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Application of supercritical carbon dioxide to improve the quality of ready-to-use carrots and pumpkins during storage

Zastosowanie dwutlenku węgla w warunkach nadkrytycznych do poprawy jakości marchwi i dyni, gotowych do użycia, podczas przechowywania

Doctoral dissertation
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Statement of dissertation supervisor

I hereby declare that this dissertation was prepared under my supervision, and I confirm that it meets the conditions required for submission in proceedings for awarding a doctoral degree.

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Abstract

Application of supercritical carbon dioxide to improve the quality of ready-to-use carrots and pumpkins during storage

This study comprehensively examined how supercritical carbon dioxide (SCCD) treatment affects enzyme activity, color, carotenoid and sugar profiles, individual phenolic compounds, and antioxidant capacity in carrot cubes of varying sizes (1 cm and 2 cm) and fresh-cut pumpkins (1 cm). In carrots, SCCD reduced polyphenol oxidase (PPO) and peroxidase (POD) activities more pronouncedly in 1 cm cubes than in 2 cm cubes, while raising a*, b*, and ΔE values in 1 cm samples. Under moderate processing conditions, carotenoid content, phenolic levels, and antioxidant activity increased in 1 cm cubes. However, at harsher process parameters, these nutritional components showed more significant degradation in 1 cm cubes compared to 2 cm cubes, emphasizing that cube size substantially influences nutrient retention. Principal component and correlation analyses revealed distinct variations between selected smaller and larger cubes regarding enzyme activities and nutrient composition following SCCD. Based on these results, 1 cm carrot cubes processed at 10 MPa, 35 °C, and 45 min emerged as the most effective parameter, achieving significant enzyme inactivation alongside high bioactive compound retention.

Regarding pumpkins, SCCD processing reduced PPO and POD activities by 21% and 18%, respectively. Similar lightness (L^*) decreased, whereas redness (a^*) and yellowness (b^*) increased. Lutein, α-carotene, β-carotene, total carotenoids, glucose, sorbitol, and other polysaccharides in SCCD-treated pumpkin displayed a fluctuating trend. Furthermore, total phenolic content, ABTS, DPPH, and superoxide radical scavenging activities increased and then decreased. Under moderate SCCD conditions, coumaryloquinic acid I and II, caffeic acid glucoside, 4-hydroxybenzoic acid, and p-coumaric acid showed marked enhancements, indicating that moderate SCCD parameters can promote the release of bound bioactive compounds from macromolecules.

SCCD effectively suppressed microbial growth while maintaining low PPO and POD activity levels throughout 3 weeks of storage, thereby delaying both enzymatic and chemical deterioration of carotenoids and phenolic compounds. Additionally, SCCD expedited sucrose hydrolysis but postponed decreases in glucose and fructose, thereby curtailing quality loss over extended storage. Overall, this study highlights the promise of SCCD, especially under moderate conditions, for maintaining high-quality, nutrient-rich produce and provides a strong foundation for its broader industrial adoption in the fresh-cut sector.

Keywords: sugar, carotenoids, phenolic compounds, polyphenol oxidase and peroxidase, microbial count, minimally processed vegetables

Streszczenie

Zastosowanie dwutlenku węgla w warunkach nadkrytycznych do poprawy jakości marchwi i dyni, gotowych do użycia, podczas przechowywania

W pracy dokonano kompleksowej analizy wpływu nadkrytycznego dwutlenku węgla (SCCD) na aktywność enzymatyczną, barwę, profil karotenoidów i cukrów, związki fenolowe oraz zdolność antyoksydacyjną w świeżo krojonych kostkach marchwi o boku 1 cm i 2 cm oraz dyni o boku 1 cm. W marchwi, SCCD skuteczniej obniżało aktywności oksydazy polifenolowej (PPO) i peroksydazy (POD) w kostkach o boku 1 cm niż w kostkach o boku 2 cm. Zaobserwowano również w nich wzrost wartości parametrów a*, b* oraz ΔE. W umiarkowanych warunkach procesu odnotowano wzrost zawartości karotenoidów, polifenoli i aktywności antyoksydacyjnej w kostkach o boku 1 cm. Intensywniejsze parametry procesu powodoway większą degradację skadników w kostkach o boku 1 cm co wskazuje, że rozmiar kostek istotnie wpływał na zachowanie badanych składników. Analiza głównych składowych oraz korelacji potwierdziły wyraźne różnice pomiędzy mniejszymi i większymi kostkami pod względem aktywności enzymów i składu chemicznego marchwi. Stwierdzono, że kostki marchwi o boku 1 cm poddane działaniu SCCD w warunkach 10 MPa, 35 °C przez 45 minut charakteryzowały się najwyższą jakością, przy skutecznej inaktywacji enzymów oraz wysokiej wartości żywieniowej.

W przypadku dyni SCCD zmniejszyło aktywność PPO i POD odpowiednio o 21% i 18%. Podobnie zaobserwowano spadek jasności próbki (L*) oraz wzrost intensywność barwy czerwonej (a*) i żółtej (b*). Zawartość luteiny, α-karotenu, β-karotenu, całkowitych karotenoidów, glukozy, sorbitolu oraz innych polisacharydów w dyni poddanej SCCD wykazywała zmienny trend. Ponadto całkowita zawartość związków fenolowych oraz aktywność przeciwutleniająca oznaczona metodami ABTS, DPPH i zdolność zmiatania rodników ponadtlenkowych początkowo wzrastały, a następnie malały. W umiarkowanych warunkach SCCD odnotowano znaczny wzrost zawartości kwasu kumarylochinowego I i II, glukozydu kwasu kawowego, kwasu 4-hydroksybenzoesowego i kwasu p-kumarowego, co wskazuje, że umiarkowane parametry SCCD mogą sprzyjać uwalnianiu związanych związków bioaktywnych z makrocząsteczek.

SCCD skutecznie hamowało wzrost mikroorganizmów, utrzymując jednocześnie niską aktywność PPO i POD przez 3 tygodnie przechowywania, co opóźniło enzymatyczne i chemiczną degradację karotenoidów i związków fenolowych. Dodatkowo, SCCD przyspieszyło hydrolizę sacharozy, ale opóźniło degradację glukozy i fruktozy, ograniczając tym samym utratę jakości podczas przedłużonego przechowywania. Podsumowując, uzyskane wyniki badań dają duży potencjał, szczególnie w umiarkowanych warunkach procesu SCCD, do utrzymania wysokiej jakości, bogatych w składniki odżywcze produktów, stanowiąc podstawę dla jego szerszego wdrożenia przemysłowego w sektorze świeżo krojonych warzyw.

Słowa kluczowe: warzywa wstępnie przetworzone, liczebność drobnoustrojów, polifenoloksydaza i peroksydaza, związki fenolowe, karotenoidy, cukry

Contents

LIST OF PUBLICATIONS CONSTITUTING THE DISSERTATION

[P1] Chen Z., Spilimbergo S., Khaneghah M. A., Zhu Z.Z., Marszałek K. (2022). The effect of supercritical carbon dioxide on the physiochemistry, endogenous enzymes, and nutritional composition of fruit and vegetables and its prospects for industrial application: An overview. Critical Reviews in Food Science and Nutrition, 64(17), 5685-5699. DOI: 10.1080/10408398.2022.2157370.

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[P3] Chen Z., Kapusta I., Zhu Z. Z., Marszałek K. (2024). Quality properties and nutritional compounds of fresh-cut pumpkin treated with supercritical carbon dioxide. The Journal of Supercritical Fluids, 206, 106147. DOI: 10.1016/j.supflu.2023.106147.

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[P4] Chen Z., Zhu Z. Z., Marszałek K. (2025). Changes in the storage quality of fresh-cut vegetables using supercritical carbon dioxide treatment. Food Chemistry, 465, 142131. DOI: 10.1016/j.foodchem.2024.142131.

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Introduction

In response to growing consumer demand for vegetables with high nutritional value and convenience, ready-to-use (RTE) vegetables have gained significant attention in the food industry. According to the Food and Agriculture Organization of the United Nations, carrot and pumpkin production has gradually increased over the years in Poland and China. China is the world's largest producer of carrots and pumpkins in 2024, accounting for 44 and 32 % of global production, respectively. Additionally, Poland is the 7th largest producer of carrots and pumpkins among European countries. Carrots and pumpkins are rich in bioactive compounds, such as phenolic compounds and carotenoids, which benefit human health. However, they are susceptible to microbial and enzymatic activity, leading to spoilage, degradation of bioactive compounds, and reduced sensory quality. Furthermore, vegetable losses due to storage problems amount to approximately 20% per year (Saranraj et al., 2012).

Heat processing is a common method for minimizing microbial contamination and inactivating enzymes, but it also promotes the degradation of nutrients and adversely affects sensory qualities. High-pressure processing (HPP) was developed to minimize bioactive compound loss, but processing at high pressures (600 MPa) often results in significant texture loss in fresh-cut vegetables. Supercritical carbon dioxide (SCCD) is an emerging food processing technique that can effectively and cost-efficiently prolong the shelf life of food products. Upon reaching 7.8 MPa and 31.7 °C, CO_2 becomes supercritical, with high solubility and diffusivity, enhancing its ability to diffuse and dissolve within food matrices, thereby effectively inactivating microbes and enzymes. With its operation at relatively mild pressure and temperature, SCCD effectively preserves nutrients and minimizes changes to sensory quality, resulting in high-quality foods. Additionally, CO_2 can be easily removed during depressurization, avoiding food safety concerns associated with solvent residues.

This study investigated the impacts of the SCCD technique on the physciochemistry properties, enzyme activities, and nutritional composition of RTE carrots and pumpkins at various parameters and storage periods. The study not only provides a comprehensive understanding of the impact of the SCCD technique on the quality of RTE foods but also offers both theoretical knowledge and practical implementation of food processing and preservation. The study largely facilitates the food industry to meet consumer demands for high-quality, convenient, and nutritious vegetables.

1. Literature Review

1.1 Supercritical carbon dioxide treatment

1.1.1 Theoretical and generation of SCCD processing

Supercritical fluids (SCFs) are defined as substances that are present above their critical temperature and pressure (Fig 1.1 (a)) (Amaral et al., 2017). These fluids have densities similar to liquids, viscosities of gases, and high diffusion coefficients (Amaral et al., 2017). SCFs have some unique properties, which contribute to their efficient penetration into food materials (cells, particles, polymers, etc.) (Brunner, 2005). As such, SCF processing can be optimised for both temperature and pressure to meet a range of industrial applications, such as in phytochemical extraction, particle engineering, polymer impregnation and microbial inactivation (Brunner, 2005; Osorio-Tobón, Silva, & Meireles, 2016). Among them, SCCD is the most used SCF in food processing because it has a relatively low critical temperature of 31.2 °C and a critical pressure of 7.38 MPa (Tab. 1.1) (Silva et al., 2020). Moreover, its chemical inertness, safety, environmental friendliness and GRAS (Generally Recognized as Safe) status make it highly suitable for application in food production (Ceni et al., 2016). Because SCCD is used under relatively low temperature and pressure conditions, it is especially well suited to the processing of thermo-sensitive food materials without damage to the product quality. SCCD processing was found to inactivate microbial and enzyme activities, improve food preservation, and maintain the nutritional compositions of the food, thus obtaining high-quality treated products. The SCCD system comprises an oven, inlet and outlet pipelines with valves, heating and cooling equipment, temperature and pressure regulator, chamber, pump, and CO_2 bottle (Fig. 1.1 (b)). Before initiating SCCD processing, the heating and cooling systems are activated to stabilize the temperature above 31.2°C. Subsequently, the samples are inserted into the chamber, and the lids are securely sealed. After opening the inlet valve and closing the outlet valve, the CO_2 bottle is turned on, and the pump is fine-tuned to keep the pressure consistently above 7.38 MPa, thus allowing CO_2 to reach its supercritical state. After the samples have remained in the chamber for a specified period, the pump is set to return to atmospheric pressure. Finally, the inlet valve is shut, and the outlet valve is opened to reduce the chamber pressure. Once the pressure returns to atmospheric conditions, the SCCD processing is complete.

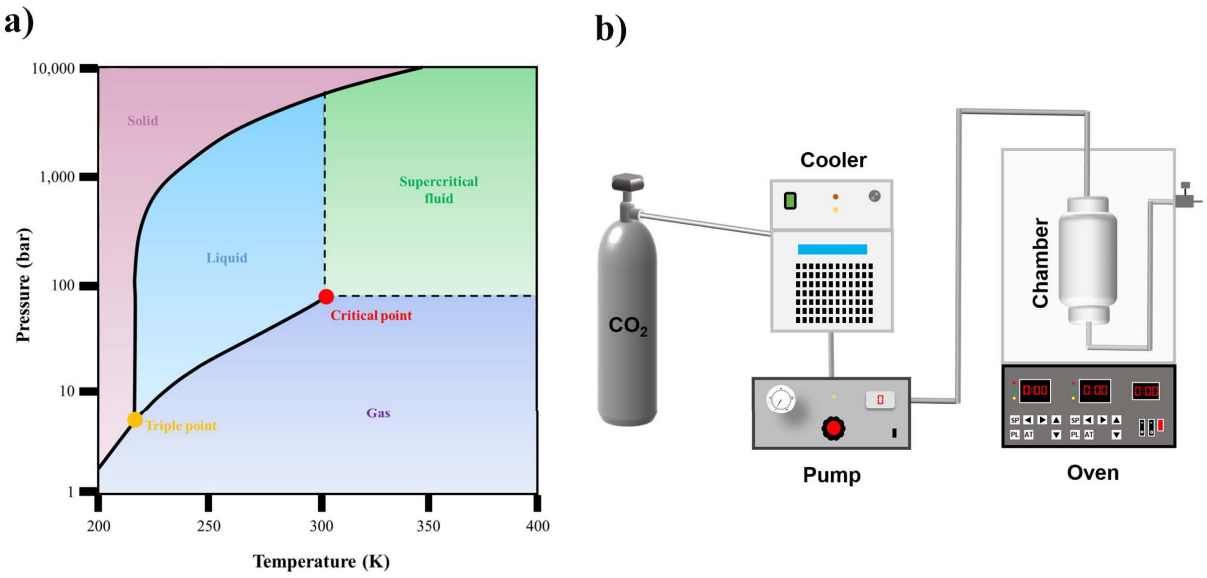


Figure 1.1. Phase (a) and schematic diagram (b) of supercritical carbon dioxide processing. (Chen et al., 2025)

Table 1.1. Physical properties of carbon dioxide at different states. (Chen et al., 2025)

State	Density (g/cm^3)	Viscosity (g/cm)	Diffusivity (cm^2/s)
Gas	0.00006-0.0002	0.0001-0.0003	0.1-0.4
Supercritical	**0.2-0.5**	**0.0001-0.0003**	**0.0007**
Liquid	0.6-1.6	0.002-0.03	0.000002-0.00002

1.1.2 Solubility of CO_2 in foods

The solubility of CO_2 is a crucial factor in influencing the effectiveness of SCCD processing. CO_2 solubility in foods is mainly influenced by pressure and temperature (Viganó et al., 2015). Some studies displayed that pressure is more effective in increasing CO_2 solubility in foods than temperature (Viganó et al., 2015; Illera et al., 2019). However, CO_2 solubility in complex liquid and solid food systems remains limited, especially for fresh-cut fruit and vegetables. Giovanna Ferrentino et al. (2010) found that glucose and sucrose reduce CO_2 solubility in model solutions and apple juice, indicating that sugar content affects SCCD efficiency. Compared to glucose and sucrose, Organic acids have minimal impact on CO_2 solubility (Giovanna Ferrentino et al., 2010). Furthermore, Illera et al. (2019) also demonstrated that high sugar concentrations decrease CO_2 solubility in apple and carrot juices.

1.1.3 Operational systems

Depending on the production scale and objective, SCCD processing can be possibly designed into three modes of operation, such as batch, semi-continuous, and continuous operation modes

(Silva et al., 2020). In the bath operation mode, the sample is placed in a closed chamber with a certain volume of CO_2 (Giacomo et al., 2009). The temperature of the chamber is controlled by a water bath (Giacomo et al., 2009). In the continuous operation mode, the sample was thoroughly mixed with CO_2 before samples were placed in the chamber, which allows fluid to flow through the entire system and provides accurate control of treatment parameters (Werner & Hotchkiss, 2006). Continuous operation mode enables samples to be processed under high pressure and short treatment time, ensuring samples' quality and safety (Fabroni et al., 2010). Moreover, the semi-continuous operation mode is an intermediate process between the bath and continuous operation mode, in which a constant flow rate of CO_2 is flowed through a series of connected pressure vessels (Porto et al., 2010). The advantages of this operation mode are more efficient pressure utilization and shorter processing times (Porto et al., 2010). In addition to these operation modes, SCCD technique is combined with other technologies to enhance CO_2 dispersion, like ultrasound, high-process homogenization, microbubbles (King, 2014; Kobayashi et al., 2016), and membranes (Sims, 2001).

1.2 Effect of SCCD processing on microorganisms

1.2.1 Microbial inactivation by SCCD processing

Microorganisms play an important role in food preservation, leading to spoilage and deterioration (Manzocco et al., 2017). The amount of molds, yeasts, and aerobic mesophilic bacteria in foods is generally used as a testing indicator to evaluate the storage quality of foods (Giovanna et al., 2017). Some studies found that SCCD treatment can effectively reduce the activities of molds, yeasts, and aerobic mesophilic bacteria, thereby extending the shelf life of foods. For instance, Pei et al., (2018) demonstrated that SCCD processing completely inactivated the molds, yeasts, and total bacteria in Hami melon juice at 30 MPa and 65 ℃ for 15 min. After 14 days of storage at 4℃, molds, yeasts, and total bacteria were undetectable in the processed samples (Pei et al., 2018).

The performance of SCCD technology for microbial inactivation in foods is affected by pressure, temperature, treatment time and CO_2 concentration (Ferrentino et al., 2009; Chen et al., 2010). For example, Ferrentino et al., (2017) found that the decrease of mesophilic microorganisms, yeasts and molds in SCCD-processed apple pieces was accelerated with a gradual increase in pressure or temperature. Furthermore, Liao at al., (2007) also reported that the enhancement in the CO_2 purity (99.5% to 99.9%) increased by 22% in the reduction of *Escherichia coli* in SCCD-treated apple juice. However, when CO_2 dissolved in foods reaches

saturation, the inactivation rate of microbes becomes slow or tends to a constant, regardless of increasing pressure, temperature or CO_2 concentration.

In addition to studies of total microorganisms, the inactivation of specific microorganisms treated by SCCD has also been studied, including *Staphylococcus aureus, Lactobacillus casei*, *Salmonella typhimurium*, *Escherichia coli*, and *S. cerevisiae*. For example, E. coli in SCCD-processed-mango syrup was completely inactivated at 20 MPa and 60 °C for 30 mins (Tang et al., 2021). Furthermore, *Lactobacillus casei* in apple juice (Silva et al., 2018), *Saccharomyces cerevisiae* in grape juice (Gunes et al., 2005) and kiwi juice (Spilimbergo & Ciola, 2010), *Lactobacillus helveticus* in orange juice (Oulé et al., 2013), *Kloeckera apiculata* (CE114) in grape juice (Gunes et al., 2005), *Candida stellata* (FAW223) in grape juice (Gunes et al., 2005), as well as *Alicyclobacillus acidoterrestris* spores in apple juice (Bae et al., 2009) exhibited significantly decreases in survival numbers after SCCD processing. Taken together, SCCD technology is an effective technology to reduce microbial activity, thereby improving food safety.

1.2.2 Inactivation mechanism of microbes by SCCD processing

The microbial inactivation mechanism in SCCD processing is a complex, multi-faceted process involving both physical and chemical interactions. As illustrated in Fig. 1.2, microbial inactivation during SCCD treatment occurs in three distinct phases: the lag stage, the rapid stage, and the resistant stage (Bi et al., 2014; Kim et al., 2007; Liao et al., 2010; Liao et al., 2008; Ortuño et al., 2014). In the lag stage, SCCD initially enters into microbial cells, modifying the surface charge and membrane permeability to facilitate the penetration of CO_2 into microbial cells (Liao et al., 2010). At this stage, microbial inactivation is minimal or absent. The subsequent fast stage is crucial for microbial inactivation. Specifically, inside the cell, SCCD dissolves in free water and dissociates into H^+, leading to a decrease in pH (Valley et al., 1927). The acidic environment destabilizes the quaternary structure of endogenous enzymes by disrupting hydrophobic interactions and breaking hydrogen bonds (Deotale et al., 2021). Furthermore, the lowered pH inhibits key metabolic activities and impairs DNA synthesis (Deotale et al., 2021). Moreover, the decline in pH further also enhances CO_2 diffusion into microbial cells, impacting the cytoplasmic environment (Daniels et al., 1985). This disruption overwhelms the cell's proton symport mechanisms and buffering capacity, causing cytoplasmic acidification (Daniels et al., 1985). Finally, in the resistant stage, numerous inactivated cells generated during the fast stage release cellular components, including intracellular materials, wall, and membrane, which adhere to the surface of surviving cells. This accumulation reduces

CO_2 accessibility to the remaining viable cells, thereby weakening the overall effectiveness of SCCD treatment.

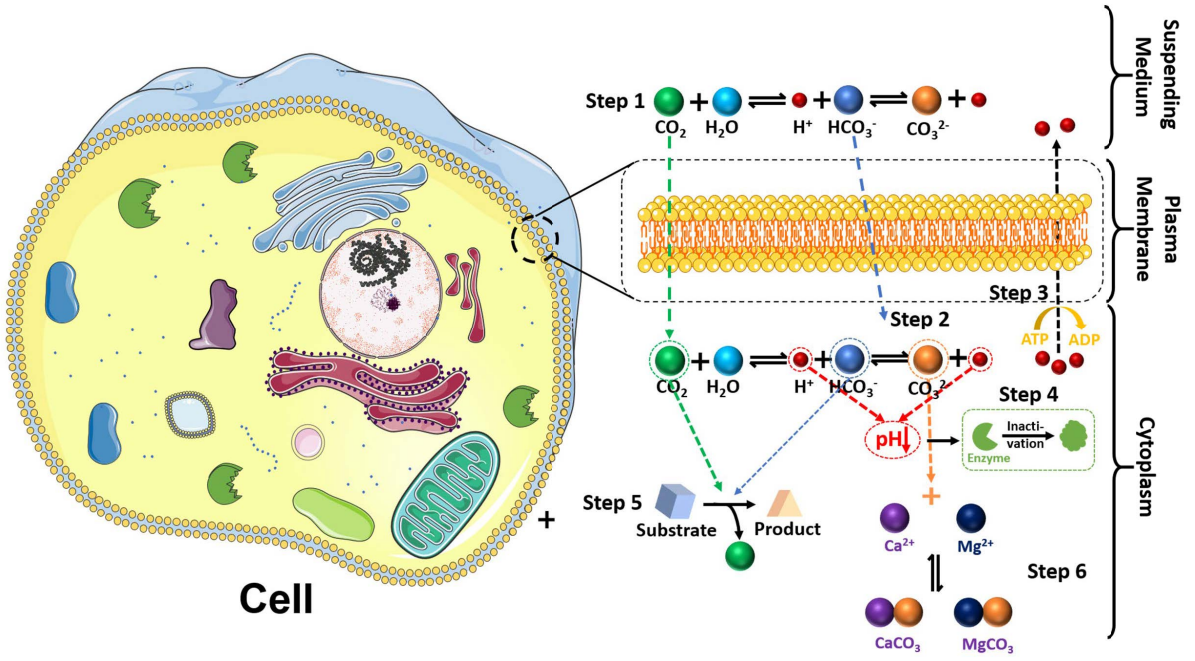


Fig. 1.2 Inactivation mechanism of microbial cells treated with SCCD treatment. (Chen et al., 2025)

1.3 Effect of SCCD processing on enzymes

1.3.1 Enzyme inactivation by SCCD processing

After postharvest processing, most endogenous enzymes in fruits and vegetables remain active, which can lead to undesirable changes in fruits and vegetables during storage (Benito-Román et al., 2019). Enzymatic inactivation is essential to prevent enzymatic browning and the formation of off-flavors (Benito-Román et al., 2019). High enzymatic inactivation contributes to maintaining the sensory attributes and nutrients of fruits and vegetables, thereby keeping them similar to fresh fruits and vegetables (Marszałek et al., 2016). SCCD technique is an effective method used to inactivate enzymes in fruits and vegetables, thus extending their shelf life. For instance, Marszalek et al., (2018) indicated that SCCD technique significantly declined the activities of peroxidase (POD) and polyphenoloxidase (PPO) in apple juice by 79% and 53% at 60 MPa and 45 °C for 30 min, respectively. Moreover, the authors found that the reduction in POD and PPO activities gradually increased with elevated pressure (Marszalek et al., 2018). Compared to POD, PPO is more susceptible to SCCD technology, which is attributed to the heat resistance of POD (Baykuş et al., 2021). Moreover, Strawberry juice (Marszałek et al., 2015), watermelon juice (Liu, et al., 2012), apple juice (Manzocco et al., 2017), orange peel (Zhang, et al., 2021), fresh-cut carrot (Spilimbergo et al., 2013), and red beetroot (Liu, et al.,

2010) exhibited significantly decreases in POD and PPO activities after SCCD processing. Apart from PPO and POD, SCCD technology is also found to reduce the activity of and polygalacturonase (PG) and pectin methylesterase (PME), thus improving the could stability of juice. For example, Illera et al., (2018) suggested that SCCD processing reduced by 100 % and 55 % of PME and PG activities in tomato juice at 60 MPa, 45 °C and 30 min, respectively. Furthermore, the fractional conversion model showed that PG is more resistant to SCCD processing than PME (Illera et al., 2018). Therefore, SCCD technology has great potential as a processing technique to inactivate these endogenous enzymes in fruits and vegetables, thereby improving the quality storage of fruits and vegetables.

1.3.2 Inactivation mechanism of enzymes by SCCD processing

Enzymes are proteins with specific structures in which conformational changes alter their function and substrate specificity (Punia et al., 2022). After SCCD treatment, enzymes undergo denaturation or conformational changes that affect their biological activity (Marszałek et al., 2019; Li et al., 2022; Iqbal et al., 2019; Oliveira et al., 2019). Several possible mechanisms for SCCD-induced enzyme inactivation have been proposed. i) CO_2 dissolved in the hydration layer generates H^+ ions, which form a highly uniform charge on the surface of the peptide chain (Iqbal et al., 2019). Electrostatic repulsive forces between peptide chains with the same charge lead to changes in protein conformation (Iqbal et al., 2018). ii) Under low-pH conditions, carbon dioxide may interact with amino acid residues in the enzyme to form a bicarbonate complex, which removes the charge from the amino acid residues, leading to a change in the conformation of the active site (Oliveira et al., 2019; Li et al., 2022). iii) During decompression, the formation of CO_2 bubbles creates a gas-liquid interface (Javad et al., 2022). Enzyme molecules with amphiphilic properties automatically migrate at the surface of the gas-liquid interface, causing amino acid residues on the enzyme molecule to rearrange at that interface, thereby resulting in the conformational changes of enzyme molecules (Marszałek et al., 2019).

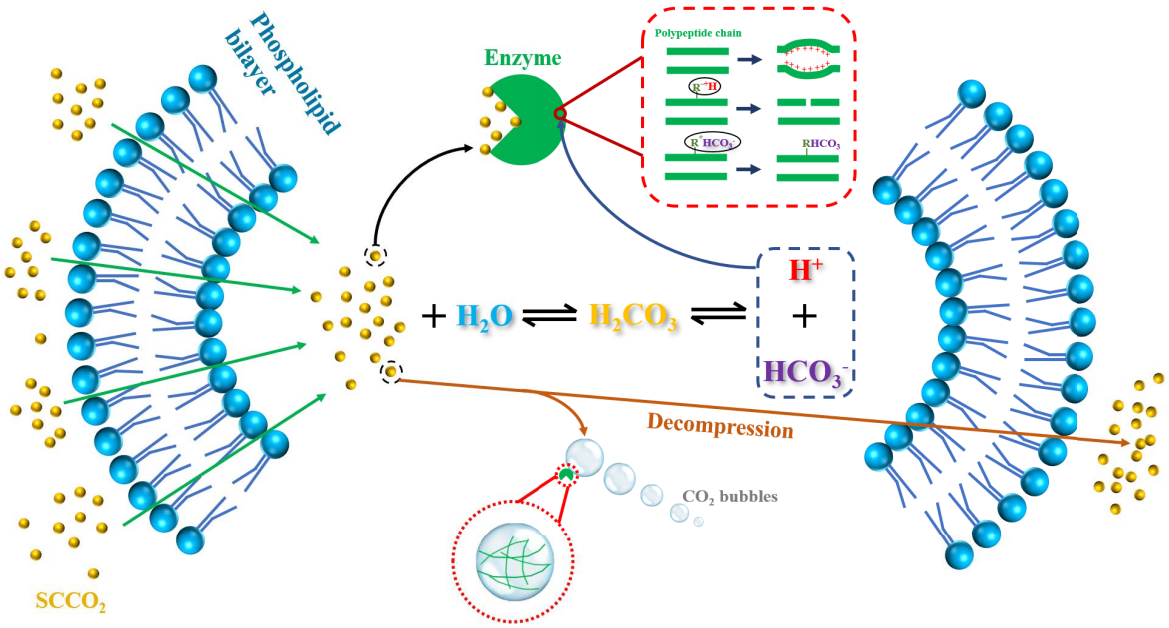


Fig. 1.3 Schematic diagram of the possible mechanisms of the SCCD technique on enzymes. (P1)

1.4 The effect of SCCD processing on the phenolic compounds

Phenolic compounds (PCs) are bioactive components that prevent oxidative damage and reduce the risk of diseases such as inflammation, hyperlipidemia, and allergies (Li et al., 2021). Changes in the stability, taste, and nutritional value of fruits and vegetables are associated with PCs (Mikołajczak et al., 2021). Some factors, such as temperature, pH, light, and PPO activity, affect the stability of PCs in fruits and vegetables (Li et al., 2020). Some studies indicate that SCCD processing did not significantly change total phenolic content (TPC) in apple juice (Illera et al., 2018), watermelon juice (Liu et al., 2012), lychee juice (Guo et al., 2011), and dietary supplement juices (Fleury et al., 2018) compared to untreated samples. Nevertheless, SCCD-treated mulberry juice exhibited higher TPC (16 %) than unprocessed samples (Darvishi et al., 2020). Additionally, SCCD treatment is found to enhance the TPC of orange peel by 109 % (Zhang et al., 2021). The increase in the TPC after SCCD processing may be attributed to the release of bound phenolic from macromolecules caused by the extraction effect of the SCCD technique. On the contrary, Marszałek et al., (2018) found that the TPC of SCCD-treated apple juice significantly reduced and gradually decreased with increasing pressure. Similar reductions in the TPC were observed in the SCCD-treated beetroot juice (Marszałek et al., 2017). The decrease in the TPC caused by SCCD processing is probably because high pressure and temperature accelerate the degradation of phenolic compounds. Furthermore, Bertolini et al. (2020) investigated the effect of storage time on the TPC of SCCD-treated pomegranate juice. The results showed that the TPC of SCCD-processed samples at day 21 was 60 % higher than

the fresh samples (Bertolini et al. 2020), suggesting that the SCCD technique is effective in preserving TPC during storage. In addition to total phenolic content, the phenolic profiles were also analyzed by Marszałek et al. (2018). These results displayed that SCCD processing led to a reduction in gallic acid, chlorogenic acid, (+) catechin, (−) epicatechin, procyanidin B_1, procyanidin B_2, and phloridzin, whereas it did not change ρ-coumaric acid content (Marszałek et al. 2018). After 10 weeks of storage, these individual phenolic contents treated with SCCD declined, and the fastest degradation was noted in procyanidin B_2 (Marszałek et al. 2018). The variability in degradation rates was attributed to differing affinities between phenolic compounds and their associated enzymes.

1.5 The effect of SCCD processing on the carotenoids

Carotenoids, the fat-soluble pigments responsible for the red, yellow, and orange hues in fruits and vegetables, are widely distributed in plants, algae, yeast, fungi, and some bacteria (Ashokkumar et al., 2023). Nevertheless, their highly unsaturated structure makes them particularly susceptible to degradation when exposed to heat, acidic conditions, oxygen, and light (Ram et al., 2020). The oxidation of carotenoids triggers a sequence of chemical reactions, thus resulting in alterations in color (Ashokkumar et al., 2023). For instance, in fresh-cut carrots subjected to SCCD processing, total carotenoid content exhibited a noticeable decline, decreasing by 6 % when compared to untreated juice (Spilimbergo, et al., 2013). Furthermore, after four weeks of storage, the carotenoid levels in SCCD-processed carrots showed no statistically significant difference from those of fresh samples (Spilimbergo, et al., 2013). The oxidation of carotenoids in SCCD-processed carrot was primarily attributed to the presence of oxygen within plant cells, which facilitated oxidative reactions leading to carotenoid degradation (Liu et al., 2021).

Lycopene, a representative carotenoid, is a red-hued lipophilic hydrocarbon compound (Li, et al., 2021). Spilimbergo, et al., (2013) investigated the impact of SCCD processing on the lycopene content. The findings demonstrated that the lycopene content in fresh-cut carrots was initially 78.6 μg/g, but after subjecting to SCCD processing and subsequent storage for four weeks, it reduced by 6 %, mainly due to the isomerization and oxidation (Spilimbergo, et al., 2013). Overall, these findings indicate that SCCD processing tends to reduce carotenoid levels in fruits and vegetables.

1.6 The effect of SCCD processing on the antioxidant activities

Fruits and vegetables are rich in various bioactive compounds that exhibit antioxidant properties, making them significant natural sources of antioxidants (Herianto et al., 2021).

These antioxidants include a diverse range of substances, such as endogenous metabolites, dietary glutathione, vitamins, carotenoids, flavonoids, and phenolic compounds (Herianto et al., 2021). Among these, phenolic compounds are the main contributors to antioxidant activity in vegetables and fruits due to their stronger ability to neutralize peroxyl radicals compared to β-carotene, vitamin E, and vitamin C (Mannozzi et al., 2019). Nevertheless, POD and PPO can alter the binding properties of polyphenols, leading to a reduction in their bioactivity, which may impair the nutritional quality of processed vegetables and fruits (Mannozzi et al., 2019).
For instance, the antioxidant activity of pomegranate juice treated by SCCD, as measured by the DPPH assay, decreased by 44.3% compared to unprocessed juice (Bertolini et al., 2020). Conversely, SCCD-processed mulberry juice exhibited a significantly enhanced antioxidant capacity when analyzed using the ferric-reducing ability power (FRAP) method in comparison to untreated samples (Illera, Sanz et al., 2018; Hui et al., 2016). Similarly, the antioxidant capacity of SCCD-processed apple juice, assessed through ABTS assay, showed a slight increase compared to untreated samples. Moreover, it was indicated that the antioxidant activity of inulin-enriched apple juice subjected to SCCD processing, as determined by the TEAC assay, was higher than that of untreated juice. The antioxidant activity is closely related to the concentration of bioactive compounds. Such variations could be attributed to the increase or decrease of certain unidentified bioactive components (Silva, et al., 2019). In the case of blackcurrant juice, its antioxidant capacity, measured by ABTS assay, initially enhanced but later declined with rising pressure (Trych et al., 2022). The reduction in antioxidant activity under high pressure may be explained by the degradation of bioactive compounds. However, SCCD-treated red beet juice displayed no statistically significant difference in antioxidant capacity when assessed using the DPPH assay (Silva et al., 2019). In conclusion, SCCD treatment has been shown to enhance the antioxidant potential of fruits and vegetables.

1.7 The effect of SCCD processing on the sugar contents

Sugar plays a crucial role as a key ingredient, contributing to its sweetness and enhancing consumer appeal (Chen et al., 2022). Consequently, assessing the influence of the SCCD technique on the sugar composition is essential for evaluating the quality of SCCD-processed products. Studies by Marszałek et al. (2018) and Cappelletti et al. (2015) found that SCCD processing did not alter the sugar contents. However, Guo et al. (2011) observed a significant increase in sugar concentration in SCCD-treated lychee juice. Specifically, the levels of glucose, fructose, and sucrose in treated samples rose by 46%, 5%, and 43%, respectively (Guo et al., 2011). Similarly, Marszałek et al. (2015) found that SCCD processing enhanced glucose

and fructose levels in strawberry juice but led to a 100 % reduction of sucrose content. Additionally, the authors examined changes in SCCD-treated samples over a 12-week storage period. Their findings indicated that glucose and fructose levels in the treated samples remained relatively stable throughout the storage period. In conclusion, SCCD processing has no significant impact on the sugar content of beverages during storage.

The above has already been published in Publication 1.

2. Hypotheses, Purpose, and Contents

2.1 Research hypotheses

1. The different cube sizes of raw material influence the effectiveness of supercritical carbon dioxide (SCCD) in food preservation.

2. The SCCD technique can inhibit oxidative enzyme activities and increase the measured concentration of bioactive compounds in carrot and pumpkin cubes.

3. The SCCD technique can inhibit microbial growth in ready-to-use carrots and pumpkins during storage and maintain their enzyme activities at a low level.

4. The SCCD technique delays the degradation of bioactive compounds such as carotenoids and polyphenols, thus maintaining the high concentration of nutrients in ready-to-use carrots and pumpkins after cold storage.

2.2 Research purpose

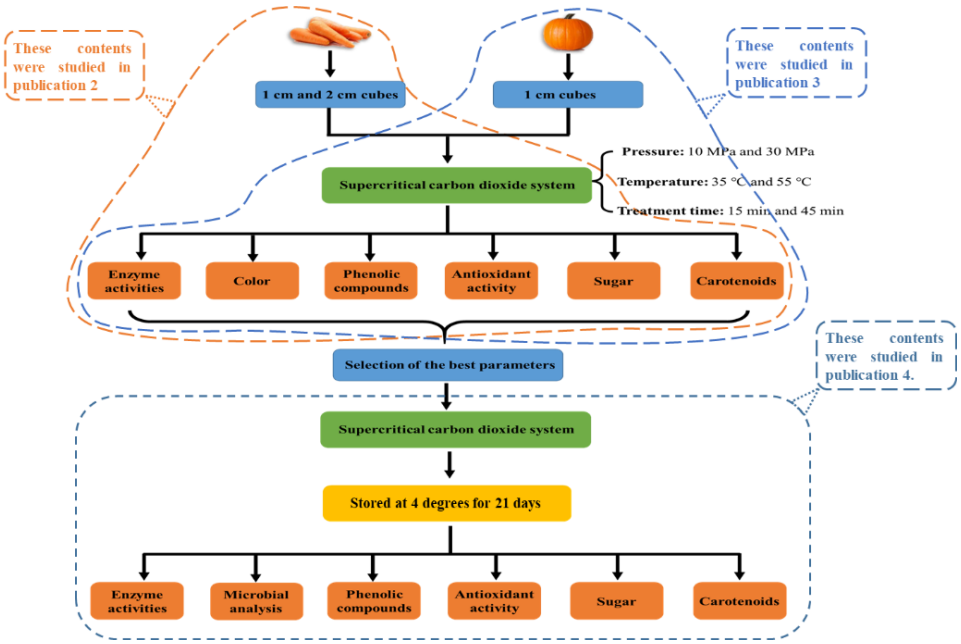
1. Assessment of changes in the enzyme activity and nutritional profile of different-sized carrot cubes treated with SCCD.

2. Evaluation of the influences of SCCD processing on the physicochemical, phenolic compounds, carotenoids and sugar profile, and antioxidant activities of pumpkin cubes.

3. Measurement of the changes in the carotenoid and sugar profile, phenolic compounds and antioxidant capacity of carrots and pumpkins during storage.

4. Revealing the quality maintenance mechanism of the SCCD technique on bioactive compounds in carrots and pumpkins during storage.

2.3 Research content



3. Materials and Methods

3.1. Materials

Pumpkin (*Cucurbita pepo*) and carrot (*Daucus carota* subsp. sativus) were purchased from a local supermarket in Warsaw, Poland. SN (sodium nitroprusside), PMS (phenazine methosulfate), PBS (phosphate buffer saline), NBT (nitroblue tetrazolium), NADH (nicotinamide adenine dinucleotide hydrogen), salicylic acid, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), Folin-Ciocalteu reagent (FCR), ρ-phenylenediamine (ρ-PLD), and catechol (CTC) were boughted from Sigma-Aldrich. Griess'reagent was bought from Fluka Analytical. Hydrogen peroxide was sourced from Avantor Performance Materials Poland. All chemicals were used as received without further purification.

3.2. SCCD processing

Carrots were washed and cut into 2 cm and 1 cm cubes, while pumpkins were cut into 1 cm cubes. To minimize variations among the cubes, all pieces of the same type were thoroughly mixed. Glass bottles containing 48 ± 0.5 g of either carrot cubes (2 cm or 1 cm) or pumpkin cubes were placed inside the SCCD chamber (SFE-4, Applied Separations, Allentown, PA, USA). The cylindrical chamber's bottom in the SCCD system was securely sealed. The glass bottle was positioned within the chamber, and the top lid was tightly closed. The air inlet was connected to the chamber's top lid, while the outlet was linked to the bottom lid. Before initiating the SCCD process, the chamber was preheated to a specific temperature, and the cooling circulation system was adjusted to maintain the ice water temperature below 0°C. The pressure pump and carbon dioxide bottle were activated to pressurize the chamber to the desired level, and these cubes were treated under these conditions for a set duration. Upon completion of the SCCD treatment, the chamber was gradually depressurized until it reached atmospheric pressure. The carrot cubes were subjected to SCCD treatment at pressures of 30 and 10 MPa, temperatures of 55 and 35°C, and treatment durations of 45 and 15 minutes. Similarly, the pumpkin cubes were processed at pressures of 30, 16, and 10 MPa, under the same temperature and time conditions. Each treatment condition was repeated three times throughout the experiment. Immediately after SCCD processing, the color variation in processed cubes was determined. The processed cubes were then placed at -20°C for further analysis. For subsequent measurements, three samples from each treatment condition were analyzed once. Therefore, each analysis was conducted three times. For the storage experiment, carrot and pumpkin were washed, peeled, and cut into 1 cm cubes. Then, forty-eight cubes of either pumpkins or carrots (48 ± 0.5 g) were treated with SCCD at 10 MPa and 45 ℃ for 20 min. When the SCCD

treatment was completed, the chamber was transferred to the clean bench. The glass bottle was then carefully removed from the chamber on the clean bench and sealed with a metal lid. Finally, the covered glass bottle was stored at 4 ℃ for 0, 3, 7, 11, 15, and 21 days.

3.3. Measurement of microbial counts

The variations in the counts of molds, yeast, and total microbial of these samples were determined following the method reported by Marszałek et al. (2015). Briefly, two grams of these samples were added to five millilitres of sterile saline and then homogenized for 2 minutes. The homogenized microbial suspension was then subjected to a serial decimal dilution process to obtain diluted microbial solutions (DMS). Next, 1 mL of DMS was added to the plates. The potato dextrose agar (PDA) and plate count agar (PCA) medium were poured into the plates containing DMS. The plates with PCA were incubated at 36 °C for 48 hours, while those with PDA were cultured at 28°C for 5 days. Finally, the number of colonies on the plates was expressed as the microbial count (CFU/g).

3.4. Measurement of enzyme activity

Peroxidase (POD) and polyphenol oxidase (PPO) activities were analyzed following the approach described by Chen et al. (2024). Specifically, these samples were grated with a blender (BRAUN, Germany) and then mixed with ten millilitres of extraction solution (w:v=1:1). Subsequently, the mixture was homogenized using a homogenizer and centrifuged with a centrifuge to obtain the supernatant. For PPO activity measurement, three millilitres of CTC solution was mixed with 0.3 mL of the supernatant and incubated at room temperature for 10 minutes. The absorbance of the resulting mixture was recorded at 420 nm using a UV–visible spectrophotometer. POD activity was determined by mixing three millilitres of PBS (fifty mM, pH 6.5) with fifty microliters of supernatants, fifty microliters of 1.5% H_2O_2, and fifty microliters of 1% ρ-PLD. The mixture was incubated at room temperature for 10 minutes, and its absorbance was measured at 485 nm.

3.5. Examination of carotenoid profiles

The carotenoid profile of the samples was analyzed using a modified approach based on the method described by Szczepańska et al. (2022). Specifically, 4.5 millilitres of hexane and 0.5 grams of BHT were added to a 50 mL centrifuge tube with 0.5 g grated pumpkin or carrot. After 3 minutes of homogenization processing, the mixture was added to 4.5 millilitres of acetone to avoid the volatilization of acetone during homogenization treatment. Moreover, the mixture was processed with ultrasound treatment and centrifugation (US+CF) to facilitate carotenoid extraction. The aqueous phase was further processed through three sequential US+CF processes

to extract all carotenoids in the samples. The extraction solutions were saturated NaCl and hexane (6 millilitres, 2:1) for the first two extractions, followed by hexane (2 millilitres) in the final step. All organic phases collected in a 50-millilitre centrifuge tube were subjected to solvent evaporation to remove the organic solvent. The dried extract was then solubilized in acetone and analyzed using an HPLC system equipped with a YMC Carotenoid column.

3.6. Total phenolic content and antioxidant capacity

The extraction of bioactive compounds from the samples followed the approach outlined by Chen et al. (2024). The extraction process involved homogenizing a mixture of ten grams of grated samples with twenty millilitres of 80% methanol at an appropriate rotational speed for three minutes. The treated solution was then subjected to three consecutive extractions using the same solvent. Ultrasound treatment and centrifugation were applied to separate the supernatant, which was collected in a 100-millilitre glass bottle. All supernatants were evaporated using a Rotavapor at thirty degrees to achieve a higher concentration of phenolic compounds. The dried extracts were subsequently reconstituted in ten millilitres of 80% methanol for further analysis.

Total phenolic content: 0.1 millilitres of supernatant was added to a 5-millilitre brown bottle containing 3.2 millilitres of FCR. After 120 min of incubation, the mixture's absorbance was determined at 765 nm. Gallic acid was used as a standard solution to calculate TPC values in both processed and unprocessed samples.

DPPH and ABST radical scavenging activity: A total of 0.05 millilitres of supernatant was mixed with 2.5 millilitres of ABST reaction reagent in a 5-millilitre brown bottle. After a reaction time of 6 minutes, the absorbance of the mixture was measured at 734 nm. Similarly, 0.1 mL of the supernatant was combined with 2 mL of DPPH radical solution in a 5 mL brown bottle, and the absorbance was recorded at 520 nm after 30 minutes of reaction. Trolox was used as a standard solution to calculate the values of ABST and DPPH radical scavenging activity in both processed and unprocessed samples.

Hydroxyl radical (·OH) scavenging radical activity: The hydroxyl (·OH) radical scavenging activity was evaluated using the salicylic acid method, as described by Zhu et al. (2022). In brief, 200 microliters of supernatant were combined with one millilitre of salicylic acid solution for analysis. Then, one millilitre of H_2O_2 solution was added to the mixture, and the reaction was conducted at 37 °C for 30 min. Finally, the absorbance was detected at 510 nm.

Superoxide ($O_2^{-\cdot}$) scavenging radical activity: The $O_2^{-\cdot}$ radical scavenging activity was measured using the approach described by Kour et al. (2023). A mixture consisting of two

millilitres of reaction solution and 0.25 millilitres of supernatants. Subsequently, 0.25 millilitres of PMSc were introduced to the mixture to initiate the reaction. After a 5-minute incubation, the absorbance was measured at 560 nm.

Nitric oxide ($NO^{\cdot}$) scavenging radical activity: The nitric oxide ($NO^{\cdot}$) radical scavenging activity was determined following the methodology described by Chen et al. (2022a). A mixture consisting of 250 microliters of PBS, 500 microliters of SN, and 250 microliters of supernatant was incubated at 37°C for 2.5 hours. After incubation, 1 mL of Griess reagent was introduced and allowed to react for 30 minutes. The absorbance of the resulting solution was then measured at 546 nm.

3.7. Determination of sugar contents

The sugar concentration in the samples was analyzed following the approach reported by Marszałek et al. (2019), with some modifications. Specifically, five grams of grated samples were mixed with ten millilitres of distilled water and homogenized at an optimal rotational speed for 3 minutes. The mixture underwent ultrasound-assisted extraction, followed by centrifugation, and the obtained supernatant was collected in a 50-millilitre centrifuge tube. Finally, the supernatant was passed through a 0.25 μm membrane filter to remove large tissue particles. An HPLC system, equipped with a Sugar-Pak I column and a Guard-Pak column, was used to determine the sugar content in the filtered samples.

3.8. Measurement of color

The grated samples were placed into a glass cuvette with a 1 cm optical path, ensuring the complete removal of air bubbles before measurement. The samples' color parameters (b^*, a^*, and L^*) were then assessed using a colorimeter. The total color difference (ΔE) chroma (C), hue angle (h), and yellow index (YI) were calculated according to equations 2, 3, and 4, respectively.

$$\Delta E = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \quad (1)$$

$$C = \sqrt{a^{*2} + b^{*2}} \quad (2)$$

$$h = \tan^{-1}\frac{b^*}{a^*} \quad (3)$$

$$YI = (142.86 \times b^*)/L^* \quad (4)$$

3.9. Statistical analysis

All tests on samples were conducted three times, including 0, 3, 7, 11, 15, and 21 days of storage. The obtained data were described as mean ± standard deviation. Origin pro-2021 was

used to prepare all Figures. Significant differences between unprocessed and processed samples over the 21-day storage period were analyzed using a one-way analysis of variance (ANOVA) followed by the Tukey test in IBM SPSS Statistics 26.

4. Results and Discussions

4.1 Color

The color variations in unprocessed and SCCD-processed carrot cubes (2 cm and 1 cm) are presented in Table 1 (P2). Compared to the untreated carrots (UC), the L^* value of SCCD-processed carrot cubes (SCCD-TC) was enhanced, indicating that SCCD efficiently prevented the browning of fresh-cut carrots (FCC). Additionally, at 55 ℃, the L^* values of 2 cm SCCD-TC were higher than those at 35 ℃, whereas the L^* values of 1 cm SCCD-TC showed a decline. This phenomenon was likely due to the increased temperature facilitating greater carbon dioxide permeation into carrot cubes. Regarding a* value, a slight increase was observed in SC13, SC11, SC3, SC2, and SC1 compared to UC. The increase may be attributed to the extraction effect of SCCD, which facilitates the release of pigment compounds. As the temperature rose to 55℃, the a^* values in 2 cm and 1 cm SCCD-processed carrot cubes declined. This reduction is likely due to pigment degradation at elevated temperatures (Marszałek et al., 2017).

In contrast, the b^* value of 1 cm and 2 cm SCCD-TC decreased at 35 ℃ with increasing pressure and extending processing time (P2), suggesting that SCCD could help prevent color darkening. However, at 55℃, the b^* value of SCCD-TC was higher than those of UC, indicating that elevated temperatures promoted browning reactions (Niu et al., 2010). For the ΔE parameter, an increase in pressure and treatment time led to a gradual rise in the ΔE value of SCCD-TC. Furthermore, the ΔE value of the 1 cm SCCD-TC was higher than that of the 2 cm cubes, which can be attributed to size differences. To further examine the color variations in the carrot cubes of different sizes, the yellowing index (YI), Hug (h), and Chroma (C) were calculated (P2). The C value of SCCD-TC at 35 ℃ displayed a declining trend with elevating pressure and treatment time, indicating that SCCD could effectively inhibit browning at an optimal temperature. Moreover, the YI and h values in SCCD-TC follow a similar trend to the C value. In conclusion, SCCD significantly impacted the color variations of smaller cubes.

Table 2 shows the influence of SCCD on the color characteristics of pumpkin cubes (P3). Compared to the untreated pumpkins (UP), pumpkin cubes processed by SCCD (SCCD-TP) showed a reduced L^* value. Moreover, as the pressure, temperature, and processing time increased, the L^* value in SCCD-TP gradually decline. In contrast, the a^* and b^* values of SCCD-TP rose progressively with the enhancement of these processing parameters. This trend can likely be explained by the fact that CO_2 under high pressure boosts the permeability of cell membranes, facilitating the migration of pigments (carotenoids) from within the cells to the

surrounding fluid (Deng et al., 2019; Nowacka et al., 2021). Additionally, the ΔE value of SCCD-TP was gradually enhanced with elevating the processing parameters. Further investigation into the C, h, and YI values of SCCD-TP demonstrated that the h values were largely unaffected by the changing parameters. Nevertheless, both the C and YI values in SCCD-TP are enhanced by increasing processing conditions. Notably, in SP12, the C and YI values were improved by 6% and 12%, respectively. Moreover, an increase was noted in the YI values. Therefore, SCCD technology had a positive impact on the appearance of pumpkins.

4.2 Microbial activity

Microbial activity is a critical factor influencing the spoilage of FCC and fresh-cut pumpkins (FCP) during storage. Table 1 shows that the total microbial count (TMC) in FCC and FCP was recorded at 2.34 and 2.59, respectively (P4). Meanwhile, yeast and mold levels in both FCC and FCP remained below 1. After SCCD treatment, the TMC in the processed carrot and pumpkin (PCP) exhibited a significant reduction, dropping to less than 1. This result indicates that SCCD effectively inhibits microbial proliferation in carrots and pumpkins. Over the 21-day storage period, the TMC in unprocessed carrots and pumpkins (PCP) continuously increased, reaching 5 in carrots and 7 in pumpkins by the end of storage. In contrast, the TMC in PCP were less than 1 for up to one week in pumpkins and 2 weeks in carrots. Even after 4 weeks, the TMC in PCP remained significantly lower than that of UCP. Furthermore, yeast levels in UCP increased evidently after day 7, reaching 5 in carrots and 5 in pumpkins by the final storage day. Throughout the entire storage period, yeast levels in PCP stayed below the detection limit, while mold levels in UCP surpassed the detection limit at 2 weeks for pumpkins and 3 weeks for carrots. In comparison, mold levels in PCP remained under 1 by the final storage day. These results suggest the effectiveness of SCCD processing in suppressing microbial growth, thereby prolonging the shelf life of FCC and FCP.

Pei et al. (2018) observed that the TMC in untreated Hami melon juice surpassed 3.50, whereas no detectable microbial presence was found in SCCD-processed samples. Their study further demonstrated that even after two weeks of storage, mold, TMC and yeast levels in the processed juices remained notably lower than those in fresh juice, suggesting that SCCD technique has a strong microbial inhibitory effect. The decline in microbial activity is primarily due to the unique characteristics of SCCD, which enable it to penetrate microbial cells, thus disrupting cellular metabolism (Chen et al., 2022).

4.3 Enzyme activity

Figure 1 shows that PPO and POD activities in SCCD-TC gradually declined as the processing parameters increased (P2). Similar trends in PPO and POD activities were noted in SCCD-TP (P3). The decline in PPO and POD activities may be attributed to the ability of SCCD to penetrate cells and dissolve in intracellular water, leading to the generation of H^+ ions (Chen et al., 2022). The accumulation of H^+ ions lowers the intracellular pH (Chen et al., 2022). This decrease in pH induces structural modifications in PPO and POD, thus resulting in their inactivation (Chen et al., 2022; Li et. al. 2023). A significant reduction in PPO activity was observed in SCCD-TC (P2) and SCCD-TP (P3) when the temperature reached 55 °C, indicating that PPO was more sensitive to heat than to variations in pressure or treatment time (Umair et al., 2022). In contrast, POD activity decreased slightly under the same condition, probably because POD is more heat-resistant than PPO (Liu et al., 2013). When comparing SC9 to SC14, the latter showed higher PPO and POD activities (P2), suggesting that cube sizes had a more pronounced influence on the enzyme activity than the parameters. Furthermore, the residual activities of PPO and POD in SCCD-TC were lower than those in 1.5 cm and 2 cm cubes, further confirming that SCCD processing reduces enzyme activity in small cubes more effectively. Two factors can explain this phenomenon. First, 1 cm cubes possess a larger specific surface area than 2 cm cubes of the same weight, allowing for greater exposure to SCCD. Second, the smaller size of 1 cm cubes means their surface is closer to the core, facilitating more efficient heat and carbon dioxide diffusion into the center.

During the 3 weeks of storage, POD and PPO activities in PCP gradually increased, with PPO peaking on day 3 and POD reaching its highest activity on day 11. The enhancement in enzyme activity was probably a response to stress induced by cutting, which can stimulate enzyme synthesis or activation (Wen et al., 2023). Compared to UCP, an earlier peak was observed in PCP, indicating that SCCD plays a role in suppressing the overall increase in enzyme activity. Similarly, Zhang et al. (2021) reported that POD activity in high-pressure carbon dioxide (HPCD)-treated water-bamboo shoots reached its maximum on day 2. In contrast, the peak occurred later in the untreated sample on day 4. During the 3 weeks of storage, PPO and POD activities in PCP were lower compared to UCP, demonstrating that SCCD technique efficiently suppresses enzymatic activity throughout storage. After one week, PPO and POD activities in PCP showed minimal variation, whereas significant fluctuations were noted in UCP. This fluctuation of enzyme activity in UCP can be attributed to the mechanical damage caused by cutting, which compromises cell integrity and promotes the release of enzymes and intracellular compounds (Zhou et al., 2022). Once exposed, these enzymes interact with oxygen, resulting

in an elevated production of high levels of reactive oxygen species (ROS) (Wang et al., 2022). The accumulation of ROS further damages cell membranes, promoting additional enzyme release and enhancing the availability of substrates and catalytic sites, ultimately causing substantial variations in POD and PPO activities (Chazarra et al., 2001). Conversely, SCCD treatment not only suppresses enzyme activity but also restricts oxygen availability within the cellular environment due to the penetration of CO_2. Although SCCD processing does not fully deactivate these enzymes, it inhibits their interaction with O_2, thus reducing ROS generation (Meitha et al., 2020).

4.4 Phenolic compounds

Table 2 and Figure 3 display the individual and total phenolic contents of carrot cubes subjected to SCCD were analyzed (P2). The total phenolic content (TPC) of 2 cm and 1 cm carrot cubes initially increased and then declined as the treatment condition changed. Compared to UC, the highest TPC were observed in SC13 and SC5, showing increases of 18 % and 36 %. Additionally, Figure 2(a) illustrates that the TPC of SP5, SP4, SP2, and SP1 exhibited a gradual increase as processing parameters elevated, reaching the highest level at SP5 (P3). Similarly, Zhang et al. (2021) reported a 21% increase in the TPC of SCCD-processed orange peels compared to the untreated samples. A comparable rise in TPC was also noted in mulberry juice subjected to SCCD (Zou et al. 2016). The enhancement of TPC in both carrot and pumpkin cubes is likely due to the efficiency of SCCD in extracting phenolic compounds, facilitating their release from macromolecular structures (Trych et al., 2022; Tang et al., 2021). Specifically, one contributing factor is that SCCD enhances the permeability of cell walls and membranes, thereby facilitating the release of intracellular phenolic compounds (Daud et al., 2022). Moreover, phenolic compounds exist in both free and bound forms. The bound form interacts with macromolecules like proteins and polysaccharides, making extraction more difficult (Wu et al., 2021). CO_2 can be associated with these macromolecules through hydrogen bonding, disrupting the hydrogen bonds between phenolic compounds and macromolecules, thus improving the efficiency of phenolic compound separation (Barbosa et al., 2020).

Conversely, when the temperature continuously rose to 55℃, the TPC of TC and TP displayed a declining trend as pressure and processing time (P2 and P3). Moreover, TPC in SC8 and SP13 was 10% lower than UC and UP (P2 and P3). The decline in TPC could be explained by SCCD-induced degradation of phenolic compounds. As the parameters intensified, CO_2 further compromised the integrity of cell walls and membranes, increasing the extent of membrane rupture. This extensive rupture facilitated the oxidase release into the extracellular fluid, where

it catalyzed reactions that reduced phenolic compound levels (Villamil-Galindo et al., 2020). Moreover, as treatment conditions elevated, increased carbon dioxide penetration into the cube increased in hydrogen ion concentration, lowering the pH and accelerating phenolic compound degradation (Osorio-Tobón, 2020).

Compared to 2 cm cubes, the TPC in 1cm cubes was higher (P2). Moreover, the degradation rate of TPC in 1cm samples at 55 ℃ compared to that in 2 cm cubes (P2). The findings indicate that the SCCD technique enhanced the extraction efficiency for smaller cubes. Moreover, the TPC of SC9 displayed a lower level than 1 cm SCCD-TC, except for SC8. However, SC9 exhibited a higher TPC than SC10, confirming that smaller cubes contribute to greater TPC retention.

To further investigate the impact of the SCCD technique on the phenolic profiles of carrot and pumpkin cubes, the UPLC-PDA-MS/MS analysis system was employed to examine the individual phenolic contents in both unprocessed and processed samples (P2 and P3). For carrot samples, the caffeic acid glucoside derivative (CAGD) content in SC13, SC9 and SC4 was higher than that of UC. Additionally, the caffeic acid glucoside (CAG) level of SC8 and SC4 showed an enhancing trend. However, as the temperature rose to 55℃, the CAGD concentration in SC17 and SC8 declined, with SC8 showing an almost undetectable level. The phenomenon indicated that temperature significantly affected the CAGD in SCCD-TC, particularly in smaller cubes. Apart from CAG and ascorbic acid, the individual phenolic contents in SC8 were lower than those of UC, whereas SC17 showed no significant differences in individual phenolics. These findings suggest that variations in processing parameters had a more significant impact on smaller carrot cubes.

In the case of pumpkin, the levels of coumaryloquinic acid I and II, CAG, 4-hydroxybenzoic acid, and ρ-coumaric acid in SP5 and SP7 were remarkably higher than in UP. Nevertheless, the concentrations of vanillic acid, quercetin 3-O-rutinoside (Q3OR), and quercetin 3-O-pentoxide (Q3OP) in SP5, SP7, and SP13 were lower, probably due to the SCCD-induced degradation of three compounds. The decrease in vanillic acid content could be attributed to the cleavage of its ether bond in an acidic environment, leading to its transformation into 4-hydroxybenzoic acid and methanol (Szczepańska et al., 2020). Moreover, our findings revealed the presence of 4-hydroxybenzoic acid in SP5 and SP7. Q3OR and Q3OP, as secondary metabolites, are prone to degradation into primary metabolites due to SCCD-induced embolism in cubes (Yang et al., 2022).

When the storage time increased from day 0 to day 3, the TPC in UCP was progressively enhanced (P4). This initial rise is probably due to the cells synthesizing phenolic compounds as a defense response to cutting, which activates a healing mechanism for the damaged tissue (Hu et al., 2022; Torres-Contreras et al., 2017). Moreover, the TPC value in PCP exhibited a gradual increase throughout 3 weeks of storage, peaking on day 13. Compared to UCP, SCCD treatment slowed phenolic compound degradation, preserving higher levels over the storage period. The elevated TPC value in PCP can be attributed to three reasons. Firstly, beyond the stress response triggered by cutting, SCCD likely induced an additional stress response, activating gene expression and enzymes related to phenolic synthesis (López-Gámez et al., 2021; Denoya et al., 2021). Secondly, CO_2 probably increase the activity of antioxidant enzymes (AEs), which prevents the interaction between phenolic compounds and free radicals (FRs), thereby inhibiting phenolic degradation (Ghasemzadeh et al., 2010; Gouda et al., 2023). Furthermore, SCCD treatment significantly decreased the activity of PPO and POD, which prevents phenolic degradation caused by oxidative enzymes, thereby maintaining a higher phenolic content.

4.5 Antioxidant activity

The impact of SCCD treatment on the radical scavenging activities of $ABTS^{\cdot+}$, $DPPH^{\cdot}$, ·OH, $O_2^{\cdot-}$ and $NO^{\cdot}$ (ABTS-RSA, DPPH-RSA, OH-RSA, O_2-RSA, and NO-RSA) in carrot and pumpkin cubes is illustrated in Figure 3 (P2) and Figure 2 (P3), respectively. After being subjected to SCCD processing, both DPPH-RSA and ABTS-RSA in carrots and pumpkins displayed a fluctuating trend as the processing parameters increased. Specifically, when the temperature was maintained at 35°C, the DPPH-RSA and ABTS-RSA of SCCD-TC and SCCD-TP gradually increased with rising pressure and extended processing time. In addition to these two indices, the O_2-RSA of SCCD-TP exhibited a similar trend to the DPPH-RSA and ABTS-RSA in the SCCD-TC and SCCD-TP. This phenomenon can be attributed to the fact that ABTS-RSA, DPPH-RSA, and O_2-RSA are mainly associated with phenolic compounds (Leja et al., 2013). Silva et al. (2019) found that although the DPPH-RSA in prebiotic-enriched apple juice remained unchanged before and after SCCD processing, the ABTS-RSA in SCCD-processed samples was higher compared to untreated ones. Conversely, as processing parameters intensified, a gradual decline was observed in O_2-RSA and NO-RSA for SCCD-TC, as well as in OH-RSA and NO-RSA for SCCD-TP. However, the OH-RSA in SCCD-TC remained comparable to that of the untreated samples. It can be seen from Figure 3 (P2) that carrot cubes exhibited significantly lower OH-RSA compared to DPPH-RSA and ABTS-RSA, which was

explained by the limited presence of bioactive compounds responsible for OH-RSA in the carrot cubes. Due to their low concentration, CO_2 penetration into the carrot cubes was insufficient to effectively with these compounds.

When the storage time increased from day 0 to day 3, the DPPH-RSA and ABTS-RSA in UCP exhibited an increasing trend (P4). This initial rise is likely due to the cellular synthesis of phenolic compounds as a defensive response to cutting (Hu et al., 2022; Torres-Contreras et al., 2017). In PCP, DPPH-RSA and ABTS-RSA steadily enhanced, reaching their highest levels by day 13. In comparison to UCP, SCCD treatment effectively slowed the phenolic degradation, ensuring their higher retention over 3 weeks of storage. The elevated DPPH-RSA and ABTS-RSA in PCP can be attributed to two key factors. Firstly, beyond the stress response triggered by cutting, the pressure applied during SCCD processing may have further stimulated enzymatic activity and gene expression associated with phenolic compounds (Denoya et al., 2021; López-Gámez et al., 2021). Secondly, the high concentration of CO_2 contributed to phenolic accumulation (Gouda et al., 2023). Additionally, CO_2 may have activated AEs, which interact with FRs to avoid phenolic degradation (Gouda et al., 2023; Ghasemzadeh et al., 2010). The combined efficacy of pressure and CO_2 improved the phenolic compounds in PCP. Furthermore, SCCD treatment significantly decreased the activities of oxidative enzymes, maintaining phenolic stability during storage, thus leading to higher retention in treated samples.

4.6 Carotenoid profile

The impacts of SCCD processing on the levels of 9-Z-β-carotene, β-carotene, β-cryptoxanthin, α-carotene, lutein, and ε-carotene are illustrated in Figure 2 (a) (P2). Compared to CU, the α-carotene content in SC1 was lower, whereas SC2, SC3, and SC4 exhibited increased α-carotene levels. This phenomenon may be attributed to the combined efficacy of degradation and extraction induced by SCCD (Zhao et al., 2019). When treated at this processing condition, the amount of CO_2 in carrot cubes was inadequate for extracting the bound α-carotene. Under this condition, the degradation rate of α-carotene caused by SCCD treatment exceeded its extraction rate, leading to a reduction in α-carotene content. As the pressure elevated to 30 MPa, the penetration of CO_2 into the carrot cubes progressively improved, resulting in a higher accumulation of CO_2 within the cubes. The elevated CO_2 effectively disrupted the bonds between macromolecules and lutein, such as proteins and carbohydrates, thereby increasing lutein content. Nevertheless, as the temperature increased to 55℃, the α-carotene content in SC8, SC7, SC6, and SC5 gradually decreased. The decline can be attributed to two factors: (i) With rising temperatures, the extraction efficiency of SCCD reached its peak. At the same time,

the α-carotene oxidation continued in the 1 cm cubes, leading to a reduction in α-carotene concentration. (ii) Higher temperatures supplied additional energy for this oxidation reaction, accelerating its rate (Zhang et al., 2021).

In the case of 2 cm carrot cubes, the α-carotene content in SC11 and SC10 gradually increased as parameters increased. However, their α-carotene levels remained lower than those observed in UC (P2). This trend was similar to the results obtained for SC1 in 1 cm cubes. Moreover, except SC11, the α-carotene levels in 2 cm cubes were lower than those of 1 cm when processed at 35℃ under identical processing conditions. This finding can be explained by the fact that, although both 1 cm and 2 cm cubes have an equal total amount of CO_2 under identical parameters, the CO_2 concentration per cubic centimeter was lower in the 2 cm cubes than in the 1 cm cubes. As a result, the SCCD-induced extraction rate in α-carotene remained lower than its degradation rate, leading to a gradual accumulation of α-carotene. However, the overall α-carotene levels in 2 cm cubes were still lower than in UC. With increasing the temperature to 55℃, the α-carotene levels in SC15 and SC14 initially rose before subsequently declining. The result can be explained by elevated temperatures enhancing the entropy of CO_2, which improved α-carotene extraction from regions closer to the center of 2 cm cubes. A similar trend was also found for β-carotene and total carotenoid content (TCC). The α-carotene levels in SC9 were positioned between SC6 and SC5 in 1 cm cubes, while in 2 cm cubes, they fell between SC15 and SC16. These findings indicate that SCCD treatment slowed the decrease of α-carotene in 1.5 cm cubes in comparison with 1 cm cubes. Additionally, the levels of 9-Z-β-carotene, ε-carotene, β-cryptoxanthin, and lutein varied with changes in processing parameters. In 1 cm cubes, carotenoid content peaked under low pressure and temperature. Nevertheless, the maximum carotenoid levels in 1 cm cubes were lower than those observed in 2 cm cubes.

For pumpkin, the effect of SCCD processing on carotenoid profiles was displayed in Table 3 (P3). At 35℃, the β-carotene, TCC, α-carotene, and lutein in SCCD-TP were enhanced with rising pressure and extended processing time. Compared to UP, SCCD processing led to an increase in β-carotene, TCC, α-carotene, and lutein. The results can be due to the extraction efficacy of SCCD technique, which facilitates the release of these bound compounds (Ndayishimiye & Chun, 2017). Firstly, SCCD disrupts the cell wall and membrane, enhancing the transfer of these compounds from the intracellular to the extracellular fluid (Yuan et al., 2022). Secondly, CO_2 can interact with macromolecules via the hydrogen group, disrupting the hydrogen bonds between carotenoids and macromolecules, thereby facilitating the release of bound carotenoids (Miękus et al., 2019). Thirdly, high pressure can alter the structure of

macromolecules, thereby improving the release of carotenoids encapsulated within them (Chen et al., 2022). As the temperature rose to 55°C, the levels of β-carotene, TCC, α-carotene, and lutein in SCCD-TP progressively decreased as pressure and processing time increased. The decline may be attributed to carotenoid degradation induced by SCCD processing. Kostrzewa et al., (2021) demonstrated that the application of the SCCD technique for carotenoid extraction in sweet paprika resulted in a lower TCC in samples treated at 45 MPa compared to those processed at 35 MPa. Moreover, the ε-carotene and β-cryptoxanthin levels in SCCD-TP consistently decreased as processing parameters were increased, which suggested that SCCD negatively affected the ε-carotene and β-cryptoxanthin contents in pumpkins.

During 3-week of storage, the levels of β-carotene, β-cryptoxanthin, 9-Z-β-carotene, α-carotene, ε-carotene, and lutein in UCP exhibited a slight increase within the first three days, then gradually declined over the period from 3 to 21 days (P4). Notably, individual carotenoid levels reached their highest point in TCP on day 11, and their concentrations remained elevated compared to FCC and FCP. These findings suggest that SCCD processing plays a role in slowing carotenoid degradation and enhancing their accumulation during storage. Typically, the breakdown of carotenoids over time is affected by both enzymatic and chemical reactions (Meléndez-Martínez et al., 2023). Exposure to oxygen can trigger the oxidation of carotenoids, forming various oxidation products (Deng et al., 2022). SCCD inhibits this oxidative process by enhancing CO_2 penetration, effectively reduces oxygen in the tissue and suppressing free radical generation, thereby minimizing carotenoid oxidation (Song et al., 2018). Additionally, SCCD significantly suppressed enzymatic activity, sustaining this inhibitory effect over the entire 21-day storage. This reduction in enzyme activity contributed to mitigating the enzymatic degradation of carotenoids (Marszałek et al., 2016).

The accumulation of individual carotenoids during storage is probably due to the biosynthesis of carotenoids regulated by SCCD. Previous studies by Abbas et al. (2020) and Rodriguez-Concepcion et al., (2013) have demonstrated that in pumpkins and carrots, lycopene undergoes cyclization, leading to the formation of β-carotene and α-carotene. Moreover, α-carotene undergoes hydroxylation to produce lutein, whereas β-carotene undergoes both hydroxylated and methylated, resulting in the formation of β-cryptoxanthin (P4) (Song et al., 2024; Xu et al., 2021). Based on this biosynthesis pathway, the levels of β-carotene, α-carotene, β-cryptoxanthin, and lutein exhibited a positive correlation with lycopene content. In SCCD-processed samples, the maximum levels of α-carotene and β-carotene were higher than those in the control group (Figure 4 in P4), suggesting that lycopene synthesis continued during storage.

Lycopene is formed from phytoene through an addition reaction (Figure 4 in P4) (Klein et al., 2015). As a key precursor in this pathway, glucose is generated via condensation reactions and glycolysis, establishing a positive correlation between sugar metabolism and carotenoid biosynthesis. The primary sources of glucose include the hydrolysis of starch and sucrose (Abbas et al., 2020).

4.7 Sugar content

Figure 2 (b) displays the sugar contents of fresh-cut carrot cubes (2 cm and 1 cm) subjected to SCCD processing (P2). The total sugar content (TSC) in 1 cm SCCD-TC exhibited a slightly decreasing trend, whereas in 2 cm SCCD-TC, TSC initially increased before declining as processing parameters intensified. The observed differences between 2 cm and 1 cm cubes can be explained by carbon dioxide penetration and heat transfer variations, which are influenced by cube size. Compared to UC, the TSC in SC13, SC12, SC11, SC10, SC9, SC4, SC3, SC2 and SC1 was higher. This increase in the TSC in SCCD-TC can be attributed to SCCD processing, which facilitates the release of the macromolecules (Cappelletti et al., 2015). However, when the temperature reached 55 °C, a slight reduction in TSC was observed in SC8, SC7, SC6, and SC5 compared to the UC, likely due to the combined efficacy of high temperature and pressure (Silva et al., 2019). Moreover, the TSC in SC8 was 5% lower than that of the UC, whereas SC17 displayed no statistically significant changes. The maximum increases in TSC observed for the 2 cm and 1 cm carrot cubes were 4.2 % and 2.6 %, respectively. Regarding individual sugar, glucose levels in SCCD-TC varied with the changes in processing parameters, while the concentrations of other sugars, including other carbohydrates, sorbitol, fructose, and sucrose, remained unchanged. These findings align with the results reported by Marszalek et al. (2018) and Cappelletti et al. (2015).

Table 4 presents the sugar contents in UP and SCCD-TP (P3). As processing parameters increased, sucrose and fructose levels in SCCD-TP progressively decreased. In comparison to UP, sucrose and fructose levels in SP13 decreased by 26% and 8%, respectively. The decline can be due to hydrolysis reactions induced by SCCD technique. Dissolving CO_2 in water creates an acidic environment, facilitating the hydrolysis of sucrose into glucose (Marszałek et al., 2015). Additionally, when the temperature was at 35°C, TSC, other polysaccharides, sorbitol, and glucose of SCCD-TP rose with elevating pressure and extending the treatment time. The rise in glucose levels further confirms that SCCD technique promotes sucrose hydrolysis. Polysaccharide levels increased due to the hydrolysis of hemicellulose, cellulose, and pectin into lower-molecular-weight polysaccharides (Marszałek et al., 2015). As the temperature

reached 55 °C, the TSC, other polysaccharides, sorbitol, and glucose in SCCD-TP exhibited a slight declining trend. Nevertheless, compared to UP, no significant changes were observed in TSC, other polysaccharides, sorbitol, and glucose in SP13. Marszałek et al. (2015) also reported that SCCD-processed strawberry juice had higher TSC, fructose, and glucose compared to the control sample. The author also found that as pressure rose to 60 MPa, TSC, fructose, and glucose levels in the processed samples further increased (Marszałek et al., 2015).

During storage, sucrose content in all samples exhibited a gradual decrease trend over time (P4), probably because of its metabolism into fructose and glucose through respiration (Wang et al., 2024). In UC, fructose and glucose levels initially enhanced from day 0 to day 3 before gradually declining. Conversely, SCCD-TC continuously increased in fructose and glucose, reaching peak levels on day 15 of storage. Several factors may account for the delayed attainment of the peak value. Firstly, the penetration of CO_2 into cubes probably displaces O_2, thereby suppressing the generation of ROS (Wang et al., 2022). A lower ROS concentration reduces the need for reducing sugars, such as fructose and glucose, to participate in free radical scavenging, thereby decreasing their consumption. Secondly, SCCD processing led to a reduction in oxidase activity within cubes, keeping it at lower levels throughout storage, which contributed to preserving nutrients. Thirdly, elevated CO_2 concentration may regulate key enzymes associated with metabolic pathways, thus slowing the respiration rate and reducing the consumption of nutritional components (You et al., 2023). When the storage time increased from day 0 to day 3, a remarkable decline was noted in fructose and glucose levels of SCCD-TP in comparison to SCCD-TC. This variation can probably be attributed to differences in sugar metabolism between carrots and pumpkins. At the start of storage, the sucrose content in carrots was higher, undergoing hydrolysis to produce fructose and glucose. However, pumpkins had a lower sucrose concentration, making starch hydrolysis the primary glucose production pathway, which was converted into fructose through isomerization. In pumpkins, this process was more energy-intensive and time-consuming than in carrots. As a result, the fructose and glucose synthesis rate was slower than their metabolic consumption, leading to a significant reduction in their levels of SCCD-TP during the initial storage period. Moreover, no sorbitol was detected in FCC and FCP either before or after SCCD processing. Nevertheless, after three days of storage, sorbitol appeared in both UCP and PCP, likely due to glucose undergoing catalytic hydrogenation to form sorbitol. During storage, sorbitol levels in UCP first enhanced and then declined slightly, whereas in TCP, sorbitol content progressively increased throughout the storage period. A higher sorbitol concentration in TCP than in UCP indicates that SCCD

processing promoted the metabolism of upstream precursors, thereby enhancing sorbitol synthesis throughout storage.

5. Observations and Conclusions

This study investigated the effects of supercritical carbon dioxide (SCCD) on the enzyme activities, physicochemistry properties, phenolic compounds, carotenoids and sugars of carrots and pumpkins at various parameters and storage periods. The results were as follows:

1. Regardless of the size of carrot cubes, PPO and POD activities gradually declined as the processing condition increased, indicating that the SCCD technique can effectively decrease enzyme activity. Moreover, the nutritional profile of 1cm and 2 cm SCCD-treated carrot cubes (SCCD-TC) exhibited fluctuations. At 35°C, the nutrient contents in 1 cm SCCD-TC increased more rapidly than those of 2 cm SCCD-TC. In contrast, as the temperature rose to 55°C, the nutrient contents in 1 cm SCCD-TC declined sharply, whereas in 2 cm SCCD-TC, it initially increased before gradually declining. Processing 1 cm carrot cubes under 10 MPa, 35°C, and 45 minutes may be the most effective condition for maximizing enzyme inactivation while maintaining nutrient contents, based on enzyme activity and nutritional profile evaluations. Additionally, for companies seeking to produce fresh-cut products using larger (2 cm) cubes, processing at 10 MPa, 55°C, and 45 minutes represents a practical parameter choice.

2. Although PPO and POD activities in SCCD-processed pumpkin cubes (SCCD-TP) decreased compared to the untreated pumpkin (UP), the reduction was not statistically significant. This minor decrease may be due to variations in enzyme types, content, and densities among raw materials. Additionally, the L value declined, while the a, b*, and ΔE values showed an increasing trend. SCCD processing significantly enhanced the total carotenoid, total sugar, glucose, β-carotene, lutein, sorbitol, other polysaccharides, and α-carotene contents in SCCD-TP at 35°C. Nevertheless, at 55 °C, SCCD treatment resulted in a noticeable decline in both carotenoid and sugar content, probably due to the degradation and extraction effects of the process. Moreover, the $ABTS^{+\cdot}$, $DPPH\cdot$, $O_2^{-\cdot}$, and total phenolic content (TPC) in SCCD-TP exhibited a fluctuation trend. The levels of coumaryloquinic acid I and II, p-coumaric acid, 4-hydroxybenzoic acid, and caffeic acid glucoside in SP7 and SP5 were notably higher than those in UP. Conversely, the levels of quercetin 3-O-rutinoside, vanillic acid and quercetin 3-O-pentoside in SP7 and SP5 were reduced.

3. During storage, the microbial load in SCCD-processed carrots and pumpkins (PCP) was significantly reduced compared to the unprocessed carrots and pumpkins (UCP), indicating that SCCD technique efficiently inhibit microbial growth during storage. Furthermore, the PPO and POD activities of PCP did not change significantly after three days of storage and maintained at low levels. The minor variations in microbial growth and enzymatic activity between

pumpkins and carrots might be linked to the differ free water distribution within extracellular and intracellular compartments. PCP exhibited higher maximum levels of carotenoids, antioxidant activity, and total phenolic content than UCP, indicating that SCCD treatment effectively slowed the degradation of carotenoids and phenolic compounds while enhancing their accumulation throughout storage. Moreover, SCCD treatment promoted sucrose hydrolysis in carrots, indicating that it may improve the metabolism of upstream substrates, thereby facilitating the synthesis of downstream compounds. In pumpkins, where sucrose levels were relatively low, starch was directly hydrolyzed as the primary upstream substrate.

6. References

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Publications

Publication 1: Chen Z., Spilimbergo S., Khaneghah M. A., Zhu Z.Z., Marszałek K. (2022). The effect of supercritical carbon dioxide on the physiochemistry, endogenous enzymes, and nutritional composition of fruit and vegetables and its prospects for industrial application: An overview. Critical Reviews in Food Science and Nutrition. Pages: 71-86.
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Publication 2: Chen Z., Kapusta I., Zhu Z. Z., Marszałek K. (2024). Enzyme activity and nutritional profile of different-sized carrot cubes treated with supercritical carbon dioxide. Postharvest Biology and Technology. Pages:87-96
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