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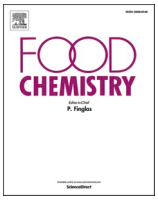

Effect of high-pressure carbon dioxide combined with modified atmosphere packaging on the quality of fresh-cut squash during storage

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ABSTRACT

The study evaluated the application of a novel high-pressure microbial inactivation method combining dense carbon dioxide with modified atmosphere packaging on organic fresh-cut squash (*Cucurbita moschata*). Approximately 4 g or 32 g of squash was packed in plastic pouches filled with CO_2 to test two different gas-to-product ratios and treated with the high-pressure method at previously optimized process conditions (45 °C, 6.0 MPa and 40 min). The products were then stored for 21 days at 4 °C and assessed for enzymatic activity, product quality, sugar content, bioaccessibility (polyphenols, DPPH antioxidant activity, and carotenoids), and sensory acceptance, with products packed in air and CO_2 serving as controls. The high-pressure treatment effectively inactivated inoculated *E. coli* to undetectable levels (inactivation >3.63 ± 0.53 Log CFU/g) and reduced the activity of the browning-responsible enzymes up to 50 %. During the shelf life, treated samples exhibited significantly higher scavenging activity for DPPH, ABTS, OH, O_2^-, and NO compared to non-treated samples, with minor exceptions at a high gas-to-product ratio. Additionally, treated samples showed increased levels of glucose and fructose and a comparable or higher bioaccessibility of antioxidants with respect to the products packed in air or in CO_2. Sensory evaluation indicated that the treatment enhanced color and smell appreciation among panelists, demonstrating the potential of this method to improve both safety and quality of fresh-cut squash.

1. Introduction

Consumers' concern regarding the possible negative health effects of food products produced with intensive farming methods has led to a great interest in the health benefits of organically-produced fruits and vegetables (Popa et al., 2019). Organic agriculture, in general, is characterized by its restriction against the use of synthetic pesticides and fertilizers, encouraging the use of fertilizers of organic origin as manure, green manure, and bone meal. Moreover, it emphasizes techniques such as crop rotation, companion planting, biological pest control, and mixed cropping (Gamage et al., 2023; Kareem et al., 2022).

Despite the diffuse consumer resistance towards new production methods, explained by Kushwah et al. (Kushwah et al., 2019) with the innovative resistance theory, the organic sector is rapidly developing. Eurostat data shows that the area used for organic agricultural production expanded from 14.7 million hectares (ha) in 2020 to 15.9

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million ha in 2021, the equivalent of 9.9 % of the total utilized agricultural area in the European Union (Statistics | Eurostat. (s.d.), *2024*).

Because of the limitation of pesticides and fungicides, many studies report a higher risk of microbial contamination in organic farming compared to the traditional one due to the action of *Escherichia coli*, mycotoxins, and parasites, leading to early quality deterioration and short shelf life of organic produce (Mditshwa et al., 2017; Rahman et al., 2021). Especially when commercialized as fresh-cut ("ready-to-eat", "ready-to-use"), post-harvest technologies are essential for enhancing the safety and extending the shelf life of fresh produce while maintaining high quality in terms of nutritional value, flavor, and freshness. Modified atmosphere packaging, passive or active, antimicrobial and antioxidant solutions and edible coatings are some of the technologies adopted to improve the product shelf life (De Corato, 2020; Ghidelli & Pérez-Gago, 2018; Kumar et al., 2023; Pratap Singh & Packirisamy, 2022; Wilson et al., 2019).

Different low-temperature technologies have been also tested to decrease the microbial load and enzymatic activity of fresh-cut products to additionally prolong their shelf life and safety. Among these, high-pressure carbon dioxide (HPCD) has been extensively studied in the pasteurization of fresh-cut vegetables (Bi et al., 2011; Spilimbergo et al., 2013), reporting promising results in terms of reduction of the activity of microorganisms and enzymes, thus improving the storage time of foods (Liao et al., 2010; Marszałek et al., 2015, Marszałek et al., 2017). However, the process was never industrially applied due to some industrialization barriers, such as the risk of post-process contamination during the packaging procedure after the treatment (Zulli et al., 2024).

A novel CO_2-based process was developed by Spilimbergo et al. (Spilimbergo et al., 2017) combining high pressures with modified atmosphere packaging (HPMAPCO_2) and exploiting the CO_2 antimicrobial power on pre-packed products. The process was tested and showed promising results for different matrices, including plant-based ones such as carrots (Barberi et al., 2021), coconut (Zambon et al., 2023), and potatoes (Zulli et al., 2023), as well as protein-based matrices like chicken breast (Santi et al., 2023, Santi et al., 2024). It resulted in enhancing the microbial safety without affecting the visual appearance and physico-chemical characteristics.

The effect of HPCD treatments was also evaluated on enzymatic activity, a critical aspect when dealing with fresh-cut food (Kong et al., 2021; Ma et al., 2022). Enzymes like Polyphenol Oxidase (PPO), Peroxidase (POD), and Pectin Methyl Esterase (PME) are key drivers of deleterious effects, such as enzymatic browning and texture degradation, that can hinder consumer acceptance. HPCD induces enzyme inactivation through multiple mechanisms, including pH lowering, conformational changes in secondary and tertiary enzyme structures, and the inhibitory effects of molecular CO_2 (Benito-Román et al., 2020; Illera et al., 2019). Such effects are particularly relevant for maintaining the freshness and sensory appeal of fresh-cut products.

Moreover, the HPMAPCO_2 process does not drastically modify the production process of fresh-cut products, which are often commercialized in modified atmospheres including CO_2, a well-established gas to extend shelf life, ensuring compatibility with current industrial practices and consumer handling. Regulatory guidelines, such as those outlined by the European Union (Regulation (EC) No. 178/2002 and Regulation (EU) No. 1333/2008), classify CO_2 as safe for use in food processing and packaging. Similarly, the U.S. Food and Drug Administration (FDA) recognizes CO_2 as Generally Recognized as Safe (GRAS), with no other constrains in applying it for innovative processes like HPMAPCO_2, except for compliance with general food safety principles and the demonstration of process efficacy.

In this study, the novel process was applied to organic fresh-cut butternut squash, a product rich in bioactive compounds, such as polyphenols, carotenoids, vitamins, and minerals (Armesto et al., 2020). Due to these bioactive compounds, squash is a good candidate product for a diet rich in components with high antioxidant potential, contributing to the reduction of the risk of developing cardiovascular diseases, cancer and diabetes mellitus (Kamiloglu et al., 2024; Kulczyński et al., 2020). However, during storage and transportation, fresh-cut squash is susceptible to microbial and endogenous enzyme activities, which can lead to spoilage, nutrient degradation and development of undesirable aromas (Armesto et al., 2020). Fresh-cut squash treated with the HPMAPCO_2 process were compared with the same products packed in air or in CO_2 during a 21-day long refrigerated storage period in terms of overall quality, enzyme activity, sugar content, antioxidant and carotenoids content, bioaccessibility and sensorial quality. The same tests were addressed by using two different gas-to-product ratios to evaluate the effect of the gas volume during both treatment and shelf life.

2. Material and methods

2.1. Sample preparation and packaging

Organic squashes (*Cucurbita moschata*) of the 'butternut' cultivar were purchased from the company "OP AGRINOVA Bio 2000" Soc. Coop (Acireale, Italy). The organic butternut products were peeled and cut into cubes with a side length of 1.5 cm and an approximate weight of 4 g. Squash cubes were packaged either in an air or 100 % CO_2 (carbon dioxide 4.0, purity >99.8 %, Nippon Gases Italia, Milano, Italy) at atmospheric pressure using a 100 %-recyclable multi-material (coextruded PE/EVOH/PE, Niederwieser, Modena, Italy) high barrier film (Niederwieser, Modena, Italy). Each package had a fixed volume of 100 mL.

Two sample-to-gas ratios were tested to study to evaluate the effect of gas volume on process performance:

1. Small scale (1:100, cube/mL): 1 cube per 100 mL, simulating an excess of gas to minimize gas limitations during the treatment and storage.
2. Medium scale (8:100, cube/mL): 8 cubes per 100 mL, reflecting a more realistic scenario for commercial applications.

Samples packaged in an air, or 100 % CO_2 atmosphere were indicated MAPair and MAPCO_2, respectively. Part of the CO_2-packed products, indicated as HPMAPCO_2, were treated with the patented method (see §2.2), and all samples were stored at 4 °C for 21 days. This duration was chosen to align with the typical shelf life of fresh-cut produce stored under refrigerated conditions, which generally ranges between 5 and 14 days, depending on the product and storage conditions.

2.2. High-pressure equipment

The HPMAPCO_2 samples, packaged in CO_2, were treated using a water-driven high-pressure equipment as described in our previous studies (Zulli et al., 2023, Zulli et al., 2024). Briefly, the high-pressure system consisted of a stainless-steel vessel (high-pressure chamber) with a volume of 4 dm^3, a storage tank with an immersion thermostat (M900-TI, MPM Instruments, Italy), and an air-driven hydraulic pump (G35LVE, Maximator, Italy). The system was designed to work at a maximum temperature and pressure of 50 °C and 20 MPa, respectively.

Before each test, the high-pressure chamber and the water in the storage tank were preheated to the desired temperature. Then, the packaged products were placed inside the chamber, which was closed, filled and pressurized with water using the high-pressure pump until the desired pressure was reached. In these conditions, the CO_2 inside the packages reach a dense state, acting as a microbial inactivation agent. Temperature and pressure were continuously monitored and maintained constant throughout the treatment duration. After the treatment, the chamber was depressurized, and the products stored at 4 °C before being processed and/or analyzed.

2.3. Optimization of processing conditions

Process conditions were selected by testing the inactivation of the

method on clinically isolated *Escherichia coli* NCTC 9901, surrogate of different pathogenic bacteria such as *Escherichia coli* O157:H7 and *Salmonella enterica*. This choice was made due to the relevance of these pathogens in food safety and the need for a well-characterized, reproducible model organism. The microbial strain was inoculated on the entire product surface by immersion as previously described in (Zulli et al., 2023, Zulli et al., 2024). The inoculated products were then packaged/treated or directly analyzed to measure the initial microbial load.

The standard plate count technique was used to determine the *E. coli* load before and after the treatment. Samples were vortexed at 2200 rpm for 90 s in a 50-mL tube together with Ringer's solution (Merck KGaA, Darmstadt, Germany) in a ratio of 1:10 in weight. The obtained solution was then serially diluted and 100 μL of the appropriate dilutions were spread-plated on Petri dishes with MacConkey agar with crystal violet (Microbiol Diagnostici, Cagliari, Italy) and then incubated at 37 °C for 24 h. Results were expressed as the decimal logarithm of the number of colony forming units (CFU) per gram of product (Log CFU/g). The under-detection limit of the used technique was 100 CFU/g (2 Log CFU/g).

2.4. Physico-chemical analysis

pH, °Brix, and color were measured for all samples throughout the storage period. Squash cubes were milled using a grinder (Moulin Broyeur, VEOHOME, France) to obtain ground samples. The °Brix of the solution obtained by squeezing the ground samples was measured using a refractometer (HHTEC, Germany). The pH of the ground samples mixed with distilled water (1:1, *w/v*) was measured using a digital pH meter (pH 1100 L, VWR, USA). The color of cubes was determined using a precision colorimeter (NR100, 3nh Technology Co., Ltd., China). The total color difference (ΔE) was calculated by using Eq. (1):

$$\Delta E = \sqrt{\left(L_1^* - L_2^*\right)^2 + \left(a_1^* - a_2^*\right)^2 + \left(b_1^* - b_2^*\right)^2}. \quad (1)$$

where L*, a* and b* represent lightness, redness, and yellowness, respectively, while 2 and 1 represent the fresh sample and the analyzed ones, respectively.

2.5. Enzyme activities

PPO and POD activities were determined using the spectrophotometric assay described by Szczepańska et al. (Szczepańska et al., 2022) with some adjustments. The extraction solution was prepared by mixing 4 g of polyvinylpolypyrrolidone (PVP), 0.1 mL of Triton X-100, 58 g of sodium chloride, and 100 mL, pH = 6.5 of sodium phosphate buffer. 5 g grated samples mixed with 10 mL of extraction solution were homogenized using a homogenizer (CAT×120, Germany) at 16,000 rpm for 3 min. The supernatants were collected after centrifugation at 11,000 rpm and 4 °C for 30 min. 0.1 mL of supernatant was added to 3 mL of 0.07 M catechol solution (pH = 6.5). After a 10-min reaction at room temperature, the PPO content was measured at 420 nm using UV–Visible spectrophotometer (Model 6705, Jenway, UK). 0.05 mL of supernatant was mixed with 0.05 mL of 1 % *p*-phenylenediamine, 0.05 mL of 1.5 % hydrogen peroxide, and 3 mL of 0.05 mol L^{-1} phosphate buffer (pH = 6.5). The reaction was carried out at room temperature for 10 min, and the absorbance was measured at a wavelength of 485 nm. Enzymatic activity was expressed as enzyme units per mg (U/mg).

2.6. Antioxidant activity

Bioactive compounds in MAPair, $MAPCO_2$, and $HPMAPCO_2$ samples were extracted through homogenization and ultrasound (Chen et al., 2024a). Specifically, 5 g of grated samples were added to a 50 mL centrifuge tube containing 10 mL of 80 % methanol. The mixture was homogenized in a 4 °C water bath at 20,400 g for 10 min, then ultrasonicated at 45 kHz, 200 W, and 25 °C for 20 min, and centrifuged at 11,000 rpm and 4 °C for 5 min. The supernatant was subsequently filtered through a 0.25 μm polytetrafluorethylene membrane for further analysis of total phenolic contents (TPC) and antioxidant capacity.

ABTS· + and DPPH· radical scavenging activities were measured according to the methodology reported by Chen et al. (Chen et al., 2024b) at 734 nm and 517 nm, respectively. The results were expressed as μmol/L Trolox equivalents per 100 g of sample.

Hydroxyl radical (·OH) scavenging was determined using the salicylic acid method (Zhu et al., 2022). Briefly, 200 μL of supernatant were mixed with 1 mL of 9 mmol L^{-1} salicylic acid solution (dissolved in 70 % ethanol). Next, 1 mL of a 9 mM H_2O_2 solution was added to the mixture, and the reaction was conducted at 37 °C for 30 min. The absorbance was measured at 510 nm.

Superoxide ($O2\cdot^-$) scavenging was measured using the method proposed by Chun et al. (Chun et al., 2003). 0.25 mL of supernatant were added to 2 mL of reaction solution (0.45 mM NADH, 0.15 mmol L^{-1} NBT, and 28 mmol L^{-1} PBS in a 1:1:4 *v*/v/v ratio). Then, 0.25 mL of 0.12 mmol L^{-1} PMSc, were added to initiate the reaction. After incubating for 5 min, the absorbance was measured at 560 nm.

Nitric oxide (NO) scavenging was assayed according to the methodology of Sueishi et al. (Sueishi et al., 2011). A mixture containing 250 μL of supernatant, 500 μL of 10 mmol L^{-1} SN, and 250 μL of PBS (pH 7.4) was incubated at 37 °C for 2.5 h. Subsequently, 1 mL of Griess Reagent was added to the mixture, which was then incubated for 30 min before measuring the absorbance at 546 nm.

The determinations of antioxidant capacity through oxygen radical absorbance capacity (ORAC) and Folin-Ciocalteu reagent (FCR) assays were also carried out. 5 g of squash samples were mixed with 50 mL of an 80 % ethanol solution containing 1 % citric acid in flasks fitted with caps. The mixture was stirred at 120 rpm for 24 h at 25 °C. After 24 h, the hydroalcoholic extract was filtered, and the residual squash was stirred again with another 50 mL aliquot of the same solvent under the same conditions. The combined extracts were then subjected to under-vacuum distillation using a rotary evaporator (Rotavapor RE111, Büchi, Flawil, Switzerland) to remove residual ethanol. The resulting extracts, rich in bioactive molecules, were used for further determinations of antioxidant activity (ORAC and FCR assays) and phenolics by HPLC.

The ORAC assay was performed as described by Cao et al. (Cao et al., 1993) and improved by Ou et al. (Ou et al., 2001) with some modifications. Briefly, measurements were performed using a Wallac 1420 Victor III 96-well plate reader (EG & Wallac, Turku, Finland) equipped with fluorescence filters (excitation 485 nm, emission 535 nm). Fluorescein (116 nmol L^{-1}) was used as the target molecule for free radical attack from AAPH (153 mM), which served as the peroxyl radical generator. The reaction was conducted at 37 °C and pH 7.0, with Trolox (1 μmol L^{-1}) as the control standard and 75 mmol L^{-1} phosphate buffer (pH 7.0) as the blank. All solutions were freshly prepared before analysis. Squash extracts were appropriately diluted with phosphate buffer (1:25–100, *v*/v) prior to analysis, and results were reported as μmol Trolox equivalents (TE) per gram of fresh sample (μmol TE/g). The Folin-Ciocalteu colorimetric method (Singleton et al., 1999) is typically used to determine total phenols but is also applied to evaluate antioxidant activity. Appropriately diluted sample extracts (1 mL) were mixed with 5 mL of FCR (previously diluted with water 1:10 v/v) and 4 mL of a 7.5 % sodium carbonate solution. The mixture was stirred for 2 h at room temperature while avoiding light exposure. The absorbance of the resulting blue solution was measured spectrophotometrically at a wavelength of 765 nm, and the concentration of total phenolics was expressed as mg of gallic acid equivalents per 100 g of fresh weight (mg GAE/100 g FW).

2.7. Analysis of phenolic and flavonoid composition

The phenolic and flavonoid compounds profile was determined by high-pressure liquid chromatography using a Waters Alliance 2695

HPLC (Waters Corporation, Milford, MA) equipped with a Waters 996 photodiode array detector and Empower software. The separation was achieved on a Luna (Phenomenex, Torrance, CA, USA) C18 analytical column (250 mm × 4.6 mm, 5 μm). The binary solvent delivery system was designed as follows:

- mobile phase A: water/formic acid (99.8:0.2, *v*/v),
- mobile phase B: acetonitrile/water/formic acid (50:49.8:0.2, v/v/v).

The gradient profile was as follows: 10–35 % B (0–30 min), 35–40 % B (30–35 min), 40–55 % B (35–40 min), 55–75 % B (40–50 min), 75–95 % B (50–55 min), 95–100 % B (55–57 min), 100–10 % B (57–60 min). The flow rate was set at 1 mL/min, the column temperature was maintained at 35 °C, and the injection volume of each sample was 20 μL. Data were analyzed at a wavelength of 320 nm for phenolic acids and 280 nm for flavonoids, respectively. All analyses were repeated three times, and quantification of the phenolic compounds and flavonoids were performed using external standard calibration curves for protocatechuic acid, catechin, syringic acid, quercetin and kaempferol and expressed as mg per kg of sample.

2.8. Determination of carotenoids

5 g of squash samples were extracted with 15 mL of a hexane/methanol/acetone (2:1:1, v/v/v) mixture. The extraction was performed at room temperature for 30 min under continuous and gentle shaking. The sample was then centrifuged (9000 rpm at 5 °C) for 5 min and the supernatant was recovered. This step was repeated twice more, and the collected organic portions were evaporated at a temperature lower than 30 °C using a rotary evaporator. The dried extracts were dissolved in 4 mL of acetonitrile and filtered (nylon, 0.22 μm) before HPLC analysis. Chromatography was carried out with a Waters Alliance liquid chromatography system equipped with a quaternary pump, a photodiode array detector, an autosampler, and Empower Manager. Carotenoids were identified using LC-DAD by comparing the retention times and UV–vis absorption spectra and chromatography with standards at λ = 450 nm for all carotenoids (Bergantin et al., 2018). Results were expressed as mg of compounds per kg of sample.

2.9. Determination of ascorbic acid

The extraction of ascorbic acid was carried out by placing 5 g of squash with 50 mL of a 3 % metaphosphoric acid solution and stirred at 120 rpm for 4 h at 25 °C. The supernatants were filtered through a 0.45-μm PTFE membrane filter, and 20 μL were injected into the HPLC using a Waters Alliance 2695 HPLC (Waters Corporation, Milford, MA) equipped with a Waters 996 photodiode array detector and Empower software. The mobile phase was 0.02 M H_3PO_4, flow rate was 1 mL/min, and the detector was set at 260 nm. Results were expressed as mg of ascorbic acid per kg of sample.

2.10. Micro- and macro-nutrients profile

Micro- and macro-elements content were determined by Inductively Coupled Plasma Spectrometry (ICP-OES Optima 2000DV, Perkin Elmer, Italy). Squash samples (5 g) were subjected to dry digestion in a muffle furnace at 550 °C for 24 h. The samples were then dissolved in a solution containing 4 mL of distilled water and 0.5 mL of concentrated nitric acid. The solutions were then poured into flasks and made up to 50 mL with distilled water before the measurements. Results were expressed in mg per 100 g of fresh sample.

2.11. Bioaccessibility

In vitro determination of bioaccessibility was carried out on freeze dried samples at day 0 and during the shelf life with the internationally accepted INFOGEST method as described by Brodkorb et al. (Brodkorb et al., 2019). Total phenolic compounds, antioxidant activity (DPPH) and total carotenoids were analyzed before digestion, in the stomach phase and in the small intestine phase. Bioaccessible fraction was separated by centrifuging the residual fraction of the intestinal phase (12,800 rpm, 30 min, 4 °C) as described by Borges et al. (Borges et al., 2019). Additionally, bioaccessibility ratio was reported as a percentage expression of the amount of the component in the bioaccessible fraction relative to the amount of the component present in the material before digestion (Reboredo-Rodríguez et al., 2021).

Total phenols, antioxidant activity and total carotenoids were determined by spectrophotometric methods. Total phenols (mg GAE/100 g FW) were determined by using Folin–Ciocalteu method according to Thaipong et al. (Thaipong et al., 2006). Antioxidant activity (mg Trolox/100 g FW) were determined by using the DPPH method of Brand-Williams et al. (Brand-Williams et al., 1995) and total carotenoids (mg/100 g FW) according to Yılmaz (Yılmaz, 2010).

2.12. Sugar contents

The sugar contents were evaluated for all sample groups throughout the whole storage, except for MAPair which was spoiled after day 9. They were measured according to the approach of Marszałek et al. (Marszałek et al., 2019) with some modifications. Specifically, 5 g grated squash was added to a 50 mL centrifuge tube containing 10 mL of ultrapure water. The mixture was homogenized in a 4 °C water bath at 16,000 rpm for 10 min and ultrasonicated at 45 kHz, 200 W, and 25 °C for 5 min. Subsequently, the mixture was centrifuged at 11,000 rpm and 4 °C for 5 min. The supernatant was analyzed using an HPLC system (Waters 2996, USA). The supernatant was eluted with 0.1 mmol L^{-1} calcium disodium EDTA by Sugar-Pak I and Guard-Pak column at 90 °C and 0.5 mL/min and detected by a refractive index detector. Results were expressed as g of sugar per L of supernatant.

2.13. Sensory attributes

The sensory attributes of fresh-cut squash before and after processing (MAP-air, MAP-CO_2, and HPMAP-CO_2) were assessed using quantitative descriptive analysis (Gaowa et al., 2023; Guan et al., 2024; Saha et al., 2023). The evaluation form included four criteria: color, appearance, smell, and texture. Each attribute was scored on a scale from 0 to 10, where 0 indicated no intensity and 10 indicated high intensity. The evaluation was conducted in a sensory analysis laboratory that complied with all requirements outlined in ISO 8589:2010/A1:2014. Sensory evaluation was performed in independent triplicates by 16 trained judges, all qualified for expert assessment in accordance with ISO 8586:2023. Each sample, prepared in equal quantities and at a consistent temperature, was placed on a plate and covered with a lid to monitor changes over a 21-day storage period. MAP-air samples were not evaluated beyond day 9 due to spoilage.

2.14. Statistical analysis

All measures were performed in triplicate and the results were expressed as mean values ± standard deviation. A two-way ANOVA test followed by Šídák's multiple comparison tests was performed using the software GraphPad Prism version 10.2.3.

3. Results

3.1. Selection of processing conditions

Process conditions were selected based on their efficacy in inactivating an inoculated microorganism, specifically *E. coli*, on the product surface. This approach was chosen because the inoculation procedure ensured a reproducible and uniform initial microbial load, which could

not be achieved using the natural microbial flora of fresh-cut squash due to its inherent variability. The use of *E. coli* NCTC 9901 as a surrogate model provided a reliable and standardized method to evaluate the treatment's efficacy under controlled conditions.

Squash samples inoculated with *E. coli* were prepared, packed, and treated according to the procedures outlined in §2.1 and §2.3. A previous study using a synthetic matrix (Zulli et al., 2024) demonstrated that a pressure of 6 MPa was most effective for microbial inactivation, with the possibility to minimize adverse effects on the product's visual quality. Consequently, the pressure was fixed at 6 MPa and the effects of temperature (35–45 °C) and time (0–60 min) were studied.

The results, presented in Fig. 1, indicate that 35 °C did not affect the inactivation of *E. coli*, even after 60 min of treatment. On the contrary at 45 °C a significant inactivation was obtained, reaching values under the detection limit of the adopted microbial count technique (2 Log CFU/g) after 40 min of treatment. Process inactivation remained below the detection limit beyond 40 min. This value is well below the maximum limit established by EU regulations for ready-to-eat vegetable products, set at 4 Log CFU/g (Commission Regulation (EC) No. 2073/2005).

For these reasons, the process conditions selected for the further qualitative studies of squash were 6 MPa, 45 °C and 40 min.

3.2. Physicochemical properties

The physicochemical properties of food products during storage are crucial indicators for monitoring the product's state and quality and are pivotal for consumer acceptance. Fig. S1 (A-D) presents changes in pH and °Brix of MAPair, $MAPCO_2$, and $HPMAPCO_2$ samples during 21 days of storage. Immediately after the high-pressure process (day 0), the pH difference between $HPMAPCO_2$ and MAPair (fresh) samples was not significant, indicating that high pressure processing did not cause a reduction in pH. In the small-scale experiments, the pH of samples packed in air (MAPair) and CO_2 ($MAPCO_2$) slightly increased throughout the shelf life, with no significant difference between them except for day 13 and 21. In contrast, the pH of $HPMAPCO_2$ samples remained lower and constant at a value close to 6. In the case of the medium-scale trials, the treatment did not immediately reduce the pH, but the value slightly increased also for $HPMAPCO_2$ samples, showing a smaller difference with the non-treated samples. This difference became not significant after the 13^{th} day of storage.

The °Brix grade of fresh-cut squash for the analyzed products in small and medium scale samples is shown in Fig. S1C and S1D. The application of the high-pressure treatment resulted in a reduction of °Brix in both scales. Following this initial reduction, the °Brix values for both MAPair and $HPMAPCO_2$ remained relatively stable throughout the 21-day storage period in both small- and medium-scale samples. The use of a 100 % CO_2 atmosphere ($MAPCO_2$), instead, led to oscillating °Brix values, fluctuating between those of MAPair and $HPMAPCO_2$ ones.

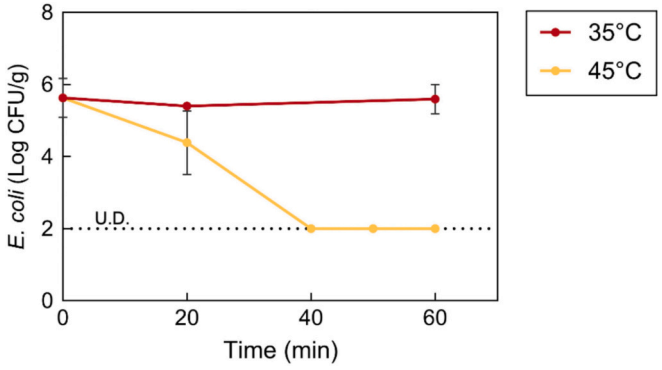

Fig. 1. *E. coli* microbial load, expressed in Log CFU/g, on squash samples treated with $HPMAPCO_2$ at 6 MPa and two different temperatures (35 and 45 °C). U.D.: under detection limit (2 Log CFU/g).

Although °Brix grade is a good indicator of dissolved solids and it is used to measure dissolved sugars in a food product, a detailed analysis of glucose, fructose, and sucrose contests was conducted to better understand the sugar behavior in untreated and treated squash samples (see §3.7).

The sample color was evaluated during the shelf life and compared with the fresh squash (day 0) by the calculating of the total color difference. The results are reported in Fig. S1 (E and F). All sample groups showed an increase in total color difference over the shelf life. However, the $HPMAPCO_2$ group exhibited the highest color difference throughout the entire storage period, that could be also observed in the pictures reported in Fig. S2. The color of the matrix was also assessed during the sensory analysis (§3.8) to determine whether this color change might be negatively perceived by a potential consumer.

3.3. Enzymatic activity

The changes in the polyphenol oxidase (PPO) and peroxidase (POD) activities of MAPair, $MAPCO_2$ and $HPMAPCO_2$ samples during 21 days of storage are displayed in Fig. 2. On the small scale, the PPO and POD activities of all samples exhibited a fluctuation and reached a maximum value at 6 days and 3 days of storage, respectively. After 9 days of storage, no significant differences were noted in the $HPMAPCO_2$ samples, while the PPO and POD activities in the $MAPCO_2$ gradually decreased with the storage time increased. Furthermore, the PPO and POD activities in $HPMAPCO_2$ samples were lower than those in MAPair and $MAPCO_2$ samples during the whole shelf life. On the medium scale, the PPO and POD activities of all samples had similar behaviors than the ones at small scale. The reduction in PPO and POD activities of fresh-cut squash after $HPMAPCO_2$ treatment (11 % and 19 %) on the medium scale was lower than on the small scale (38 % and 43 %). However, in this case after 9 days, POD activity of $HPMAPCO_2$ and $MAPCO_2$ were comparable, while PPO activity was still lower in the $HPMAPCO_2$ group. Overall, $HPMAPCO_2$ processing effectively reduced PPO and POD activities in fresh-cut squash and maintained them at lower levels during storage. The ratio of raw material and CO_2 can significantly influence the performance of $HPMAPCO_2$ processing.

3.4. Antioxidant activity

The antioxidant activity of MAPair, $MAPCO_2$, and $HPMAPCO_2$ samples during storage is displayed in Figs. 3, 4, and 5.

The CO_2-based treatment did not reduce the ABTS activity, but higher values were observed for $HPMAPCO_2$ samples after day 3 of storage. Treated samples displayed indeed a slight peak at day 9 with a slight decline after that point. MAPair and $MAPCO_2$ samples instead showed a peak at day 3, with a rapid decrease after that. The trends of the ABTS radical scavenging activity in squashes on the medium scale (Fig. 3B) were comparable to that on the small scale, with all samples showing an initial increase. Then, while MAPair and $MAPCO_2$ samples showed a decline in activity over time, $HPMAPCO_2$ values remained stable throughout the entire storage. Thus, the $HPMAPCO_2$ treatment maintains higher activity levels of ABTS for a longer period compared to the other two.

In contrast, the results obtained for DPPH radical scavenging activity were quite different between small scale (Fig. 3C) and medium scale (Fig. 3D). In small-scale trials, the treatment led to a significant reduction of DPPH activity. Then, all samples displayed a peak around day 9 followed by a decline until the end of the storage period. However, $MAPCO_2$ showed a steeper decline with respect to $HPMAPCO_2$, reaching similar levels after day 13. Differently, in the medium-scale trials, the process effect on DPPH activity was minimal and the $HPMAPCO_2$ samples showed higher values during the shelf life.

The MAP-air treatment demonstrated a sharp decline in OH radical scavenging activity over time in both scales (Fig. 3E and F). In contrast, $MAPCO_2$ and $HPMAPCO_2$ samples showed a more gradual decline after

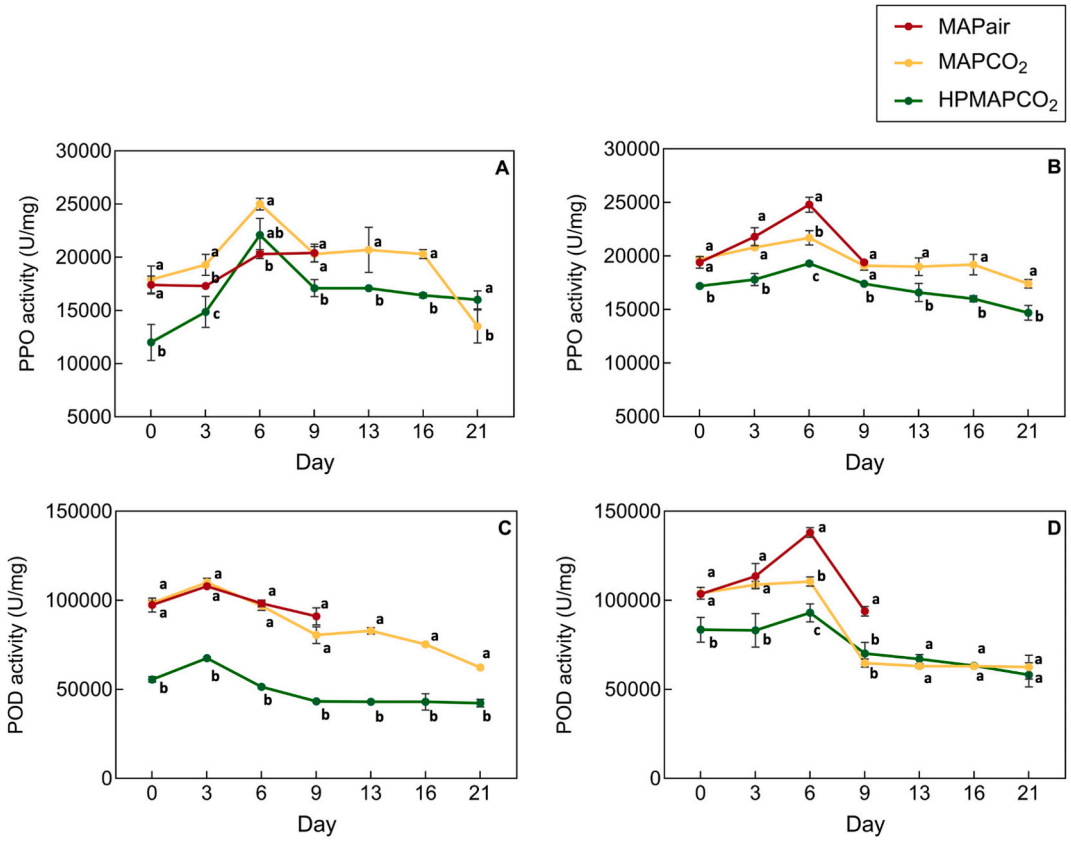

Fig. 2. Changes in enzyme activity of MAPair (red), $MAPCO_2$ (yellow), and $HPMAPCO_2$ (green) samples during 21 days of storage. A) PPO activity on a small scale; B) PPO activity on a medium scale; C) POD activity on a small scale; D) POD activity on a medium scale. Different letters indicate statistically different values ($\alpha = 0.05$) in the same time point. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

an initial increase, with the $HPMAPCO_2$ treatment maintaining higher levels of OH radical scavenging activity in the medium scale but slightly lower values in the small scale.

Fig. 4 A showed a general decline in $O2^-$ radical scavenging activity over time for all treatments on a small scale. While MAP-air sample's values almost immediately decrease their value, $MAPCO_2$ and $HPMAPCO_2$ showed an incremental peak at day 6 followed by a reduction. However, $HPMAPCO_2$ values were always higher at the end of the storage. Similarly, results in medium-scale trials (Fig. 4B) show a higher $O2^-$ radical scavenging activity for $HPMAPCO_2$ samples with a higher peak at day 13 of storage.

Finally, the NO radical scavenging activity showed a peak between day 6 and 9 for all treatments in both small scale (Fig. 4C) and medium scale (Fig. 4D). After the peak, all the value decreased but the $HPMAPCO_2$ treatment maintained higher scavenging activity.

Results obtained by the ORAC assay are reported in Fig. 5A and B. Regarding the small-scale trial, ORAC units, expressed in μmol TE/g of sample, reported oscillating values ranging between 1195.50 ± 44.19 ($MAPCO_2$ squash samples after 21 days) and 1957.50 ± 376.53 (MAPair squash samples after 6 days of refrigerated storage). Small differences were observed in the small-scale trials without a specific trend. In the medium-scale treatments, the ORAC unit value was constant during the storage with no differences observed between the three samples for each time-point.

The results of the Folin-Ciocalteu reagent assay are reported in Fig. 5C and D. In the small-scale trial, TPC was increased by about 33 % compared to the fresh samples as a result of the treatment with high pressure CO_2. $HPMAPCO_2$ samples displayed the highest values of phenolic content compared to both MAPair and $MAPCO_2$, while the lowest value was observed for MAPair after 13 days of storage (89.65 ± 3.13 mg GAE/100 g FW). In the medium-scale trial no differences were recorded immediately after the treatment and the same behavior was noted until 6 days of refrigerated storage. After 13 days of storage the MAPair squashes showed the lowest phenolic content equal to 80.5 ± 0.1 mg GAE/100 g FW.

3.5. Phenolic compounds, carotenoids, ascorbic acid, micro- and macro-nutrients

The individual phenolic compounds and flavonoids detected in squash samples in small- and medium-scale trials are shown in Table S1. The main detected phenolic components were protocatechuic acid, catechin, and syringic acid while the main flavonoids were represented by quercetin and kaempferol. As a general consideration, both in the small scale and in the medium scale, free individual compounds showed a slight decline during the refrigerated storage, presumably linked to polymerization reactions that might have occurred between phenolic acids and free sugars or other organic acids naturally present in the squash tissue (Shahidi & Yeo, 2016; Zhang et al., 2020). The statistical analysis performed for each timepoint of the refrigerated storage showed that in the small-scale trial $HPMAPCO_2$ samples retained significantly higher levels of protocatechuic acid (at day 6), catechin (at day 21), syringic acid (at day 21), quercetin (at day 6 and 21) and kaempferol (at day 6) compared to MAPair and $MAPCO_2$ samples. No statistically significant differences were instead recorded for the medium-scale trial.

The results of the HPLC determination of ascorbic acid content are reported in Fig. 6A and B. As seen before, squash samples showed differential behavior in the small-scale and the medium-scale trial. Ascorbic acid content was demonstrated to be sensitive to the high-pressure CO_2 treatment in the small-scale treatment with lower values recorded for the $HPMAPCO_2$ samples compared to the other two treatments

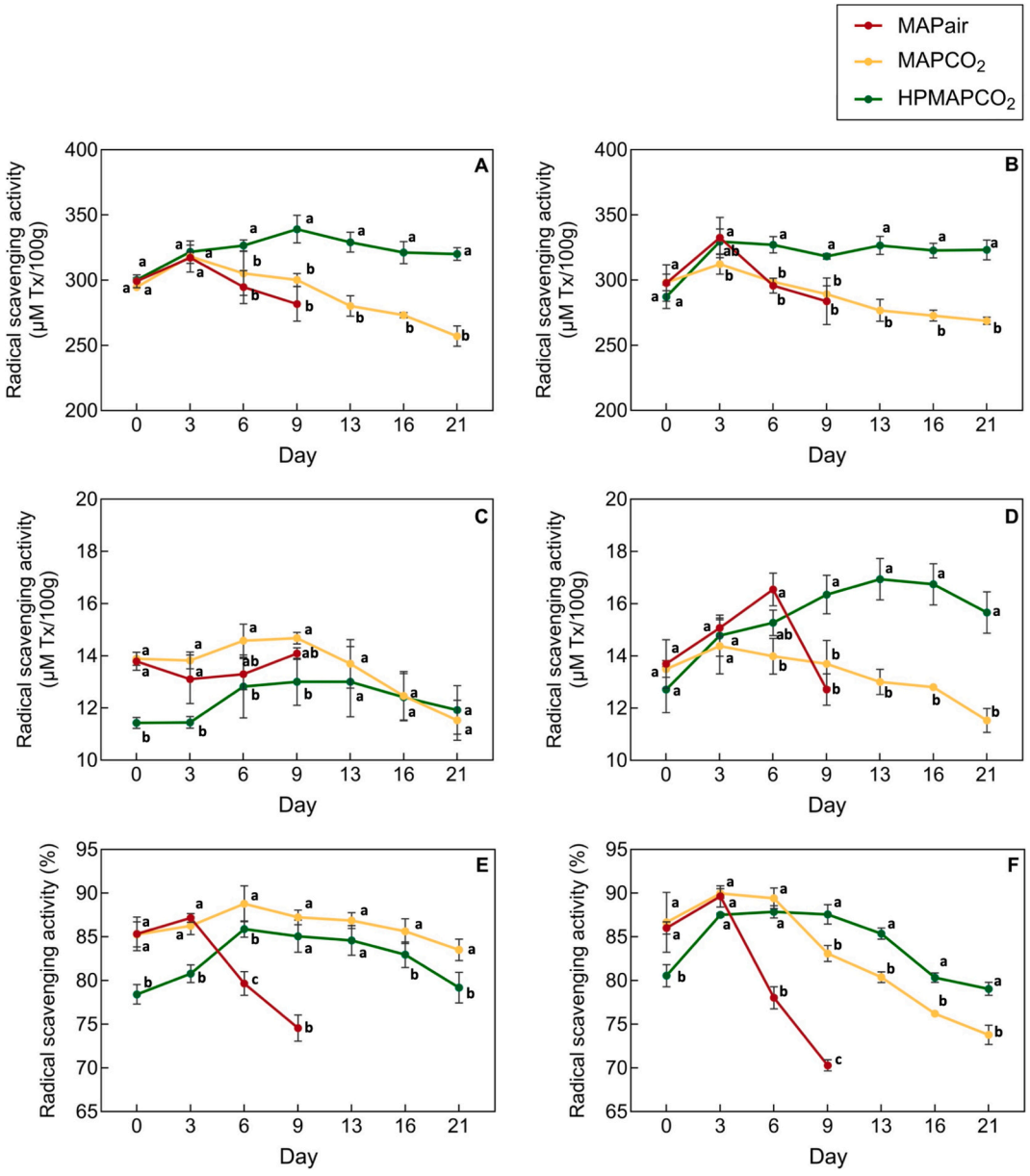

Fig. 3. Antioxidant activity during a 21-day storage period for fresh-cut squash samples MAPair (red), $MAPCO_2$ (yellow), and $HPMAPCO_2$ (green). A) ABTS radical scavenging activity on a small scale; B) ABTS radical scavenging activity on a medium scale; C) DPPH radical scavenging activity on a small scale; D) DPPH radical scavenging activity on a medium scale; E) OH radical scavenging activity on a small scale; F) OH radical scavenging activity on a medium scale. Different letters indicate statistically different values ($\alpha = 0.05$) in the same time point. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

immediately after the treatment (day 0) and along the refrigerated storage. On the other hand, in the medium-scale trial, its values showed an increase due to the treatment, remaining then constant along the refrigerated storage.

Total carotenoids content was reported in Fig. 6C and D, while ascribed individual components, specifically α-carotene, β-carotene, and lutein, were determined through HPLC analysis, and results are reported in Fig. S3. In the small-scale trial, the total and individual carotenoid content showed an increase as a result of the treatment (day 0), recording a decrease only after 6 days of cold storage. At the end of cold storage (day 21) the α-carotene and β-carotene contents were higher in the $HPMAPCO_2$ samples compared to the other two treatments. As far as the medium-scale trial is concerned, a different behavior was evidenced, with a decrease in total and individual carotenoids in $HPMAPCO_2$ squashes immediately after the treatment (day 0) and lower or intermediate values, with respect to the other two treatments, along the refrigerated storage till day 13.

Macro- and micro-nutrients were determined by ICP-OES and results are shown in Table S2. Both in the small-scale and in the medium-scale trial, K, P, Ca, Na and Mg resulted as the most represented minerals. In the small-scale trial, slight differences among the three samples with lower values being recorded for Cu and Zn and higher values for P in the $HPMAPCO_2$ samples. The medium scale trial showed that after 13 days of storage Na and Fe levels were significantly lower in $HPMAPCO_2$ compared to $MAPCO_2$ samples while P levels in $HPMAPCO_2$ samples were higher. However, it can be stated that the high-pressure treatment did not relevantly affect the micro and macro elemental quali-quantitative pattern of the squash samples which maintained adequate levels of these elements during the refrigerated storage.

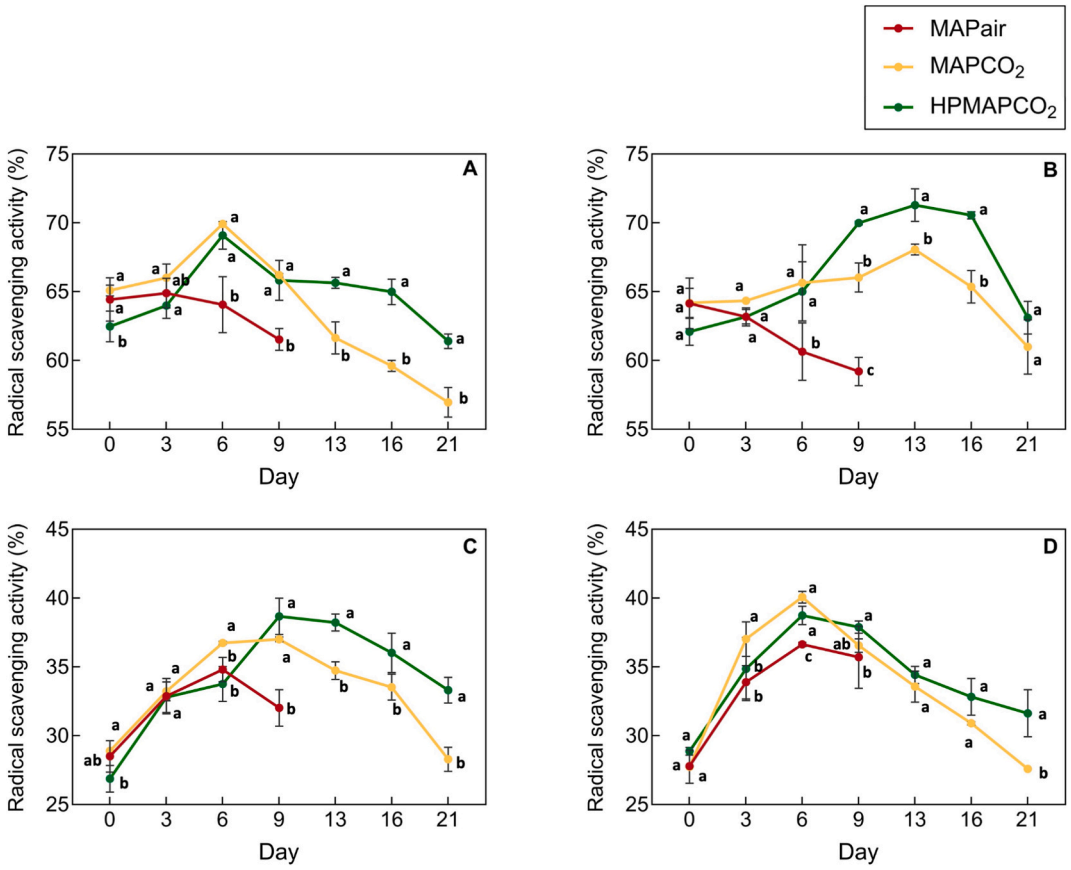

Fig. 4. Antioxidant activity during a 21-day storage period for fresh-cut squash samples MAPair (red), $MAPCO_2$ (yellow), and $HPMAPCO_2$ (green). A) O_2^- radical scavenging activity on a small scale; B) O_2^- radical scavenging activity on a medium scale; C) NO radical scavenging activity on a small scale; D) NO radical scavenging activity on a medium scale. Different letters indicate statistically different values ($\alpha = 0.05$) in the same time point. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.6. Bioaccessibility

Bioaccessibility studies were conducted during the shelf life of three samples (MAPair, $MAPCO_2$, and $HPMAPCO_2$) for total polyphenols, DPPH antioxidant activity, and total carotenoids. Their contents in the freeze-dried samples, in the stomach phase, in the intestine phase, and in the bioaccessible fraction are shown in Table S3 of the Supplementary materials, while the bioaccessible fractions are shown in Figs. S4A, S4C, and S4E for small-scale trials, and Figs. S4B, S4D, and S4F for medium-scale trials.

It is possible to observe how the bioaccessible ratio of treated samples is similar or higher than that of fresh samples, for both small and medium scales. This can be noted during the whole shelf life for polyphenols, antioxidants, and carotenoids: while MAPair and $MAPCO_2$ had similar bioaccessibility ratios, the value for treated samples was usually higher.

3.7. Sugar contents

In this section, sugar contents, specifically glucose, fructose, and sucrose, were analyzed and results are shown in Fig. 7.

Both glucose and fructose (Fig. 7A, B, C, and D) showed an increasing trend throughout the shelf life, while sucrose (Fig. 7E and F) showed a decreasing trend in a similar manner between small and medium scale and for all samples.

Regarding glucose, the treatment did not lead to any immediate variation in glucose concentration. However, $HPMAPCO_2$ samples generally showed the highest glucose content compared to MAPair and $MAPCO_2$.

Very similar trends were observed for fructose, with the only difference being that in small-scale trials the process application led to an increase of the fructose content in $HPMAPCO_2$ samples, resulting also in a higher value during the storage. This difference was not noted in medium-scale treatment.

The sucrose content of MAPair, $MAPCO_2$, and $HPMAPCO_2$ showed a decreasing trend in both scales. $HPMAPCO_2$ sucrose content was slightly lower than the others, but this difference was not significant in many time points of the storage, especially compared to $MAPCO_2$ samples. These trends are line with °Brix results presented in Figs. S1C and S1D.

3.8. Sensory attributes

Consumer acceptance of the squash samples was evaluated through panel tests conducted over the shelf life of the product. The sensory attributes assessed included color, appearance, smell, and texture for both small scale and medium scale samples. Results are shown in Table 1.

On the small scale, MAPair scores for all sensory attributes decreased from day 0 to day 9 of storage. $MAPCO_2$ scores for color declined over time, reaching the lowest point at 21 days of storage. Appearance, smell, and texture also exhibited a gradual decrease throughout the storage period. Compared to MAPair samples, color scores were similar, while appearance and texture received lower ratings from the panelists. Smell scores were instead higher. However, it should be noted that MAPair samples were not evaluated after day 9 due to spoilage.

$HPMAPCO_2$ samples had color scores that remained stable until day 9 of storage and then rapidly declined. Appearance scores decreased significantly, especially from 6 to 21 days of storage. Smell scores

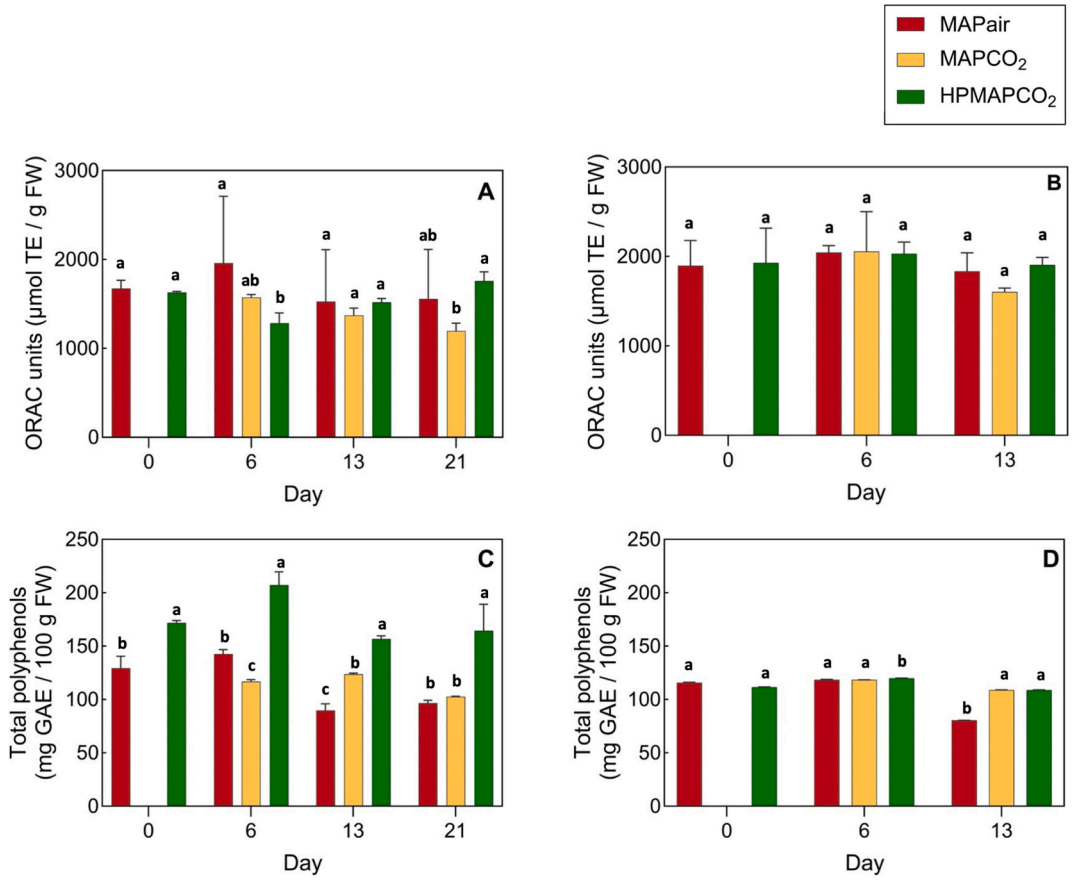

Fig. 5. ORAC units and total polyphenols (FCR essay) during a 21-day storage period for fresh-cut squash samples MAPair (red), $MAPCO_2$ (yellow), and $HPMAPCO_2$ (green). A) ORAC units on small scale; B) ORAC units on medium scale; C) Total polyphenols on small scale; D) Total polyphenols on medium scale. Different letters indicate statistically different values ($\alpha = 0.05$) in the same time point. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

decreased slightly over time, while texture showed a significant drop after 6 days of storage, with a further decline by 21 days of storage. Overall, the results showed that the sensory attributes of fresh-cut squash tend to decrease over time during storage, with variations depending on the storage condition. $HPMAPCO_2$ tended to maintain the smell quality better than other treatments, while MAPair generally showed a more considerable decline in quality over time.

On the medium scale, MAP-air showed a decline in all attributes over time. $MAPCO_2$ showed fluctuations in color, with the lowest score at 6 days of storage and a slight increase by 21 days. Appearance and texture both declined steadily over time, while smell scores fluctuated without a clear trend. $HPMAPCO_2$ initially had relatively high scores for color, with a slight decrease by 21 days of storage. Appearance scores were lowest at the beginning of the shelf life, increased slightly, and then declined again towards the end of the storage period. The smell remained high throughout the period, while texture gradually declined.

The texture was maintained better on the medium scale compared to the small scale across all treatments.

4. Discussion

This study provides a comprehensive analysis of the effects of a novel high-pressure microbial inactivation method for fresh-cut squash. Initially, process conditions were selected to achieve inactivation of the inoculated bacterium *E. coli* to undetectable levels. Notably, the achieved inactivation was higher than that observed using the same process on a synthetic matrix (agar). Specifically, less than 3 Log CFU/g of the same *E. coli* strain were inactivated after 40 min at 45 °C and 6 MPa (Zulli et al., 2024). This discrepancy may be attributed to differences in structure (*e.g.* porosity, density) between the two matrices, but further comparative studies are needed to confirm this hypothesis.

The treated squash samples ($HPMAPCO_2$) were then compared with non-treated products packed in air (MAPair) or in CO_2 ($MAPCO_2$) during a 21-day refrigerated storage. The three sample groups were evaluated in terms of quality, enzymatic activity, antioxidant capacity, sugar content, bioaccessibility, and sensorial acceptance.

The findings indicate that $HPMAPCO_2$ is generally more effective in preserving quality and extending the shelf life of fresh-cut squash compared to MAPair and $MAPCO_2$ treatments. The process significantly inactivated PPO and POD; their activities peaked earlier (3 days of storage) in MAPair and $MAPCO_2$ samples compared to $HPMAPCO_2$, where peaks occurred at 6 days of storage. This suggests that $HPMAPCO_2$ may be more effective in delaying enzymatic browning, a major factor in the visual and sensory deterioration of fresh-cut products. The best results were obtained for PPO on a small scale, with activity reduced by nearly 50 %. As reported by different studies, enzyme denaturation by supercritical CO_2 could be induced by different mechanisms. These include the disruption of hydrogen bonding and hydrophobic interactions, leading to the unfolding of the enzyme's polypeptide chain. Additionally, CO_2 may interact with amino acid residues at the enzymes' active sites, further contributing to their denaturation (da Veiga et al., 2024). However, this reduction was much lower in the medium-scale trials, highlighting the potential effect of the gas-to-product ratio, as previously observed for microbial inactivation in our earlier study (Zulli et al., 2024).

Interestingly, the reduction in enzymatic activity was greater than

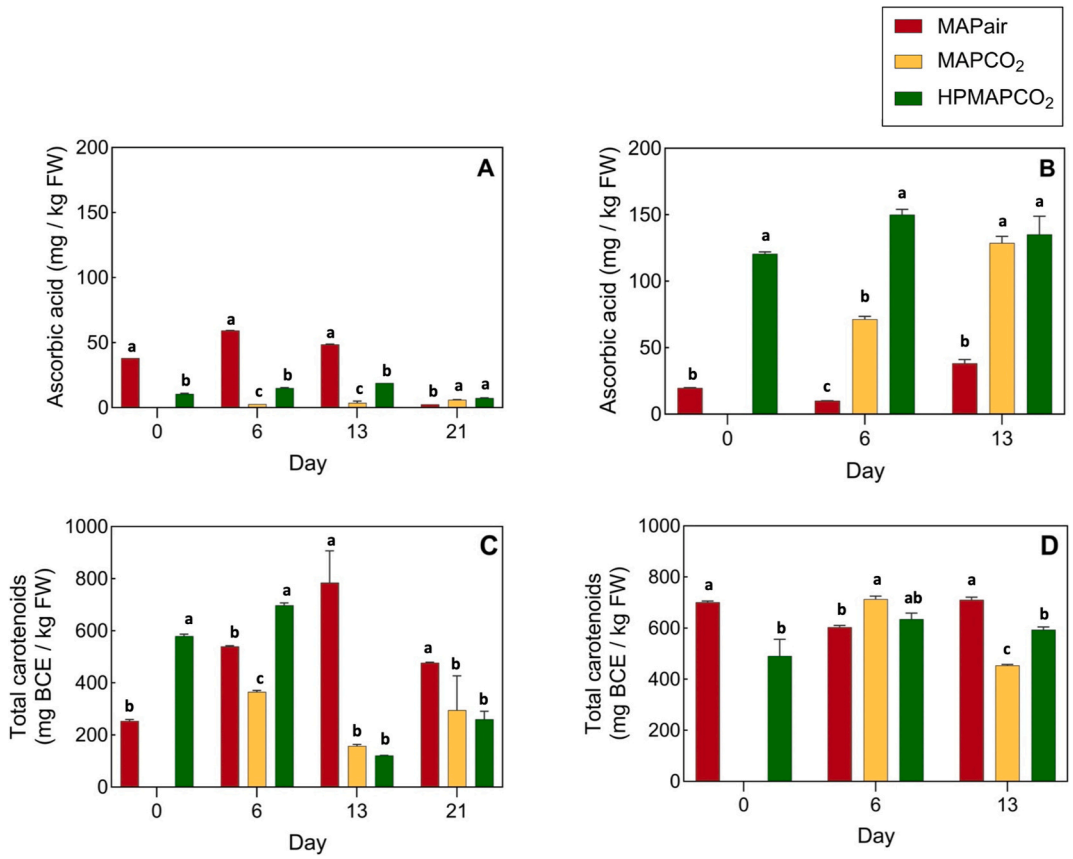

Fig. 6. Ascorbic acid, and total carotenoids levels during a 21-day storage period for fresh-cut squash samples MAPair (red), $MAPCO_2$ (yellow), and $HPMAPCO_2$ (green). A) Ascorbic acid on small scale; B) Ascorbic acid on medium scale; C) Total carotenoids on small scale; D) Total carotenoids on medium scale. Different letters indicate statistically different values ($\alpha = 0.05$) in the same time point. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

that obtained through direct supercritical CO_2 treatment on pumpkin (*Cucurbita pepo*), even at harsher conditions. The maximum reduction achieved was 21 and 18 % for PPO and POD, respectively, at 30 MPa and 55 °C for 45 min (Chen et al., 2024b). Conversely, much higher inactivation levels of the same enzymes have been reported in other studies. For instance, Illera et al. (Illera et al., 2019) achieved more than 90 % reduction of PPO activity and complete inactivation of POD in a more complex food matrix, such as shrimps (*Litopenaeus vannamei*), highlighting the strong matrix dependency of CO_2-based processes.

The antioxidant capacity of fresh-cut squash was assessed throughout the shelf life of the three different sample groups to determine the potential of the high-pressure treatment. Results showed that the antioxidant capacity, as determined by the radical scavenging activities of ABTS, DPPH, OH, O_2^-, and NO, was generally higher and sustained for a longer period under $HPMAPCO_2$ treatment. This suggests that $HPMAPCO_2$ could better preserve the bioactive compounds responsible for antioxidant activities in fresh-cut squash. This preservation prevents oxidative degradation, which can adversely affect color, flavor, and nutritional quality, thus the acceptance of the products.

However, this higher antioxidant capacity was not observed when using the ORAC assay. This discrepancy may be attributed to the fact that the ORAC assay specifically measures the ability of antioxidants to scavenge peroxyl radicals, which might not fully reflect the overall antioxidant potential or the stability of certain bioactive compounds under high-pressure conditions. The ORAC assay's sensitivity to different types of antioxidants and the specific conditions of the assay may account for this variation.

Similar results were obtained when evaluating the different bioactive compounds, specifically carotenoids, phenolics, and ascorbic acid (vitamin C). In the small-scale trials, an increase in total polyphenols and total and individual carotenoids was observed in the $HPMAPCO_2$ sample group. On the other hand, ascorbic acid was sensitive to the treatment. In the medium-scale trials, polyphenols and ascorbic acid were not significantly affected by the treatment, while a slight decrease in total carotenoids was observed.

About micro and macro-element content, the $HPMAPCO_2$ treatment did not significantly affect the qualitative or quantitative profile of the squash samples, which maintained adequate levels of these elements during the refrigerated storage. K, P, Ca, Na, and Mg were the most represented minerals.

To the best of the authors' knowledge, research studies specifically focusing on the maintenance of antioxidant capacity and bioactive molecule content in squash during shelf life are lacking. However, numerous studies have demonstrated that a controlled atmosphere packaging (MAP) can maintain high levels of phenolic content and antioxidant activities in various fruits and vegetables, such as peach (Dong et al., 2022), cherry (Khorshidi et al., 2011) and leafy vegetables (Yang et al., 2022).

Other post-harvest technologies to further improve products' shelf life, such as irradiation and high hydrostatic pressure (HHP) treatment, have also been tested in terms of product quality retention. Despite variations in analytical methods, units of measurement, product varieties, and experimental conditions, studies consistently report that products treated with HHP better preserve antioxidant compounds and capacity compared to thermal treatments (Pérez-Lamela et al., 2021).

The bioaccessibility and bioavailability of naturally-present bioactive molecules in fruits and vegetables are strongly influenced by their chemical characteristics, including solubility, hydrophobicity,

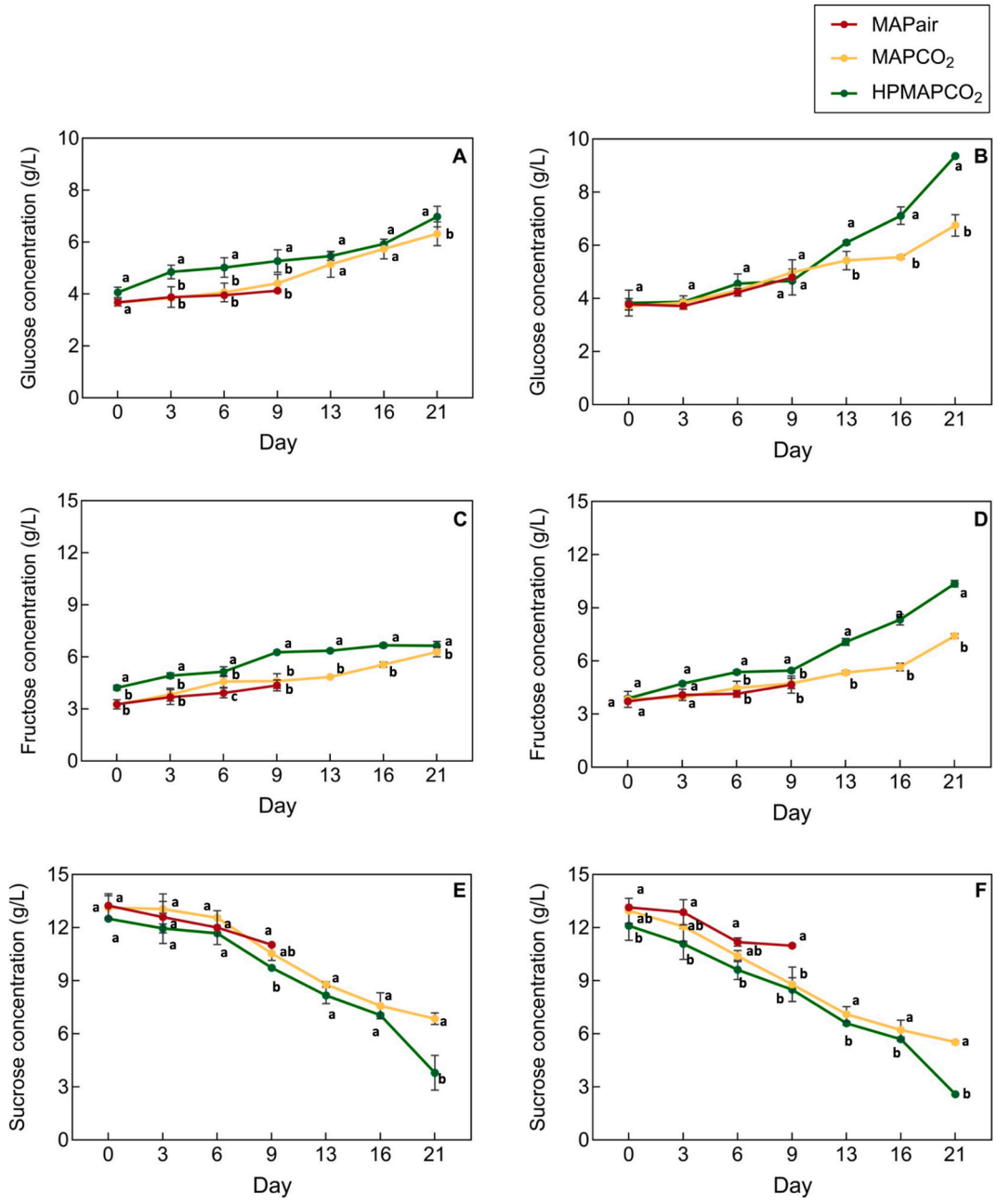

Fig. 7. Sugar contents during a 21-day storage period for fresh-cut squash samples MAPair (red), $MAPCO_2$ (yellow), and $HPMAPCO_2$ (green). A) Glucose on small scale; B) Glucose on medium scale; C) Fructose on small scale; D) Fructose on medium scale; E) Sucrose on small scale; F) Sucrose on medium scale. Different letters indicate statistically different values ($\alpha = 0.05$) in the same time point. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

molecular weight and spatial configuration (Barba et al., 2017). These properties can be altered by thermal and non-thermal treatment for shelf-life prolongation, thereby affecting their bioaccessibility. Simulating the digestion process can provide valuable insights into how treatments may modify bioactive compounds and their overall impact. In the studied process, the bioaccessibility of total polyphenols, antioxidants (measured by DPPH assay), and total carotenoids during the shelf life of fresh-cut squash was only slightly modified, and in some cases even improved, compared to traditionally packed products. This indicates that the $HPMAPCO_2$ treatment can be adopted for this product without negatively affecting its composition or beneficial effects. The slight improvement observed also suggests that the treatment could enhance the availability of certain bioactive compounds during digestion.

Regarding the sugar content (glucose, fructose, and sucrose) of the analyzed squash samples, results showed a general increase in glucose and fructose levels and a decrease in sucrose content during storage across all conditions. However, the $HPMAPCO_2$ treatment led to a higher increase and better maintenance of sugar levels, suggesting potential preservation of taste and nutritional quality. These sugars contribute to the organoleptic profile of the product, enhancing its perceived freshness and consumer appeal compared to untreated samples.

Sensory attributes such as color, appearance, smell, and texture were also monitored. The results indicated that all sensory attributes tended to decline over time during storage, with variations depending on the treatment. Notably, $HPMAPCO_2$ maintained smell quality better than other treatments, while MAPair generally exhibited a more significant decline in overall quality over time. Moreover, sensory evaluation

Table 1
Sensory attributes of fresh-cut squash of the compared treatments in small scale (MAPair, $MAPCO_2$, and $HPMAPCO_2$) during the cold storage of butternut squash. Different letters indicate statistically different values ($\alpha = 0.05$) for the same parameter in the same time point.

Scale	Sample	Parameter	Storage time (days)						
			0	3	6	9	13	16	21
Small	MAPair	Color	6.00 ± 0.00 ^{a}	6.00 ± 0.00 ^{a}	4.88 ± 0.35 ^{a}	4.38 ± 0.52 ^{a}	–	–	–
		Appearance	9.75 ± 0.46 ^{a}	9.88 ± 0.35 ^{a}	9.38 ± 0.52 ^{a}	8.00 ± 0.00 ^{a}	–	–	–
		Smell	5.25 ± 0.71 ^{b}	4.75 ± 2.55 ^{b}	4.00 ± 0.00 ^{c}	3.88 ± 0.35 ^{b}	–	–	–
		Texture	10.00 ± 0.00 ^{a}	10.00 ± 0.00 ^{a}	9.88 ± 0.35 ^{a}	8.75 ± 0.46 ^{a}	–	–	–
	$MAPCO_2$	Color	6.00 ± 0.53 ^{a}	6.00 ± 0.00 ^{a}	5.00 ± 0.00 ^{a}	4.38 ± 0.52 ^{a}	4.13 ± 0.35 ^{a}	4.13 ± 0.35 ^{a}	3.80 ± 0.45 ^{a}
		Appearance	9.50 ± 0.76 ^{a}	9.38 ± 0.52 ^{a}	8.63 ± 0.74 ^{ab}	7.00 ± 0.00 ^{b}	6.80 ± 0.45 ^{a}	6.67 ± 0.82 ^{a}	5.13 ± 0.35 ^{a}
		Smell	7.25 ± 1.04 ^{a}	6.63 ± 0.74 ^{b}	6.00 ± 0.53 ^{b}	6.75 ± 0.71 ^{a}	6.38 ± 0.52 ^{b}	6.40 ± 0.55 ^{a}	6.17 ± 0.41 ^{b}
		Texture	9.75 ± 0.46 ^{ab}	9.50 ± 0.53 ^{a}	8.00 ± 1.20 ^{b}	7.63 ± 0.52 ^{b}	5.50 ± 0.53 ^{a}	5.40 ± 0.55 ^{a}	5.00 ± 0.00 ^{a}
	$HPMAPCO_2$	Color	6.00 ± 0.53 ^{a}	6.38 ± 0.52 ^{a}	5.00 ± 0.00 ^{a}	5.38 ± 0.74 ^{a}	4.75 ± 1.16 ^{a}	3.25 ± 0.46 ^{b}	3.20 ± 0.45 ^{a}
		Appearance	8.38 ± 1.30 ^{a}	7.38 ± 0.52 ^{b}	7.75 ± 0.46 ^{b}	5.00 ± 0.00 ^{c}	5.40 ± 0.89 ^{b}	5.83 ± 0.41 ^{a}	4.38 ± 0.74 ^{a}
		Smell	8.63 ± 0.52 ^{a}	8.63 ± 0.92 ^{a}	8.00 ± 0.53 ^{a}	7.88 ± 0.99 ^{a}	7.75 ± 0.71 ^{a}	7.60 ± 1.34 ^{a}	7.33 ± 0.52 ^{a}
		Texture	9.13 ± 0.35 ^{b}	7.88 ± 0.35 ^{b}	7.88 ± 0.35 ^{b}	5.25 ± 0.71 ^{c}	5.25 ± 0.71 ^{a}	5.67 ± 0.52 ^{a}	4.60 ± 0.55 ^{a}
Medium	MAPair	Color	6.75 ± 0.46 ^{b}	6.00 ± 0.00 ^{b}	6.13 ± 0.35 ^{b}	4.00 ± 0.53 ^{c}	–	–	–
		Appearance	9.75 ± 0.71 ^{a}	9.88 ± 0.35 ^{a}	9.38 ± 0.52 ^{a}	7.00 ± 0.00 ^{b}	–	–	–
		Smell	5.00 ± 0.00 ^{c}	3.88 ± 0.35 ^{c}	4.00 ± 0.00 ^{c}	4.38 ± 1.06 ^{c}	–	–	–
		Texture	10.00 ± 0.00 ^{a}	10.00 ± 0.00 ^{a}	9.88 ± 0.35 ^{a}	8.63 ± 0.52 ^{a}	–	–	–
	$MAPCO_2$	Color	6.88 ± 0.35 ^{b}	7.25 ± 1.39 ^{ab}	4.88 ± 0.35 ^{c}	5.63 ± 0.52 ^{b}	5.75 ± 1.28 ^{b}	5.00 ± 0.00 ^{b}	6.00 ± 0.00 ^{b}
		Appearance	9.38 ± 0.74 ^{a}	9.00 ± 1.07 ^{a}	9.38 ± 0.52 ^{a}	7.83 ± 0.41 ^{a}	7.80 ± 0.45 ^{a}	6.25 ± 0.71 ^{a}	5.25 ± 0.46 ^{a}
		Smell	7.50 ± 0.53 ^{b}	6.38 ± 0.74 ^{b}	6.13 ± 0.35 ^{b}	6.63 ± 0.74 ^{b}	6.50 ± 0.76 ^{b}	6.40 ± 0.55 ^{b}	6.17 ± 0.41 ^{b}
		Texture	9.75 ± 0.46 ^{a}	9.38 ± 0.74 ^{a}	8.17 ± 0.41 ^{b}	7.63 ± 0.52 ^{a}	7.38 ± 1.60 ^{a}	6.38 ± 0.52 ^{b}	5.60 ± 0.55 ^{b}
	$HPMAPCO_2$	Color	8.75 ± 0.46 ^{a}	8.25 ± 0.46 ^{a}	8.75 ± 0.46 ^{a}	8.17 ± 0.41 ^{a}	8.13 ± 0.35 ^{a}	7.88 ± 0.83 ^{a}	7.80 ± 0.45 ^{a}
		Appearance	6.50 ± 1.60 ^{b}	6.38 ± 0.52 ^{b}	6.75 ± 0.46 ^{b}	5.00 ± 0.00 ^{c}	5.60 ± 1.14 ^{b}	5.00 ± 0.63 ^{a}	4.50 ± 1.07 ^{a}
		Smell	8.63 ± 0.52 ^{a}	8.63 ± 0.74 ^{a}	8.00 ± 0.53 ^{a}	8.38 ± 0.92 ^{a}	8.63 ± 0.52 ^{a}	8.20 ± 1.10 ^{a}	8.33 ± 0.52 ^{a}
		Texture	9.13 ± 0.35 ^{a}	8.17 ± 0.41 ^{b}	7.88 ± 0.35 ^{b}	7.88 ± 1.25 ^{a}	7.25 ± 0.71 ^{a}	7.80 ± 0.45 ^{a}	7.25 ± 0.46 ^{a}

confirmed that the observed differences in color in the treated products were not perceived negatively by consumers. This suggests that $HPMAPCO_2$ may be more effective in preserving the sensory qualities of fresh-cut squash, contributing to a better consumer experience during its shelf life.

5. Conclusion

A novel industry-oriented high-pressure microbial inactivation process ($HPMAPCO_2$) was applied to organic fresh-cut squash, and its quality was evaluated during refrigerated storage, comparing it with traditionally packed products. The results demonstrated that $HPMAPCO_2$ can effectively extend the shelf life by achieving microbial and enzymatic inactivation while maintaining the overall quality of squash in terms of bioactive compound content, antioxidant activity, bioaccessibility and sensorial acceptance. Future studies will focus on further investigating the microbiological status of the product throughout its shelf-life. This will provide a deeper understanding of the long-term effects of $HPMAPCO_2$ treatment on the safety and quality of fresh-cut squash, ensuring its suitability for commercial applications. Moreover, the application to different types of matrices will be evaluated to gain a broader understanding of its effectiveness for different food categories.

Ethical permission and panelist consent

The sensory research addressed in this work complied with relevant laws and institutional guidelines and was approved by the ethics committee of the Wroclaw University of Environmental and Life Sciences (Doc#: N0N00000.0020.1.6.4.2024). Human participants were required to sign a consent form that declared "I agree to participate in this sensory evaluation" before undertaking the sensory evaluation.

CRediT authorship contribution statement

Riccardo Zulli: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Zhe Chen:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Fabio Santi:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Urszula Trych:** Writing – review & editing, Methodology, Investigation, Formal analysis, Conceptualization. **Justyna Szczepańska-Stolarczyk:** Writing – review & editing, Methodology, Investigation, Formal analysis, Conceptualization. **Magdalena Cywińska-Antonik:** Writing – review & editing, Methodology, Investigation, Formal analysis, Conceptualization. **Pietro Andrigo:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Margherita Amenta:** Writing – review & editing, Methodology, Conceptualization. **Gabriele Ballistreri:** Writing – review & editing, Validation, Methodology, Conceptualization. **Giusy Maria Platania:** Writing – review & editing, Methodology, Formal analysis. **Nicolina Timpanaro:** Writing – review & editing, Methodology, Conceptualization. **Susanna Aurora Tortorelli:** Writing – review & editing, Methodology, Investigation, Formal analysis, Conceptualization. **Zineb Benmechernene:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Yasin Ozdemir:** Writing – review & editing, Visualization, Validation, Resources, Project administration, Funding acquisition, Conceptualization. **Alessandro Zambon:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Methodology, Investigation, Funding acquisition, Conceptualization. **Simona Fabroni:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Krystian Marszalek:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Sara Spilimbergo:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Sara Spilimbergo reports financial support was provided by Italian Ministry of Agriculture, Food Sovereignty and Forests. Krystian Marszalek reports financial support was provided by National Center for Research and Development. Zineb Benmechernene reports financial support was provided by Ministry of Higher Education and Scientific Research (Algeria). Yasin Ozdemir reports financial support was provided by General Directorate of Agricultural Research and Policies of the Ministry of Agriculture and Forestry of the Republic of Türkiye. Sara Spilimbergo reports administrative support was provided by H2020 ERA-NETs SUSFOOD2 and CORE Organic Cofunds. Simona Fabroni reports financial support was provided by Italian Ministry of University and Research. Sara Spilimbergo reports financial support was provided by Italian Ministry of Enterprises and Made in Italy. Fabio Santi reports financial support was provided by Italian Ministry of University and Research under the PNRR, Mission 4. Sara Spilimbergo has patent #IT10201700009804 issued to University of Padova. Alessandro Zambon has patent #IT10201700009804 issued to University of Padova. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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The graphical abstract was made by Biorender.com.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2025.142882.

Data availability

Data will be made available on request.

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