important to highlight that sample A4 was added with the NBP and not added with potassium sorbate, a common preservative used in the food industry for prolonging the shelf life of food products. Sample A4 showed the same positive results in keeping the microbial quality of the pulp during 45 days of shelf life with no growing of the microorganisms studied (under the limit of detection), which shows the impact of this ingredient positively as a natural preservative in combination with HPP. Additionally, HPP alone (100% pulp, with no ingredients) could not inactivate all the contaminants, consequently brining a shorter shelf life (15 days) when compared to the actual study (45 days).

Sample A5 was added with NBP in combination with sorbate potassium reduced by 50% compared with the samples A2 and A3. As it can be observed by the results, no growing was observed, showing that in this product the use of potassium sorbate could either be reduced by 50% or even totally replaced by the NBP extracted from the pumpkin-peel, since in sample A4 no microbial growing was observed.

Prior to the shelf-life determination, the raw pumpkin-pulp (100% pulp, with no industrial mix or NBP) had its microbiota evaluated and showed an initial contamination of 2.9 log/g for mesophilic bacteria and 2.3 log/g for *Enterobacteriaceae*. Thus, the inactivation as well as the inhibition of microbial growth during the 45 days of shelf life could be attributed to the processes as well as its combinations with the ingredients used. Besides that, it is important to highlight that in a previous study, raw pumpkin-pulp (not added with any ingredient) treated by the same HPP conditions showed a considerable contamination at day 20 and at day 34, (4.7 log/g for total aerobic mesophilic and 3.12 log/g for *Enterobacteriaceae*). On the other hand, thermal process (same conditions as for the sample A2) at day 34 showed 4.2, 4.3, and 4.5 log/g of total aerobic mesophilic, *Enterobacteriaceae* and molds and yeast, respectively. This result clearly shows a positive correlation between the process applied and the ingredients used, since a shelf life of 45 days was achieved with no microbial growth.

High pressure is recognized as a cold pasteurization technique, with great ability to inactivate non-spore bacteria at ambient temperature (Inanoglu et al., 2022; Silva, 2023). Besides HPP, the industrial mix used contained acid citric and citrate, both ingredients with an antimicrobial activity, thus, it is believed that the microbial inactivation is due to a combined effect of HPP, NBP

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TABLE 4 pH variations during 45 days of pumpkin-pulp shelf life.

Sample	Days		
	1	21	45
A1	4.96 ± 0.01^{Aa}	4.94 ± 0.01^{Cb}	4.97 ± 0.01^{Ca}
A2	4.98 ± 0.01^{Ba}	5.01 ± 0.00^{ABb}	5.04 ± 0.01^{Ac}
A3	5.02 ± 0.01^{Ca}	5.02 ± 0.01^{Aa}	4.98 ± 0.01^{Cb}
A4	4.93 ± 0.01^{Da}	5.00 ± 0.01^{Bb}	5.03 ± 0.01^{Ac}
A5	5.01 ± 0.01^{Ea}	5.02 ± 0.02^{ABa}	5.01 ± 0.01^{Ba}

Different capital letters in the same column means diff statistic ($p < 0.05$) in the same day of shelf life for different samples. Different small letters in the same line means diff statistic ($p < 0.05$) for the same sample in different days of shelf life. a-d are just statistical tests.

and industrial mix (with and without potassium sorbate). Besides the great browning control (Zou et al., 2023), acid citric also prevent contamination due to the pH decay, metal chelating, so combining acid citric with other decontamination methods can significantly impact the microbial inactivation in fresh foods (Książek, 2024). Similarly to the present study, Zou et al. (2023) showed that the combination of citric acid (CA&PEN) and HPP (500 or 600 MPa, 5 min) also promoted a synergistic effect and inhibited the total aerobic bacteria in banana puree up to 3 months. The NBP, characterized in previous work under standard extraction conditions, showed notable antimicrobial activity against five bacterial and two fungal strains associated with foodborne contamination, at concentrations between 10 and 2.5 mg/mL (Leichtweis et al., 2025). This appears as a positive result, mainly by the ones where the potassium sorbate could be avoided or replaced by the NBP (sample A1, A4 and A5). Additionally, the use of potassium sorbate is controlled by different international laws, the *Codex Alimentarius* and European legislation on food additives have established a maximum level of 1,000 ppm for benzoate and sorbate in various products, such as sauces, exceeding this amount not only infringes regulatory guidelines but also represents a danger to human health (Yazdanfar et al., 2023).

pH and color evaluation

Table 4 shows the pH values measured during days 1, 21, and 45 of shelf life. As can be observed, the pH results are in accordance with the microbiological results, showing any or very small variation, and being stable during 45 days of shelf life. Besides the significant difference shown by the statistical evaluation, those differences are possibly negligible since less than ≤1.9% of variation was observed between the samples, representing, in general, for all samples a very similar pH ~5 (≤5.03 and ≥4.93). The pH of the untreated pumpkin-pulp was 6.5, and after addition of the mix, NBP and process application (HPP and pasteurization) an initial (day 1) and final (day 45) value of pH 5 was observed and this may be explained by the ingredients added to the pulp (NBP, potassium sorbate, citric acid and citrate), which was able to reduce the contamination, and consequently not promoting any alteration in pH values. Additionally, the mix contains citrate, a common salt used as a pH controller in the food industry, due to its buffering properties (Behera et al., 2021). These results are in agreement with the findings of Rinaldi et al. (2023), with no changes in pH for pumpkin (cv. *Violina rugose*) processed by HPP (100–600 MPa).

Table 5 shows the color evaluation of pumpkin-pulp, in general, no significant color changes were observed. Usually, samples processed by pasteurization (sample A2) presented a darker color, as it can be observed by the lower value in L* parameter. For the other samples (samples A1, A3, A4 and A5), the same trend was observed over the 45 days of shelf life, a slight increase in the L* value, representing a lighter color for the pulps. As expected, HPP did not change the color of the samples. Same results were previously reported by Contador et al. (2014) in pumpkin-puree (*Cucurbita moschata* var. Butternut) treated by HPP (400–600 MPa), according to the authors, the HPP treatment presented an advantage compared with thermal treatment, with less influence on color modifications. Pumpkin cubes (*Cucurbita moschata* var. Butternut) showed reductions in their L*, a*, and b* values after thermal treatments, consistent with this study's findings (except for b*). According to Pimpaporn et al. (2007), the reduction in L* may be influenced by starch gelatinization due to its clarity-like characteristic, what could also apply for this study once in the industrial mix added to the pumpkin-pulp, starch was one of the ingredients.

Texture profile analysis (TPA)

Table 6 shows the TPA analysis for the pumpkin-pulp during shelf life, represented by the parameters of firmness, elasticity, cohesion, gumminess, chewiness, and stickiness. The sample A2 presented the most different characteristics in terms of firmness when compared with all the other samples (≤361% higher than the other samples), showing a significant impact of thermal treatment compared to HPP treatment. This could be attributed to the effect of elevated temperature on starch properties, since starch gelatinization begins at 60 °C (Rittenauer et al., 2021), and it will consequently bring considerable food texture modifications resulting from physicochemical changes in cell-wall materials, as the gelatinization of starch (Kadam et al., 2015). On the other hand, samples A1, A3, A4 and A5 processed by HPP were not exposed to elevated temperature (≤30 °C), showing the lower firmness properties. High pressure can in fact reduce the firmness/hardness of plant tissue, led by cell wall breakdown and rupture, degradation of pectin and loss of turgor (Dhenge et al., 2023). Similarly, Rinaldi et al. (2023) found that pressure levels at

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TABLE 5 Color evaluation during 45 days of shelf life of pumpkin-pulp.

Days	Sample	L*	a*	b*	RGB
1	A1	47.09 ± 0.58^{Aa}	42.70 ± 0.31^{Aa}	53.80 ± 0.15^{ABa}	
	A2	43.82 ± 0.81^{Da}	42.80 ± 0.52^{Aa}	54.10 ± 0.74^{BCa}	
	A3	47.05 ± 0.33^{Ca}	41.31 ± 0.06^{Ba}	56.05 ± 0.38^{Aa}	
	A4	48.75 ± 0.77^{BCa}	40.45 ± 0.86^{Ba}	54.06 ± 0.36^{ABa}	
	A5	49.38 ± 1.31^{Ab}	39.03 ± 0.85^{Ca}	51.03 ± 1.34^{Ca}	
		L*	a*	b*	RGB
21	A1	50.6 ± 0.95^{Bb}	39.86 ± 0.34^{Bb}	52.25 ± 0.65^{Cb}	
	A2	48.88 ± 0.77^{Cb}	43.49 ± 1.18^{Aa}	58.12 ± 0.65^{Aa}	
	A3	49.67 ± 1.09^{BCb}	39.90 ± 0.32^{Ba}	54.78 ± 0.62^{Ba}	
	A4	50.83 ± 0.52^{Bb}	37.26 ± 0.27^{Cb}	51.72 ± 0.53^{Cb}	
	A5	55.10 ± 0.76^{Aa}	37.04 ± 0.36^{Cb}	48.87 ± 0.63^{Db}	
		L*	a*	b*	RGB
45	A1	52.26 ± 0.78^{Ab}	37.3 ± 0.34^{Cc}	49.12 ± 0.43^{Cc}	
	A2	48.22 ± 0.51^{Cb}	39.2 ± 0.45^{Ab}	56.75 ± 0.61^{Ab}	
	A3	51.52 ± 0.57^{ABb}	38.12 ± 0.25^{Bb}	52.47 ± 0.41^{Bb}	
	A4	51.26 ± 0.55^{Bb}	35.00 ± 0.31^{Dc}	49.00 ± 0.54^{Cc}	
	A5	50.52 ± 0.57^{Bb}	34.78 ± 0.39^{Dc}	47.57 ± 0.60^{Db}	

Different capital letters in the same day of shelf life in between different samples means difference statistic ($p < 0.05$).
Different small letters in different days of shelf life and for the same samples means difference statistic ($p < 0.05$).
a-d are just statistical tests.

200–600 MPa affected the texture of pumpkin cubes, the firmness was reduced significantly ($p < 0.05$) in levels of 19%, 29%, 45%, 51% and 55%, respectively at 200, 300, 400, 500, and 600 MPa.

It is widely known that during heating of food starch, the granules undergo of gelatinization, a process when starch granules swell up in the presence of water and heat (Balakrishna et al., 2020) and this process will strongly influence not only the properties and structural organization of the starch (Yan et al., 2024) but consequently the texture properties (firmness, stickiness, gumminess) of food that contains starch.

Another important parameter in the texture properties of food is the chewiness, which quantifies the energy required for chewing a sample until it can be swallowed, and it is strongly influenced by firmness (Xu et al., 2020). Similarly for firmness, gumminess and chewiness of samples A2 (pasteurized) showed greater values compared with the HPP samples, being ≤360% and 493% higher, respectively. For cohesion and elasticity, no difference was observed during shelf life and in between the different samples. Stickiness refers to the force required to peel off the fraction of product adhering to the interior of the oral cavity (Kouassi et al., 2021). Similarly, for firmness and chewiness, samples processed thermally (sample A2) presented a very much higher value when compared with the HPP samples, showing values ≤670% higher.

In general, for all TPA parameters tested, samples processed by HPP showed less influence when compared with pasteurization (sample A2). HPP has influence in starch gelatinization (Paciulli et al., 2019) but in very different pathways compared to heat, what can result in different gel texture properties. For wheat starch, for example, pressure gelatinization showed a smaller degree of granule swelling when compared with heat treatment (Liu et al., 2020), what can result in different functional properties as well as suggested in this present study for TPA characteristics.

Oxidative analysis

The mass spectrometric analysis of the extracted pumpkin-pulp samples revealed a decrease in ß-carotene, lutein and γ-tocopherol content over time. Sample A5 showed the lowest reduction in carotenoid and γ-tocopherol content. However, sample A3 exhibited the highest decrease in lutein and γ-tocopherol contents at 71.98% and 84.42%, respectively. The 45-day storage period significantly affected the γ-tocopherol content, with losses ranging from 62.26% to 84.42%. The decrease in β-carotene content ranged from 42.73% to 66.16%, with the lowest values found in sample A2, which was lower than all other groups, even at the initial content (54.98 ± 7.95 mg/100 g dw) (Table 7). Sample A2 has a significantly lower carotenoid content than samples A1 and A3. These samples differ mainly in their treatment (HPP vs. pasteurization). While sample A2 was pasteurized, samples A1 and A3 were treated with high pressure. These results suggest that pasteurization itself has a strong influence on the degradation of carotenoids.

The TPC in the pumpkin-pulp samples at the beginning of the experiment was between 38.01 ± 2.61 and 52.55 ± 1.69 mg GAE/

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TABLE 6 Texture profile parameters from pumpkin-pulp during 45 days of shelf life.

Day 1	A1	A2	A3	A4	A5
Firmness	2.59 ± 0.4^{Cab}	8.78 ± 0.73^{Aa}	4.53 ± 1.28^{Ba}	2.3 ± 0.11^{Ca}	2.3 ± 0.14^{Ca}
Elasticity	0.98 ± 0.06^{Aa}	0.93 ± 0.24^{Aa}	1.1 ± 0.12^{Aa}	1.04 ± 0.04^{Aa}	1.04 ± 0.05^{Aa}
Cohesion	0.23 ± 0.26^{Ab}	0.38 ± 0.14^{Aa}	0.14 ± 0.55^{Aa}	0.48 ± 0.19^{Aab}	0.48 ± 0.25^{Aab}
Gumminess	0.60 ± 0.64^{Ba}	3.38 ± 1.46^{Aa}	0.67 ± 0.93^{Ba}	1.09 ± 0.4^{ABa}	1.09 ± 0.48^{ABa}
Chewiness	0.58 ± 0.73^{Aa}	3.27 ± 1.93^{Aa}	0.71 ± 1.05^{Aa}	1.14 ± 0.43^{Aa}	1.14 ± 0.52^{Aa}
Stickiness	4.28 ± 1.38^{Ba}	29.57 ± 1.45^{Aa}	4.50 ± 2.00^{Ba}	3.79 ± 0.74^{Ba}	3.79 ± 1.08^{Ba}
Day 21	**A1**	**A2**	**A3**	**A4**	**A5**
Firmness	2.82 ± 0.3^{Cb}	9.33 ± 0.58^{Aa}	4.21 ± 0.41^{Ba}	2.47 ± 0.2^{Ca}	3.06 ± 0.19^{BCa}
Elasticity	1.06 ± 0.04^{Aa}	1.11 ± 0.12^{Aa}	1.01 ± 0.11^{Aa}	1.01 ± 0.09^{Aa}	0.88 ± 0.15^{Aa}
Cohesion	0.27 ± 0.15^{Ab}	0.38 ± 0.10^{Aa}	0.29 ± 0.08^{Aa}	0.35 ± 0.13^{Ab}	0.19 ± 0.09^{Ab}
Gumminess	0.76 ± 0.41^{Ba}	3.49 ± 1.05^{Aa}	1.23 ± 0.3^{Ba}	0.89 ± 0.39^{Ba}	0.58 ± 0.30^{Ba}
Chewiness	0.80 ± 0.40^{Ba}	3.90 ± 1.38^{Aa}	1.24 ± 0.33^{Ba}	0.98 ± 0.42^{Ba}	1.96 ± 0.38^{Ba}
Stickiness	3.15 ± 0.58^{Ba}	22.63 ± 4.8^{Ab}	3.85 ± 0.56^{Ba}	3.03 ± 0.88^{Ba}	3.2 ± 1.47^{Ba}
Day 45	**A1**	**A2**	**A3**	**A4**	**A5**
Firmness	3.76 ± 0.42^{Cb}	9.81 ± 0.24^{Aa}	4.68 ± 0.28^{Ba}	2.71 ± 0.14^{Da}	2.13 ± 0.09^{Da}
Elasticity	1.34 ± 0.19^{Aa}	1.10 ± 0.00^{Ba}	1.03 ± 0.03^{Ba}	0.98 ± 0.05^{Ba}	1.06 ± 0.05^{Ba}
Cohesion	0.75 ± 0.17^{ABa}	0.59 ± 0.25^{ABCa}	0.24 ± 0.08^{Ca}	0.36 ± 0.21^{BCa}	0.87 ± 0.18^{Aa}
Gumminess	2.81 ± 0.72^{ABa}	5.87 ± 2.54^{Aa}	1.17 ± 0.42^{Ba}	0.99 ± 0.66^{Ba}	1.85 ± 0.39^{Ba}
Chewiness	3.76 ± 1.1^{ABa}	6.42 ± 2.78^{Aa}	1.22 ± 0.46^{Ba}	0.91 ± 0.69^{Ba}	0.52 ± 0.40^{Ba}
Stickiness	5.22 ± 2.52^{Ba}	20.53 ± 3.97^{Ab}	4.89 ± 0.51^{Ba}	3.64 ± 1.02^{Ba}	3.57 ± 0.66^{Ba}

Different capital letters in the same line means statistical difference ($p < 0.05$) in between the different samples for each parameter evaluated. Different small letter in the column means statistical difference ($p < 0.05$) for the same sample and same parameter evaluated.
a-d are just statistical tests.

100 g (Table 8). These values were lower than those reported in the literature for *Curcubita moschata* (Piepiórka-Stepuk et al., 2023; Armesto et al., 2020). The extraction method, fruit maturity, agricultural processes, environmental conditions during storage (e.g., temperature, light, oxygen) or the time between harvest and processing may explain these differences (Kamiloglu et al., 2024; Kulczyński et al., 2020). Samples A4 and A5 exhibit significantly higher TPC compared to the other samples, probably due to the addition of NBP extracted from peels, which are rich sources of phenolic compounds (Li, 2020). A non-significant decrease in TPC after 45 days was observed in samples A2, A3, and A5, with sample A5 showing the lowest decrease (3.92%). The phenolic compounds, therefore, appear to be stable over this period. In samples A1 and A4, however, significantly lower values were found after 21 days of storage, although the further decrease in TPC by the end of the experiment was no longer significant. Applying high pressure or pasteurization to pumpkin-pulp does not seem to have a detrimental effect on the phenolic compounds. González-Cebrino et al. (2016) investigated the impact of high pressure processing (400, 500, and 600 MPa) on various parameters in pumpkin-purée, such as color or bioactive compounds. They concluded that there was no significant difference in carotenoid and phenolic content after the treatment, which resulted in a consistent nutritional quality (González-Cebrino et al., 2016). The slight decrease in TPC may also indicate low enzyme activity in the pumpkin-pulp samples, which can influence the phenolic compounds. Pretreatment processes like high-pressure treatments or food additives (e.g., citric acid) may affect the activity of polyphenol oxidase (PPO), which causes browning or discoloration of fruits and vegetables and thus has a direct influence on the shelf life or product quality of food (Jukanti, 2017; González-Cebrino et al., 2016; Li et al., 2024).

The highest FRAP values over the entire storage period were found for samples A4 and A5 (Figure 1). The higher TPC could explain the higher antioxidant capacity of these samples. Studies have shown that antioxidant capacity is positively correlated with phenolic content (Mokhtar et al., 2021; Wu et al., 2010; Li, 2020).

In contrast, the β-carotene of the samples did not increase noticeably after the addition of pumpkin peel extract. This may be explained by the extraction method used, as the peel was extracted with water, whereas carotenoids such as β-carotene are lipophilic compounds that are typically extracted using organic solvents (Saini and Keum, 2018). Consequently, the higher

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TABLE 7 Changes in β-carotene, lutein and γ-tocopherol content in freeze-dried pumpkin-pulp samples after different treatments.

Storage time (d)		Treatment				
		A1	A2	A3	A4	A5
β-carotene (mg/100 g dw)	0	150.01 ± 7.45^{a}	54.98 ± 7.95^{b}	138.01 ± 10.05^{ac}	123.32 ± 5.33^{c}	91.1 ± 5.33^{d}
	21	111.08 ± 27.65^{a}	48.88 ± 9.38^{b}	100.58 ± 5.70^{ac}	102.47 ± 4.39^{ac}	84.46 ± 3.49^{c}
	45	52.06 ± 1.65^{a}	ND	47.28 ± 6.75^{a}	41.74 ± 9.68^{a}	52.17 ± 17.32^{a}
	D %	*65.30*		*65.74*	*66.16*	*42.73*
Lutein (mg/100 g dw)	0	1.57 ± 0.07^{a}	1.21 ± 0.32^{b}	3.74 ± 0.11^{c}	1.46 ± 0.01^{ab}	0.87 ± 0.10^{d}
	21	1.17 ± 0.12^{ad}	1.53 ± 0.11^{b}	2.52 ± 0.16^{c}	1.40 ± 0.31^{ab}	0.99 ± 0.15^{d}
	45	0.54 ± 0.05^{a}	0.58 ± 0.09^{a}	1.05 ± 0.02^{b}	0.90 ± 0.07^{c}	0.63 ± 0.07^{a}
	D %	*65.81*	*51.87*	*71.98*	*38.09*	*27.35*
γ-tocopherol (mg/100 g dw)	0	0.131 ± 0.008^{a}	0.132 ± 0.003^{a}	0.186 ± 0.002^{b}	0.137 ± 0.02^{a}	0.080 ± 0.020^{c}
	21	0.084 ± 0.004^{a}	0.093 ± 0.003^{b}	0.077 ± 0.003^{c}	0.062 ± 0.005^{d}	0.064 ± 0.002^{d}
	45	0.031 ± 0.002^{a}	0.037 ± 0.001^{b}	0.029 ± 0.001^{c}	0.030 ± 0.002^{ac}	0.030 ± 0.001^{ac}
	D %	*76.13*	*72.27*	*84.42*	*78.10*	*62.26*

ND: not detectable, dw: dry weight, *D* %: degradation percentage; Different letters indicate significant differences within a row (ANOVA, $p < 0.05$, Tukey-HSD).
a-d are just statistical tests.

TABLE 8 Changes in total phenolic content (TPC) in freeze-dried pumpkin-pulp samples after different treatments.

Storage time (days)		Treatment				
		A1	A2	A3	A4	A5
TPC (mg GAE/100 g dw)	0	48.86 ± 1.13^{Aa}	42.52 ± 2.02^{Aa}	38.01 ± 2.61^{Ab}	52.49 ± 4.20^{Ac}	52.55 ± 1.69^{Ac}
	21	42.89 ± 1.78^{Ba}	40.54 ± 2.66^{Aa}	35.48 ± 1.12^{Ab}	48.75 ± 2.58^{ABc}	52.48 ± 1.87^{Ad}
	45	41.95 ± 1.55^{Bac}	38.91 ± 3.00^{Aab}	35.05 ± 0.77^{Ab}	45.87 ± 2.97^{Bc}	50.49 ± 2.73^{Ad}
	D %	14.14	8.49	7.79	12.61	3.92

TPC: total phenolic content, GAE: gallic acid equivalent, dw: dry weight, *D* %: degradation percentage; Different small letters indicate significant differences within a row, different capital letters indicate significant differences within a column (ANOVA, $p < 0.05$, Tukey-HSD).
a-d are just statistical tests.

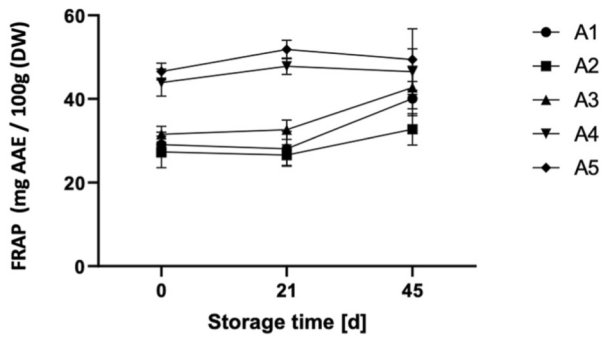

FIGURE 1
Estimation of antioxidative capacity in freeze-dried pumpkin-pulp samples during shelf life (Dw: dry weight, AAE: Ascorbic Acid Equivalent).

antioxidant capacity observed in samples A4 and A5 is likely mainly associated with phenolic compounds present in the peel extract. In addition to phenolic compounds and carotenoids, pumpkin peel may also contain other antioxidant compounds such as ascorbic acid, which could further contribute to the higher FRAP-values in samples A4 and A5 (Gavril et al., 2024).

While the antioxidant capacity of sample A5 increased significantly after 21 days, no significant changes were observed in the other samples at this time point. A notable difference between the treatment groups is that samples A4 and A5 did not differ significantly from their initial values after 45 days of storage, which may indicate a stabilizing effect of the added pumpkin peel extract. The presence of total phenolic compounds may have been sufficient to maintain antioxidant capacity and prevent the formation of additional reducing degradation products. A similar stabilizing effect of phenolic compounds has also been reported in other food systems (Oliveira et al., 2025).

TABLE 9 Proximate composition of the formulations.

		Proteins	Crude fat	Carbohydrates	Ash	kcal
Storage time (ST)	0 Days	5.1 ± 0.7	0.41 ± 0.03	90.4 ± 0.9	4.2 ± 0.4	385 ± 2
	45 Days	5.0 ± 0.8	0.35 ± 0.04	90.5 ± 0.8	4.2 ± 0.4	385 ± 2
p-value (n = 15)	Students T Test	0.014	<0.001	0.117	0.871	0.168
Preservative type (PT)	A1	4.8 ± 0.2	0.38 ± 0.05	91.2 ± 0.2	3.6 ± 0.1^{d}	387.6 ± 0.7^{a}
	A2	4.7 ± 0.3	0.36 ± 0.02	91.0 ± 0.4	4.0 ± 0.1^{c}	385.9 ± 0.5^{b}
	A3	3.97 ± 0.08	0.43 ± 0.01	90.9 ± 0.2	4.7 ± 0.2^{a}	383.3 ± 0.6^{d}
	A4	5.5 ± 0.1	0.37 ± 0.03	89.7 ± 0.3	4.4 ± 0.2^{a,b}	384.2 ± 0.8^{c,d}
	A5	6.07 ± 0.03	0.38 ± 0.09	89.4 ± 0.2	4.1 ± 0.2^{b,c}	385.3 ± 0.7^{b,c}
p-value (n = 6)	Tukey's test	<0.001	<0.001	<0.001	<0.001	<0.001
ST × PT (n = 30)	*p*-value	<0.001	<0.001	0.014	0.623	0.878

Different letters within the same column indicate significant differences between preservative treatments according to Tukey's or Tamhane's T2 *post hoc* tests ($p < 0.05$). The effect of storage time was evaluated by Student's t-test ($p < 0.05$).
a-d are just statistical tests.

In contrast, pumpkin-pulp samples A1, A2 and A3 showed a significant increase in antioxidant capacity after this storage period. Corleto et al. (2018) also reported a higher antioxidant capacity when storing arugula juice at different temperatures for 32 days. According to the authors, it may be attributed to the degradation of higher phenolic or flavonoid glucosides to lower phenolic components or aglycone moieties, respectively. Therefore, the increase observed in the samples A1-A3 likely reflects compositional changes rather than enhanced protection against oxidative deterioration. The observed changes in antioxidant capacity during storage reflect dynamic alterations in the phytochemical composition of plant-based foods. Previous studies have shown that antioxidant capacity may fluctuate over storage time, with temporary decreases followed by increases (Piljac-Žegarac and Šamec, 2011). The present results suggest that the antioxidant capacity and TPC do not deteriorate after 45 days at 5 °C. This indicates that the two different treatments (HPP, pasteurization) and the added substances (NBP and industrial mix) contribute to maintaining the oxidative stability of pumpkin-pulp.

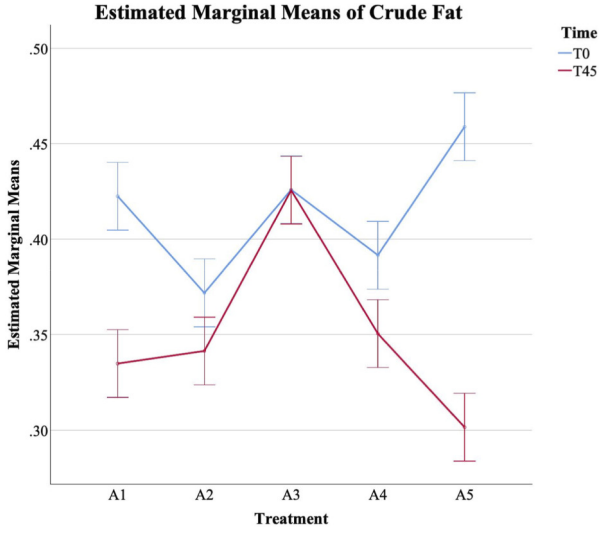

FIGURE 2
Estimated marginal means (EMM) of crude fat content for the different preservation treatments during storage (0 and 45 days). Error bars represent standard errors.

Proximate and chemical composition

Table 9 shows the proximate composition evaluated at two storage times (0 and 45 days) across the different treatments. Carbohydrates were the most abundant macronutrient, followed by proteins, as expected by a previous study (Leichtweis et al., 2023). A significant interaction was sought for proteins, crude fat, and carbohydrates. Thus, only general tendencies could be extracted from the EMM for crude fat, shown in Figure 2. In the figure, it is clear that there was a higher amount of fat at T0 than after 45 days, except for A3, where there were no differences among the samples. The lowest amount of fat was found for A5 at 45 days. This variation can be explained by the very low-fat content in the pulp. Table 10 contains the different soluble sugars found in the samples, totalling four individual sugars, of which the most abundant was sucrose and the least prevalent was trehalose. In terms of the statistical analysis, a significant interaction was found for all sugars, including the sum of these carbohydrates. Neither the EMM nor the PES allowed for any tendencies to be extracted, meaning that both storage time and the incorporation of preservatives and processing methods highly influenced the results.

Table 11 shows the 13 individual fatty acids found in the third analysis. Of these, nine were saturated fatty acids, while two were monounsaturated and another two were polyunsaturated fatty acids. The most abundant was C16:0 (palmitic acid), followed by C18:1 (linoleic acid). C10:0 (capric acid), was the least abundant fatty acid. Overall, as for Table 10, in Table 11, a significant interaction was sought between the two factors, not allowing for individual

TABLE 10 Soluble sugars detected.

		Fructose	Glucose	Sucrose	Trehalose	Total
Storage time (ST)	0 Days	10 ± 4	9 ± 4	20 ± 9	1.6 ± 0.5	40 ± 3
	45 Days	11 ± 3	10 ± 3	21 ± 3	2.4 ± 0.5	45 ± 5
p-value (n = 15)	Students T Test	<0.001	<0.001	<0.001	<0.001	<0.001
Preservative type (PT)	A1	12 ± 4	11 ± 3	22 ± 3	2.2 ± 0.7	48 ± 4
	A2	8 ± 2	8 ± 2	23 ± 3	2.0 ± 0.5	41 ± 1
	A3	9 ± 3	9 ± 4	20 ± 3	1.9 ± 0.8	40 ± 4
	A4	9 ± 2	8 ± 2	25 ± 1	2.2 ± 0.7	44 ± 4
	A5	12 ± 4	13 ± 4	13 ± 10	1.6 ± 0.5	39 ± 2
p-value (n = 6)	Tukey's test	<0.001	<0.001	<0.001	<0.001	<0.001
ST × PT (n = 30)	*p*-value	<0.001	<0.001	<0.001	<0.001	<0.001

Different letters within the same column indicate significant differences between preservative treatments according to Tukey's or Tamhane's T2 *post hoc* tests ($p < 0.05$). The effect of storage time was evaluated by Student's t-test ($p < 0.05$).

TABLE 11 Individual fatty acids detected in the formulations.

		C10:0	C12:0	C14:0	C15:0	C16:0	C18:0	C18:1n9t	C18:1n9c	C18:2n6c	C18:3n3	C20:0	C22:0	C24:0
Storage time (ST)	0 Days	0.2 ± 0.3	1.3 ± 0.3	1.9 ± 0.4	0.1 ± 0.3	43 ± 4	7 ± 3	8 ± 2	14 ± 7	6 ± 4	3.3 ± 0.7	0.7 ± 0.9	2.5 ± 0.9	3 ± 2
	45 Days	n.d	1.0 ± 0.3	1.4 ± 0.3	0.1 ± 0. 2	46 ± 4	7 ± 3	7 ± 2	19 ± 3	7 ± 4	3.4 ± 0.8	0.8 ± 0.7	2.0 ± 0.4	3 ± 2
p-value (n = 15)	Student's t-test	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.002	0.265	<0.001	<0.001	<0.001	<0.001
Preservative type (PT)	A1	0.4 ± 0.4	1.2 ± 0.4	2.0 ± 0.9	0.5 ± 0.1	43.6 ± 0.1	8.8 ± 0.1	6.0 ± 0.7	15 ± 10	11 ± 1	3.9 ± 0.6	0.6 ± 0.6	2.0 ± 0.4	n.d
	A2	n.d	1.37 ± 0.06	1.88 ± 0.09	n.d	42 ± 18	1.5 ± 0.2	9 ± 1	15 ± 1	7 ± 1	3.2 ± 0.4	n.d	1.72 ± 0.03	1.71 ± 0.06
	A3	n.d	1.2 ± 0.3	1.6 ± 0.3	n.d	43 ± 3	8.0 ± 0.7	6.2 ± 0.5	20 ± 1	1.6 ± 0.2	4.2 ± 0.2	n.d	1.9 ± 0.5	4 ± 3
	A4	n.d	0.79 ± 0.05	1.3 ± 0.2	n.d	42 ± 4	8.2 ± 0.4	10 ± 2	18.9 ± 0.6	8.8 ± 0.6	2.7 ± 0.5	1.21 ± 0.02	2.9 ± 0.8	4 ± 2
	A5	n.d	1.3 ± 0.2	1.7 ± 0.1	n.d	43 ± 5	8.6 ± 0.2	6.6 ± 0.5	13 ± 8	7 ± 4	2.6 ± 0.3	1.9 ± 0.4	2.9 ± 0.6	3 ± 1
p-value (n = 6)	Tukey's test	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
ST × PT (n = 30)	*p*-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

n.d.: not detected. Different letters within the same column indicate significant differences between preservative treatments according to Tukey's or Tamhane's T2 *post hoc* tests ($p < 0.05$). The effect of storage time was evaluated by Student's t-test ($p < 0.05$).

classifications. Neither the EMM nor the PES allowed for tendencies to be obtained.

Although statistical differences were observed, it is possible to verify that neither the change in preservatives nor the storage time substantially impacted the nutritional quality of the formulations. It is possible to verify that the quantities of nutrients are very similar and, in general, the composition profiles are not altered. This is a condition that supports the NBP use as a food additive, as the Codex Alimentarius specifies that an additive should not alter the nature or qualities of the product (Laganà et al., 2017). In contrast, pumpkin

powders, rather than extracts, do alter the nutritional and sensory characteristics of products, even if these changes are positive (Mala et al., 2018; Minarovicova et al., 2017).

Conclusion

Throughout the 45 days, samples demonstrated excellent microbial stability, with no detectable growth of microorganisms under all sample conditions. The shelf-life extension is attributed to the process treatments and their combinations with the NBP and the industrial mix. Notably, sample A4, which contained the NBP but no potassium sorbate, demonstrated the NBP effectiveness as a preservative when combined with high pressure processing (HPP) as well as with the industrial mix, thereby enhancing shelf life. This result is significant because previous studies showed that HPP alone could not achieve the same level of microbial inactivation, resulting in a shorter shelf life compared to 45 days in this study. Besides that, sample A5, which contained the NBP and a 50% reduction in potassium sorbate, also exhibited no microbial growth. Suggesting that potassium sorbate could be reduced by 50% or fully replaced by the NBP (sample A4), without compromising the product's microbial stability. Besides that, quality attributes also positively demonstrated that the combinations of HPP as a nonthermal technique have a potential effect when combined with natural-based preservative, color kept stable during shelf life, oxidative stability throughout antioxidant capacity showed that samples added with the NBP (sample A4 and A5) had significantly higher FRAP values when compared to the ones without NBP. Additionally, the presence of the NBP in the samples, along with the processes, did not compromise the nutritional quality of the pumpkin pulp. The two-factor analysis did not explain a significant influence of either the NBP or the storage time on the evaluated quality parameters.

Data availability statement

Data can be made available upon request.

Author contributions

LRR: Writing – review and editing, Formal Analysis, Writing – original draft, Data curation, Investigation, Methodology. ML: Data curation, Investigation, Formal Analysis, Writing – original draft. EL: Formal Analysis, Data curation, Writing – original draft, Investigation. BS: Writing – original draft, Formal Analysis, Investigation, Data curation. MA: Methodology, Writing – original draft, Investigation. CP: Software, Writing – review and editing, Supervision. MC: Software, Writing – original draft, Writing – review and editing. LB: Project administration, Funding acquisition, Supervision, Writing – review and editing. OS: Project administration, Funding acquisition, Resources, Writing – review and editing, Supervision.

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Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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